Agricultural practices impact on soil quality and health : case study of slovenian irrigated or organic orchards

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Influence de différentes pratiques agricoles sur la qualité et la santé des sols

Etude de cas sur des vergers slovènes irrigués ou en agriculture biologique

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Agricultural practices impact on soil quality and health
Case studies of Slovenian irrigated or organic orchards

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SUMMARY

Underestimation of soil properties and poor understanding of soil conditions can have many negative consequences, which results in quality or quantity of yield, soil degradation or even environmental pollution. According to importance of agricultural practices, our study focused on their impact on soil quality and health. The research took place from November 2003 to October 2007 in apple orchards in northeaastern Slovenia where two frequent agricultural practices were investigated: (i) drip irrigation on Calcaric Cambisol and its effects on structural stability and microbial biomass at Gačnik experimental station and (ii) combination of organic fertiliser (Campo guano) and liming in organic farming to enhance microbial biomass and nitrogen nutrition at Pohorshi dvor on Dystric Cambisol. The presence of faecal pathogens in the soil due to irrigation or organic fertilizer was also investigated.

Water potential was measured during two seasons in both locations. Structural stability according Bartoli method, organic mater characteristics (including grain size organic matter fractionation and isotopic signature of organic carbon origin), and microbiological parameters were analysed as potential indicators of soil quality in irrigation practice comparing an irrigated (IR) to a non-irrigated (NIR) row. In organic farming, mainly pH and microbiological parameters were followed according treatments on an experimental blocks comparison. According to hilly terrain and land levelling in Gačnik, we were dealing with two groups of soil differing in thickness, organic matter, and calcium carbonate contents: one at upslope and another at mid and downslope. Considering soil characteristics, slope effect was more expressed than irrigation effect. According to slope, water gravimetric content (W), organic matter (OM), microbial biomass (MB), and respiration (R) increased towards downslope while total carbonates (Ca) and structural stability (SS) decreased. According to irrigation, W, OM, and SS contents decreased, while MB and R increased from NIR to IR rows. No difference was observed for Ca between treatments. According to slope, higher carbonate content was as an important factor for higher structural stability as organic matter pool. According to irrigation, lower W in IRR row could be explained by modification in root distribution due to drip irrigation. Irrigation leads to an increase of soil microbial biomass and its activity (as a short-time effect) and decrease of OM (as a long-term effect); moreover, a decrease of OM originating from the marl bedrock was observed in IRR row and attributed to microbial mineralization. Lower SS of IRR row is related to the OM reduction. Seasonal variations of structural stability show complex trends resulting from the combination of climatic conditions and biological activity. In organic fertilising study, the interaction of Compo guano and lime together was not clear, but in long term this is probably the best solution because it had positive consequences on both soil pH and available nitrogen, while preserving fair levels of MB and labile organic matter (LOM). Irrigation water and Compo guano were considered as eventual sources of faecal coliforms which remains in soils. From our study it was concluded that OM, MB, R and faecal coliforms can be treated as general useful indicators in assessing soil quality. According to agricultural practice, SS should be emphasized as an important quality indicator in irrigation practice and pH in organic farming.

Key words: Calcaric Cambisol, Dystric Cambisol, soil quality, soil health, orchards, drip irrigation, slope, organic fertilizing, liming, structural stability, fresh and sedimentary organic matter, microbial biomass, pathogens.

RÉSUMÉ ÉTENDU

Une mauvaise connaissance des propriétés des sols et de leur fonctionnement peut avoir de nombreuses conséquences néfastes sur le rendement et la qualité des récoltes, sur la dégradation des sols et sur une pollution de l'environnement. En raison de l'importance des pratiques agricoles, notre étude s'est focalisée sur leur impact sur la qualité et la santé des sols. La recherche s'est effectuée de novembre 2003 à octobre 2007 sur des vergers de pommiers implantés sur des collines dans le nord-est de la Slovénie. Deux pratiques agricoles fréquentes dans cette région ont été suivies : (i) une irrigation localisée au goutte à goutte sur des Calcaric Cambisol (CALCOSOL) développés sur marnes, et ses effets sur la stabilité structurale des sols et leur biomasse microbienne à la Station expérimentale de Gačnik et (ii) la combinaison d'un engrais organique (Compo guano) et d'un amendement calcaire dans un verger conduit en agriculture biologique à Pohorski dvor sur un District Cambisol (ALOCRISOL) développé sur schistes. La présence de microbes pathogènes fécaux dans le sol, dus à l'irrigation ou à l'apport d'engrais organiques animaux a aussi été recherchée sur les deux sites.

Le régime hydrique du sol a été suivi durant deux étés par des relevés tensiométriques hebdomadaires sur les deux sites. A la station expérimentale de Gačnik, un rang irrigué a été comparé à un rang non irrigué. La teneur en matière organique totale, son fractionnement granulométrique et la signature isotopique des différentes fractions permettant de discuter de leur origine et leur turn over ont été mesurés. La biomasse microbienne et son activité ont été caractérisées au printemps et à l'automne en 2004 et 2005. La stabilité structurale a été mesurée selon la méthode de Bartoli à l'automne 2004 et au printemps 2005. Sur le verger conduit en agriculture biologique à Pohorski dvor seul le pH et les paramètres microbiologiques ont été suivis selon la même périodicité en comparant les différents traitements dans une expérimentation par blocs. Enfin, sur les deux sites, une quantification des champignons, des bactéries (aérobies, anaérobies, coliformes fécaux) et des virus présents dans le sol a été réalisée.

Incidence de l'irrigation par goutte à goutte sur la qualité du sol sur le site de Gačnik sur la qualité physique du sol- Les sols de ce verger, argilo-limoneux et carbonatés, varient fortement de l'amont à l'aval de la parcelle située sur une pente de 15%. le sol est peu épais à l'amont, la marne altérée apparaissant dès 60 cm tandis qu'à mi-pente et à l'aval le sol est épais >1 m et la marne plus fortement altérée. L'observation des profils pédologiques et l'historique de la parcelle montrent que le sol a été fortement remanié sur les 60 premiers centimètres préalablement à la plantation du verger. Le passage d'une plantation en terrasses à une plantation dans le sens de la pente a conduit à l'effacement des terrasses suivi d'un labour profond dont en voit encore la trace à 60 cm de profondeur à l'aval de la parcelle *(Fig. 3.3 & Tab. 3.9)*. Un échantillonnage systématique de la teneur en carbone organique de l'horizon de surface, selon un pas de 6 m, montre un accroissement selon la pente suivant une forme en zig-zag reflétant la trace des anciennes terrasses *(Fig. 3.19)*. Dans les 30 premiers centimètres la teneur en matières organiques, le rapport C/N et la capacité d'échange cationique augmentent de l'amont vers l'aval tandis la teneur en carbonates de calcium décroît *(Fig. 3.15)*. Le pH reste stable entre 8 et 8, 4. A la surface du sol dans les rangs de plantation traités par désherbage chimique, une croûte alguaire se forme sur le côté ombragé du rang.
L'effet de la pente va être dominant sur les caractéristiques fonctionnelles du sol : la teneur en eau pondérale (W) *(Tab. 3.25 & 3.26)*, la biomasse microbienne (MB) *(Fig. 3.42)* et sa respiration (R) augmentent vers l'aval ; tandis que la stabilité structurale (SS) diminue *(Tab. 3.25 & 3.26; Fig. 3.44)*. L'effet de l'irrigation se perçoit cependant : en comparant le rang non

irrigué au rang irrigué, on observe une baisse de la teneur en eau pondérale *(Fig.3.27 à 3.30)* et de la stabilité structurale *(Fig. 3.45)* tandis que seule la fraction grossière de la teneur en matière organique et son rapport C/N augmentent *(Tab. 3.17 & 3.18)* de même que la biomasse *(Fig. 3.31, 3.33, 3.40, 3.42)* et la respiration microbienne *(Tab. 3.23 & 3.24)*. L'irrigation en revanche n'a pas d'influence sur la teneur en carbonates de calcium *(Tab. 3.16)*.

La variation de la stabilité structurale en fonction de la pente s'explique par les contributions respectives du carbonate de calcium et de la matière organique qui se compense entre l'amont et l'aval. En fonction de l'irrigation, la teneur en eau pondérale plus faible en surface dans le rang irrigué pourrait s'expliquer par la modification de l'architecture du système racinaire du pommier provoqué par l'irrigation au goutte à goutte. L'irrigation conduit à une augmentation de la biomasse microbienne et de son activité à court terme et à long terme, à une réduction de la teneur en matières organiques. Nous avons de plus constaté, grâce à la signature isotopique du carbone sur les différentes fractions granulométriques *(Tab. 3.31et Fig. 3.24)* (i) qu'une partie du pool de matière organique du sol provenait de l'altération de la marne, (ii) que cette matière organique d'origine sédimentaire était majoritairement présente dans la fraction granulométrique la plus fine et dominait à l'aval du versant et enfin (iii) que l'irrigation, en augmentant la biomasse microbienne et son activité, conduisait à une minéralisation d'une partie de ce stock de carbone d'origine sédimentaire. La stabilité structurale plus faible du rang irrigué doit être reliée à la baisse de la teneur en matière organique de ces rangs. Les variations saisonnières de la stabilité structurale en fonction de la position dans le versant et du traitement irrigué ou non sont complexes et résultent de la combinaison de l'effet du climat de l'année de l'activité biologique.

Incidence de la fertilisation organique et du chaulage - Concernant la fertilisation organique, l'apport conjoint de Compo Guano et d'un amendement calcaire sur ce sol limono-argileux acide est vraisemblablement le meilleur compromis sur le long terme sur la qualité chimique et biolobique du sol parce qu'il présente des effets positifs à la fois sur le pH et sur l'azote disponible tout en maintenant à un bon niveau la biomasse microbienne et la teneur en matière organique labile.

L'irrigation par des eaux de mauvaise qualité biologique et l'apport de Compo Guano doivent être considérés comme des sources de coliformes fécaux, voire de virus qui vont se maintenir ensuite dans le sol.

De notre étude, nous pouvons conclure que la teneur en matières organiques, la biomasse et la respiration microbienne, et la présence de coliformes fécaux dans le sol peuvent être de bons indicateurs de la qualité des sols. Concernant les deux pratiques agricoles étudiées, la stabilité structurale devrait être considérée comme un indicateur pertinent concernant l'irrigation et le pH en agriculture biologique sur des sols acides.

Mots clés : CALCOSOLS, ALOCRISOLS, qualité des sols, santé des sols, vergers, irrigation au goutte à goutte, agriculture biologique, chaulage, stabilité structurale, matière organique sédimentaire, biomasse microbienne, bactéries pathogènes

POVZETEK

Podcenjevanje talnih lastnosti in slabo poznavanje kondicije tal lahko negativno vplivajo na kakovost in količino pridelka ter prispevajo k degradaciji tal ali celo k onesnaževanju okolja. Glede na pomembnost agrotehničnih ukrepov, smo se v raziskavi osredotočili na njihov vpliv na kakovost in zdravje tal. Raziskava je potekala od novembra 2003 do oktobra 2007 v nasadu jablan v severovzhodni Sloveniji, kjer smo v raziskavo vključili dva pogosta agrotehnična ukrepa: (i) kapljično namakanje tal na evtričnih karbonatnih tleh in vpliv le-tega na strukturno stabilnost tal in mikrobno biomaso na poskusni lokaciji Gačnik in (ii) kombinacijo organskega gnojenja (Compo guano) in apnenja na distričnih rjavih tleh v ekološkem nasadu Pohorski dvor, z namenom povečati mikrobno biomaso in prehrano z dušikom. V namakalni vodi oz. v organskem gnojilu smo ugotavljali prisotnost fekalnih patogenov. Na obeh lokacijah smo v dveh sezonah merili vodni potencial tal. Analizirali smo strukturno stabilnost tal po Bertolijevi metodi, določili vsebnost organske snovi (vključno s fizikalno frakcionacijo organske snovi ter z določanjem izotopskega značaja izvora organskega ogljika) in mikrobiološke parameter kot potencialne indikatorje kakovosti tal v namakalni praksi, s primerjavo namakane (IRR) in nenamakane (NIR) vrste. V ekološkem nasadu smo spremljali predvsem pH tal in mikrobiološke parametre glede na različna obravnavanja v bločnem poskusu. Glede na hriboviti teren in opravljeno izravnavanje tal v Gačniku, smo imeli opravka z dvema različnima skupinama tal glede na globino, vsebnost organske snovi in kalcijevega karbonata: (i) prvo skupina na vrhu pobočja in (ii) drugo skupino na sredini ter ob vznožju pobočja. Naklon terena je imel močnejši učinek na talne lastnosti kot namakanje. Glede na naklon terena, so se gravimetrična vsebnost vode (W), vsebnost organske snovi (OM) in mikrobne biomase (MB) ter nivo mikrobnega dihanja (R) večali v smeri proti vznožju pobočja, medtem ko so se skupni karbonati (Ca) in stabilnost strukturnih agregatov (SS) večali proti vrhu pobočja. Namakanje (IRR) je v nasprotju s kontrolo (NIR) povzročilo zmanjšanje W, OM in SS vrednosti ter porast MB in R. V vsebnosti Ca med IRR in NIR nismo opazili razlik. Glede na naklon terena, je za večjo strukturno stabilnost zraven organske snovi pomembna tudi vsebnost karbonatov. Manjšo W v namakanih tleh (IRR) lahko pojasnimo s spremembno v razporeditvi koreninskega sistema kot posledico kapličnega namakanja. Namakanje povzroča porast MB in njene aktivnosti (kratkotrajni učinek) ter zmanjšanje skupne OM (dolgotrajni učinek), nadalje pa vpliva tudi na zmanjšanje OM, ki izvira iz lapornate matične podlage in je povezano z mikrobno mineralizacijo. Nižja SS v IRR vrsti je povezana z redukcijo OM. Sezonska variabilnost stabilnosti strukturnih agregatov kaže na kompleksni potek, odvisen od kombinacije klimatskih razmer in biološke aktivnosti. V raziskavi z organskim gnojenjem, interakcija Compo guana in apnenja ni bila pojasnjena, vendar dolgoročno najverjetneje predstavlja najboljšo rešitev zaradi pozitivnega učinka na pH tal terdostopni dušik ob ohranjanju zadovoljivih vrednosti MB ter labilne organske snovi (LOM). Namakalna voda in Compo guano predstavljata potencialna vira za fekalne koliformne bakterije, ki ostajajo v tleh. V naši raziskali smo ugotovili, da lahko OM, MB, R in fekalne koliformne bakterije uporabimo kot primerne splošne indikatorje za ocenjevanje kakovosti tal. Glede na vrsto agrotehničnih ukrepov, je treba kot pomemben kazalec kakovosti tal v namakalni praksi pudariti SS, medtem ko je v ekološki pridelavi pomemben pH.

Ključne besede: tla, evtrična rjava tla, distrična rjava tla, tla, kakovost tal, zdravje tal, nasadi, kapljično namakanje, naklon terena, organsko gnojenje, apnenje, stabilnost strukturnih agregatov, sveža in sedimentirana organska snov, mikrobna biomasa, patogeni.

ABBREVIATIONS

AFNOR - Association Française de NORmalisation
APHA – American Public Health Association
AW - Available Water
bl - block
BOD - Biochemical Oxygen Demand
Corg - organic Carbon
CEC - Cation Exchange Capacity
CFU - Colony Forming Units
CIVC - Le Comité Interprofessionnel du Vin de Champagne
Cmin - CO_2, released by microbial respiration
cmol – centimol
COD - Chemical Oxygen Demand
cv. – cultivar
e – void ratio
e_c - clods void ratio
e_T - total void ratio
EC – Electrical Conductivity
E. coli - Escherichia coli
EDTA - Ethylenediaminetetraacetic acid
EEC - Hi Crome Selective agar
ENESAD - Etablissement National d'Enseignement Supérieur Agronomique de Dijon
FAO - Food and Agriculture Organization
FC - Field Capacity
HEM - hemicellulose
HEV - Hepatitis E
hPa - hectoPascal
INRA - Institut National de la Recherche Agronomique
IRR - irrigation, irrigated
ISO - International Organization for Standardization
kPa – kiloPascal
LOM - Labile Organic Matter pool
MB – Microbial Biomass
mM – millimol
MPN - Most Probable Number
M9 - dwarfing tree rootstock
NIR - non irrigated
Nmin - mineral Nitrogen
Norg - organic Nitrogen
OC – organic Carbon
OM - Organic Matter
ÖNORM – Austrian national standard
PDA - Potato Dextrose Agar
PDB – Potato Dextrose Broth
pF - cologarithm of water tension expressed in water height pressure in cm

r - correlation coefficient
R^2 - coefficient of determination
RR - rainfall quantity
S - degree of saturation
S - sum of basic cations
S/T - base saturation rate
SAR - Sodium Adsorption Ratio
spec. resp. - specific respiration
SOC – Soil Organic Carbon
SOL – Soluble organic matter fraction
SOM - Soil Organic Matter
SWRC - Soil Water Retention Curve
TBS - tetrabromo-2-benzotriazole
TOC - Total Organic Carbon
USEPA - United States Environmental Protection Agency
W - gravimetric water content
WHO - World Health Organization
WP - Wilting Point
WSA – Water Stable Aggregates
γ_d - total bulk density
γ_{dc} - clod bulk density
Ψ- water potential
σ - standard deviation
υ - water ratio

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General introduction

GENERAL INTRODUCTION

The research, which took place from November 2003 to October 2007, was focusing on the impact of different agricultural practices on soil quality in apple orchards in North-East Slovenia. For this reason, two important agricultural practices - irrigation and organic fertilising - were chosen, in different apple productions - integrated and organic and on two different soil types - Calcaric and Dystric cambisol. For improving the yield, irrigation is almost necessary agricultural practice in Slovene fruit production, while organic farming represents an alternative way to produce more natural food with low chemical input. Both of these practices are supported by Slovene government and the extent of both is getting bigger every year. Both have some advantages and disadvantages for fruit production and for the environment, especially if they are not performed well. The main idea was to study two different practices with completely different soil types to have a wider view of soil behaviour in orchards and try to find the most appropriate solution of applied agrotechnical techniques on soil quality. Instead of focusing on yields, as in most agricultural studies, we focused on soil, with emphasis on basic soil microbiological properties. In our study we are trying to make more comprehensive assessment for soil quality by searching for some useful soil indicators for orchards.

In the first part we are dealing with irrigation practice including slope and their effects on soil properties, especially on soil structure and soil microbes. The second part is dealing with the possibility of enhancing microbial biomass and nitrogen nutrition with different combinations of organic fertiliser and liming.

In both experiments, micro organisms play the main role in soil quality and soil health, which might affect both yield and environment. Also their role in food safety and human health is important, because, as already stated, irrigation water or organic fertilizers can be the potential sources of some dangerous pathogens. The challenge is therefore to improve knowledge of soil science and soil microbiology in agricultural practices, not just for scientific purposes but also to share both basic and practical knowledge with farmers and consumers.

Despite the different problems, we have just one common goal: to remain good soil condition for sustainable plant production and minimise the risk to produce infected fruit by pathogens (food safety). Both aspects of the study are complex, so the main objective of this thesis is to initiate a huge work and to issue some guidelines for further investigation.

By analysing some physical and microbiological properties in one realistic field experiment with irrigation system, it is possible to show some new perspectives to the producers and more attention might be paid to some important soil facts, which were overlooked before. For improving benefits of irrigation systems, we need a more comprehensive approach for estimating soil quality. This evaluation requires focusing not only on chemical (fertility) considerations, but also on the dynamic soil conditions – a combination of physical, biological, and chemical characteristics, which are directly affected by recent and current land use decisions and practices.

In relation with irrigation, the aims of our project are:

- to define the effect of irrigation and slope on soil physics, chemistry and soil microbiology;
- to determine the connections between microbial indicators and (especially) physical properties of soils, with the interpretation of the results in the case of irrigation;
- to propose microbial indicators which could be useful for assessing soil quality, health and sustainability in the case of irrigation;
- to contribute to the knowledge of side-effects of irrigation on soil ecology;
- to enable a comprehensive approach, including soil physical and microbial properties;
- to control irrigation water and soil for possible water or soil-born pathogens.

We will intend to examine the following hypotheses:

1. Irrigation and slope affect soil microbial biomass and microbial activity.

2. Irrigation and slope affect soil physical parameters (bulk density, structural stability, water regime), and organic matter dynamics.

3. The relationships between soil physical properties and soil microbial parameters are controlled by water supply and slope.

4. Microbial and physical soil indicators are appropriate for assessing soil quality in case of irrigation.

5. Irrigation water can represent a potential source of pathogens.

To improve nitrogen nutrition in organic plant production, it is important to focus on microbes, which enable the release of available nitrogen forms in soil. In organic production, the use of inorganic nitrogen fertilisers is not allowed, so plant nitrogen nutrition represents one of the important problems in organic plant production. Organic farmers are replacing service with direct available forms of nitrogen in artificial fertilisers with the application of organic fertilisers, which have indirect an affect on nitrogen nutrition via microbial mineralization processes. To optimise the effectiveness of these processes, the basic conditions (pH, humidity, aeration, C/N) for microbial activity should be improved. Our attention should be focused first on basic soil properties and their improvement, in view of getting as good as possible conditions for successful microbial activity. Another important thing is to apply the appropriate form(s) of organic fertilisers: fertilisers obtained from treated organic matter are safer in sense of having dangerous pathogens.

In relation with organic fertilising, the aims of our project are:

- to elucidate the effect of applied organic matter on nutrient cycling, soil chemistry and soil microbiology (microbial biomass and microbial activity);
- to elucidate the effect of organic mater (used as fertilizer) and liming on soil pH and mineralization processes;
- to propose microbial indicators which could be useful for assessing soil quality and soil health in orchards;
- to compare behavior of microbes under organic fertilizing and liming in two different soil types according to water conditions;
- to control presence of some pathogens in soils.

We will intend to examine the following hypotheses:

1. Organic fertilisers and liming increase soil microbial biomass and its activity.
2. Organic matter and liming affect soil pH and mineralization process.
3. Organic fertilising and liming have different effect on soil microbes in different soil types (according to water conditions).

Within the framework of co-mentorship between France and Slovenia, my research program has been carried out in these two countries. It involved the University of Burgundy (Université de Bourgogne, Dijon) and the University of Maribor (Univerza v Mariboru). This project was supported by grant from the French Embassy in Slovenia, INRA-Dijon, University of Burgundy and AgroSup Dijon in France, the University of Maribor, Faculty of Agriculture and Life Sciences in Slovenia.

Chapter 1

Literature review

Chapter 1: Literature review

Interest in evaluating the quality and health of our soil resources has been stimulated by increasing awareness that soil is a critically important component of the earth's biosphere, functioning not only in the production of food and fibre but also in ecosystems function and the maintenance of local, regional, and global environmental quality (Glanz, 1995). With the suspected increasing degradation of agricultural soils, there is a great need for sustaining the soil resource and enhancing soil quality. An assessment of the impact of changing soil management should therefore ideally include some measure of soil health or quality, as this is inseparable from issues of sustainability (Doran and Safely, 1997). In this way, soil quality has become a tool for assessing the sustainability of soil management systems (Schloter *et al*., 2006). Soil quality is increasingly proposed as an integrative indicator of environmental quality (National Research Council, 1993), food security (Lal, 1999) and economic viability (Hillel, 1991). Therefore, soil quality assessment is also a useful indicator of sustainable land management.

I. Definitions of soil quality and soil health

The soil is a basic source of agricultural production. It represents at the same time the area of the important life processes and the living place for many different living organisms (Atlas and Bartha, 1997). Being a complex medium, soil is quite difficult to evaluate.

In the past the identification of fertility was based on the capacity to support agricultural production. Nowadays, soil quality includes a broad range of functions and services it performs. A variety of definitions have been proposed for the term of soil quality by soil scientists in the last decades (de Han *et al*., 1990; Chaussod, 1996; Máté and Tóth, 1996; Bouma, 1997; Doran and Safley, 1997; Karlen *et al*., 1997; Sojka and Upchurch, 1999; Davidson, 2000; Doran and Zeiss, 2000; Loveland and Thompson, 2001; Tóth *et al*., 2007), ranging from a purely agricultural point of view to a more environmental perspective. The Soil Science Society of America (SSSA) and Karlen *et al*. (1997) had proposed a definition, which presents an integration of scientific knowledge with practical approach. The description of soil quality by SSSA as "The capacity of a specific kind of soil to function, within natural or managed ecosystems boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitations" can be regarded as one of the most comprehensive definition. Soil quality can be briefly defined as the sustainability of a soil for a specific use (Gregorich *et al*., 1994).

Soil quality is an increasingly popular concept, especially for its biological aspects, and "soil health" is even used as a synonym in some countries. The biological characteristics of soils are in close interaction with physical and chemical properties on one hand, and are more or less modified by human activities on the other (Chaussod, 2002). For cultivated soils, it is possible to identify four main aspects of quality: 1) fertility (ability of the soil to provide

crops with nutriments and other growth factors), 2) sanitary aspects (contamination by pathogens, pests or weeds), 3) environmental aspects (impact of soil functioning on other compartments of the ecosystem – e.g. effect on water and air quality) and 4) resilience (ability to resist and recover all biological activities after a physical or chemical stress) (Chaussod, 1996).

According to Tóth *et al*. (2007), soil quality refers to its ability to provide ecosystem and social services and to maintain such functions under changing conditions. The concept of soil quality expressed by this definition allows practical applications with regards to targeted social and/or ecosystem services. The evaluation scheme has to consider the two basic elements of soil quality: (1) functional ability and (2) response properties. These two elements reveal the (1) capacity to perform a function under given conditions and the (2) range of the functioning capacity under changing conditions. Soil quality evaluation must therefore be performed with special regards to the goal of the assessment (Toth, 2008).

The term soil health is preferred by some (Doran *et al*., 1996; Doran and Safley, 1997), because it portrays soil as a living, dynamics system whose functions are mediated by diversity of living organisms. Good management and conservation practices are required because soil health, biodiversity, and soil resilience are sensitive to anthropogenic disturbance (Freckman and Virginia, 1997). Balance between soil function for productivity, environmental quality, and plant and animal health is needed for optimal soil health (Doran, 2002). The concept of soil health includes the ecological attributes of the soil which have implications, beyond its quality, on its capacity to produce particular crops. These attributes are chiefly those associated with the soil biota: diversity, food web structure and activity for a range of functions (Pankhurst *et al*., 1997). Soil biodiversity *per se* may not be a soil property that is critical for the production of a given crop, but it is a property that may be vital for the continued capacity of the soil to support that crop (Pankhurst *et al*., 1997). Current technologies for increased agricultural production have largely ignored this vital management component.

Soil health focuses more on the biotic components of a soil, reflecting the maintenance of soil organisms and their proper functioning as regulators of nutrient cycling and soil fertility. In the related literature both terms are intermingled and it can be considered that soil quality encompasses soil health (Anderson, 2003). Soil biota can increase or reduce agricultural productivity, depending on its composition and the targets of its different activities (Blume *et al*., 2002; Degens *et al*., 2000). Conversely, farming practices modify soil life, including the total number of organisms, the diversity of species and the activity of these organisms, including the formation of aggregates by soil biota. These changes can be beneficial or detrimental to the soil biota and its functions and its regenerative capacity. The improved management of soil biota could play a vital role in maintaining soil quality (or health) and in achieving the goals of agricultural production and food security through sustainable land use and land resource management (Convention on Biological Diversity, 2001). Soil quality assessment, and especially the identification of key soil properties which can serve as indicators of soil health, became a major issue for land managers and for food producers throughout the world.

II. Indicators of soil quality and soil health

The success of management in maintaining soil quality depends on our understanding of how soil responds to agricultural use and practices over time (Gregorich *et al*., 1994). Therefore, methods to quantify soil quality must assess changes in selected soil attributes over time. However, soil quality cannot be measured directly from the soil alone, but is inferred from soil characteristics and behaviour under defined conditions. Furthermore, there is no single measurement that can quantify soil quality (Stewart, 1992), but there are certain soil properties that could be good indicators when considered together.

Understanding the response of soils to agricultural practices over time helps to evaluate whether the investigated practices maintain or improve soil quality. Traditionally, the quality of soil has been mainly associated with its productivity (Hornik, 1992), but nowadays the concept of soil quality is much more comprehensive. Soil quality depends on a large number of chemical, physical and biological properties, and its characterization requires the selection of properties most sensitive to changes in management practices (Yakovchenko *et al.*, 1996).

Good indicators of soil quality must be related to ecosystem processes, integrating physical, chemical, and biological properties. They must be sensitive enough to management and allow analytical accessibility and practical utility to agricultural specialists, producers, conservationists, and policy makers (Doran and Parkin, 1996). Initially, the use of a basic set of indicators to assess soil quality in various agricultural management systems was proposed. While many of these key indicators are extremely useful to specialists (i.e. researchers, consultants, extension staff, and conservationists), many of them are beyond the expertise of the producer (Hamblin, 1991). However, the use of simple indicators of soil quality which have meaning to farmers and other land managers will likely be the most fruitful means of linking science with practice in assessing the sustainability of management practices (Romig *et al*., 1995). Although soils have an inherent quality as related to their physical, chemical, and biological properties within constrains set by climate and ecosystems, the ultimate determinant of soil quality and health is the land manager. As such, the assessment of soil quality and direction of change with time is a primary indicator of sustainable management (Doran, 2002; Karlen *et al*., 1997).

To assess soil quality, indicators (soil properties) are usually linked to a specific soil function (Howard, 1993; Larson and Pierce, 1994; Karlen *et al*., 1996; Doran *et al*., 1996) e.g., as a medium for plant growth, and reflect changes over various spatial and temporal scales. To perform as good indicators, the selected soil properties should be sensitive, easy to measure, verifiable, and well-related to land management and environmental transformation (Carter *et al*., 1997; Seybold *el al*., 2001; Erkossa *et al*., 2007).

In our research, particular soil properties were chosen as the potential indicators according to different agricultural practices.

II.1 Physical indicators

Bulk density, water retention and transfer parameters and structural stability were chosen as basic physical soil characteristics and to study the effects of irrigation.

Soil bulk density (and porosity) varies according to soil texture, structure, and organic matter content, but within a given soil type, it can be used to monitor degree of soil compaction and puddling. Changes in bulk density affect other properties and processes that influence water and oxygen supply (Schoenholtz *et al.*, 2000).

Water soil and transfer parameters are universally important for monitoring all soil functions. Available water holding capacity and saturated hydraulic conductivity are the two most frequently found in minimum data set (MDS) of physical soil quality indicators. Available water holding capacity measures the relative capacity of a soil to supply water and saturated hydraulic conductivity is both an indicator of drainage rate and water/air balance in soil (Schoenholtz *et al.*, 2000).

Aggregate stability describes the ability of the soil to retain its arrangement of solid and void space when exposed to different stress (Kay, 1990). Structural stability of soil is an essential parameter, influencing many soil physical properties such as water filtration and water-air ratio, but also erodibility, biological activity and plant growth (Lynch and Bragg, 1985). Soil structure as such is not a plant-growth factor, but it influences practically all plant-growth factors: it determines the depth that roots can penetrate, the amount of water that can be stored in the soil (soil water distribution, movement and retention), availability of plant nutrients (nutrient recycling), aeration, movement of soil fauna and microbial activity (Hermawan and Cameron, 1993; Langemaack, 1999; Rampazzo *et al*., 1998; Pardo *et al*., 2000).

Stability characteristics are generally specific for a structural form and the type of stress being applied. A measure of aggregate stability could serve as a surrogate for soil structure, which is critical for development of root systems (Kay and Grant, 1996). To evaluate the impact of management practices on the soil environment it is necessary to quantify the modifications to the soil structure (Danielson and Sutherland, 1986). Since crop management systems generally have a strong influence on soil structural characteristics, aggregate stability is considered as a key indicator to assess soil structure (Six *et al*., 2000) and also soil quality (Albiach *et al.*, 2001). The decline in soil structure is increasingly seen as a form of soil degradation (Chan *et al*., 2003) and it is often related to land use and soil/crop management practices.

II.2 Chemical indicators

Among chemical indicators for soil quality, soil reaction (pH) is obviously important in the case of liming. This basic factor is known to influence nutrient availability and microbiological activity.

Soil organic matter (SOM) is one of the most important parameter of soil quality for both scientists and farmers (Romig *et al*., 1995). Soil organic matter is a nutrient sink and source, enhances soil physical and chemical properties, and promotes biological activity (Doran and Parkin, 1994; Gregorich *et al.*, 1994). The content of soil organic matter changes very slowly and many years are generally required to detect changes resulting from disturbance (Kandeler *et al*., 1993). It is well known that cultivation of the natural land resources induces SOM losses, which in turn directly affects the soil chemical, physical, and biological properties, and finally resulting in loss of crop production capacity (Stevenson and Cole, 1999). Soil organic carbon and total nitrogen are arguably the most significant single indicators of soil quality and productivity (Larson and Pierce, 1991; National Research Council, 1993; Cannell and Hawes, 1994).

II.3 Microbiological indicators

Soil organic matter (SOM) levels may vary within years, whilst active SOM-fractions like macro- and light fraction-organic matter, soil microbial biomass and microbial functions may change within shorter periods of time (Smith *et al*., 2000). Soil microorganisms have been shown to be potentially useful (early and sensitive) indicators of soil health, because they respond to soil management in time scales (month/years) that are relevant to land management (Torsvik *et al*., 1994; Hewitt and Sparling, 1998; Sparling and Schipper, 1999; Pankhurst *et al.*, 1997; Kandeler *et al*., 1993). Soil microbiota, existing in extremely high density and diversity, rapidly modify the energetic performance and activity rates to changing environmental conditions (Schloter *et al*., 2003). Some biological measurements (such as enzymatic activities) are not useful measures of soil quality because they are too much affected by both seasonal and spatial variations (Nannipieri, 1994). National programs for monitoring soil quality are now generally based on microbial biomass and respiration measurements and are sometimes extended also to nitrogen mineralization, microbial diversity and functional groups of soil fauna (Bloem *et al*., 1998). One of the major difficulties in the use of soil organisms *per se*, or of soil processes mediated by soil organisms, as indicators of soil health has been methodological: what to measure, how and when to measure it, and how to interpret changes in term of soil function (Visser and Parkinson, 1992; Pankhurst *et al*., 1997; Kandeler *et al*., 1993; Sparling, 1997).

Despite their small volume in soil (<0.5 %), microorganisms represent a very important component of soil organic matter (Paul and Clark, 1996; Sylvia *et al.*, 1999). Soil microorganisms are involved in many biochemical processes and particularly C turnover (Buckley and Schmidt, 2003). They have an important role in soil fertility (especially decomposition of organic matter and recycling nutrients for plants) and decontamination of soils, especially degradation or bioaccumulation of toxic residues (Soulas and Lors, 1999). They also form symbiotic associations with roots, facilitating nitrogen fixation or phosphate uptake. They act as antagonists to pathogens, influence the weathering and solubilization of minerals (Silver *et al*., 1996) and contribute to soil structural stability (Emerson *et al*., 1986). Thereby, soil microorganisms and biological activity also affect water holding capacity, infiltration rate, crusting, erodibility, and susceptibility to compaction (Elliot *et al*., 1996).

These services are not only essential to the functioning of natural ecosystems, but also constitute an important resource for sustainable agricultural systems. Thus soil quality (or soil health) evaluations need to focus not only on chemical (fertility) considerations, but on the

dynamic soil conditions as well. This supposes to consider a combination of physical, biological and chemical characteristics, which are directly affected by recent and current land use decisions and practices.

The soil microbial biomass can be defined as organisms living in soils that are generally smaller than approximately 10μm. Most attention is given to fungi and bacteria and they are generally dominating within the biomass. These two groups of microbes are the most important with reference to energy flow and nutrient transfer in terrestrial ecosystems (Richards, 1987). It has been suggested that the microbial biomass content is an integrative signal of the microbial significance in soils because it is one of the few fractions in soil organic matter that is biologically meaningful, sensitive to management or pollution and finally measurable (Jenkinson & Powlson, 1996; Powlson, 1997). However, it must be also realized that between different soil samples different biomass may occur without direct correlation to soil quality (Martens, 1995; Dilly and Munch, 1998). Although microbial biomass is generally acknowledged to represent only a very small proportion of total carbon in the soil (0.1–5%), it is characterised by its rapid turnover compared to the other components of organic matter (Chaussod *et al.*, 1988).

Soil microbial respiration, measured through carbon dioxide production is a direct indicator of microbial activity and indirectly reflects the bio-availability of organic matter (Parkin *et al*., 1996; Gomez *et al*., 2001). Soil microbial activity leads to the liberation of nutrients available for plants but also to the mineralization or mobilization of pollutants and xenobiotics. Thus microbial activity is of crucial importance in biogeochemical cycling. Microbial activities are mostly regulated by nutritional conditions, temperature, water availability, pH and oxygen supply (Schloter *et al*., 2003).

Soil pathogens. Microbial pathogens are widespread in the natural environment and diffuse pathogen pollution is chronic in rural environments. Soils and sediments are identified as having a critical role as transport pathways and reservoirs of pathogenic organisms. Despite this, important gaps remain in our knowledge of pathogens interactions with physically and biogeochemically heterogeneous soils environments. In particular, nonlinear and dynamic drivers of soil pathogen interaction and pathogen transport are under-researched because soils are complex and subsurface environments difficult to study (Centre for Sustainable Water Management, 2007-2008).

Pathogens that have the potential to infect humans can be divided into the categories of bacteria, protozoans, and viruses. Difficulties and expenses involved in the testing for specific pathogens, however, have generally led to the use of indicator organisms of enteric origin to estimate the persistence and fate of enteric pathogens in the environment (Crane *et al*., 1981). Faecal coliforms (FC) are the most commonly used indicator organisms. *Escherichia coli* are the most common FC and although most *E. coli* strains are non-pathogenic, some strains, such as *E. coli* O157:H7, pose a serious health risk to humans. Infectious viruses found in water systems include Enterovirus, Rotavius, Hepatitis A, and Retrovirus (USEPA, 2001).

II.4 Soil quality index (SQI)

Assessment of complex soil quality and health requires a minimum data set of physical, chemical and biological parameters (Gregorich *et al*., 1994; Doran and Safely, 1997) which need to be aggregated to provide an overall index of soil quality (Burns *et al*., 2006). Comparison of individual indicators against reference sites is one way of assessing soil quality (Bucher, 2002; Carey *et al*., 2009; Nelson *et al*., 2009), but individual indicators are often interdependent or may show functional redundancy (Hunt and Wall, 2002), so combining them meaningfully into a single index may enhance the assessment (Bucher, 2002; Andrews *et al*., 2002). The values of the selected indicators need to be converted into scores before they are integrated into an index. This requires establishment of a functional relationship between the soil function in question and the indicators (Erkossa *et al*., 2007).

III. Agricultural practices and their impact on soil quality

Soil is under pressure and its quality is suspected to decrease. The European Commission (2002) recognized soil degradation in Europe as a serious problem which is driven by human activities such as inappropriate agricultural practices, urban and industrial sprawl, industrial activities, construction, and tourism. Alteration of soil characteristics by anthropogenic impact changes functional capacities of the soil. Agricultural technologies and current practices like monocropping, residue management, mineral fertilization, overuse of pesticides, heavy agricultural machinery, inadequate management practices of soil and irrigation, can significantly affect soil quality by changing physical, chemical, and biological properties (Fauci & Dick, 1994). Long-term human impact (e.g. sealing), as well as short-term soil management (e.g. irrigation) modifies material and energy flows. Erosion, a decline in organic matter content and biodiversity, contamination, sealing, compaction, salinization, and landslides were identified as the main soil threats (Andrews and Carroll, 2002; European Commission, 2002). Conventional horticultural cropping, due to continuous soil removal and intensive use of pesticides and fertilizers, is the main activity leading to deterioration of soil physical, chemical and biological properties (Albiach *et al*., 2000). These modifications result in transformation of the soil processes to smaller or greater extent. When these processes are traceable, controllable, soil-use and soil quality remains sustainable on the long run (Toth, 2008). It is important to be aware that soil is a finite and non-renewable resource, because regeneration of soil through chemical and biological weathering of underlying rock requires geological time (Huber *et al*., 2001).

In our study, some agricultural practices are involved in alteration of soil properties in different ways and levels. Preliminary mechanical interventions on soils such as terracing, land levelling and deep ploughing are the roughest and they change soil profiles from inherent to anthropogenic. These changes are leading to major landscape modifications and land degradation (Borselli *et al*., 2006). In addition, new management techniques with more intensive production are used after the abandonment of traditional practices (García-Ruiz *et al*., 1996; Zalidis *et al*., 2002). Heavy machines such as bulldozers are being used for large scale soil movements in order to create new terracing systems for vineyards and orchards. These movements are not always controlled by law or technical guidelines and are determined by the needs of the owner or the person on charge of the machinery. These works modify the soil surface characteristics, which influence the infiltration properties at the surface (Poesen *et*

al., 1990; Léonard and Andrieux, 1998; Malet *et al*., 2003) and interact with other geomorphologic processes such as erosion (Lundekvam *et al*., 2003) and mass movements, mainly during extreme precipitation events (Abreu, 2005). The spatial variability created by all these operations leads to heterogeneous infiltration and runoff responses on hill-slopes. The soil redistribution also modifies the soil slope stability and the stability of the terraces, increasing the risk of surface mass movements. Some studies have pointed out the spatial variability of soil properties along hill-slopes (Agbenin and Tiessen, 1995; Bartoli *et al*., 1995) and with the slope degree (Janeau *et al*., 2003).

III.1 Irrigation

Irrigation is one of the most common agricultural practices in orchards and its positive effect on crop production is well known. The benefits of irrigation may include: better and improved crop (apple) quality, earlier crop production, greater yields, efficient nutrient distribution, less plant stress and reduced yield variability (Cetin *et al.*, 2004). Although irrigated agriculture has some benefits such as yield increase (Bilgehan, 1998), it brings about some problems such as increased drainage rates, salinisation-alkalinization and degradation of soil structure (Çullu *et al*., 2002). Irrigation is directly linked with soil water conditions what could potentially affect soil structure and thus soil water-air ratio, plant nutrition, soil microbial biomass and activity. Inappropriate production technologies have resulted in soil quality deterioration, leading to soil organic matter losses and structure degradation, affecting water, air and nutrient flows, and consequently plant growth (Golchin *et al.*, 1995). Other externalities such as crusting, runoff, surface- and groundwater pollution and increased CO_2 emissions are also influenced by irrigation. Species biodiversity can also be affected by management practices: generally high-input agricultural practices decrease biodiversity (Munyanziza *et al.*, 1997; Lupwayi *et al*., 2001). The effects of freshwater irrigation on soil are primarily physical, including increased drainage and nutrient transport. Wastewater irrigation can have more significant chemical and biological effects on soil properties. Among the potential risks associated with irrigation with waste treated water is degradation of soil structure, e.g. aggregate stability deterioration, a decrease in soil hydraulic conductivity, surface sealing, runoff and soil erosion problems, soil compaction, and a decrease in soil aeration (Bhardwaj *et al*., 2007). In addition to the impact of irrigation heterogeneity on the distribution of percolation in the field, it is often believed that intensive irrigation leads to rapid movement of nitrate below the root zone (Endelman *et al*., 1974) since nitrate is carried down through the profile with the percolating water.

It can be concluded that, depending on various parameters such as water quality, soil, agricultural techniques, fertilization and other chemical treatments, crop and climate, irrigation may sometimes severely damage the soil (Miller and Donahue, 1995; Tedeschi and Dell'Aquilla, 2005). The initial increase in crop yields becomes unsustainable, and in some circumstances severe chemical, physical, and biological fertility problems appear (Sun *et al.*, 2003), which can eventually compromise the agricultural activity itself (Porta et al., 1994). The question is whether irrigation is capable of continuing the high level of agricultural production in the longer term without damaging the environment (Pereira *et al*., 1996). There are several examples of large areas in the world that formerly were very productive and now are almost abandoned (Nunes *et al*., 2007).

The primary sustainability goal for soil is the maintenance of productivity. Irrigation should be managed so that it has minimal adverse effects on the quality of the soil. This will ensure that the soil is healthy and remains productive in years to come. Proper management of irrigation water and wastewater as fertiliser can result in enhanced productivity. By using the correct indicators of soil productivity, the effects of irrigation can be gauged, and thus optimised. Soil productivity is affected directly and indirectly by the type of crop, management practices and soil quality, which in turn is affected by moisture, pH, organic matter, heavy metal content etc. Many of these soil characteristics are interdependent - changes in one characteristic result in changes in another. This means that monitoring a subset should highlight any changes in soil productivity. The effect of some agricultural practices such as irrigation on soil structure will depend both on the soil natural properties (particle-size distribution, organic matter content, etc.) and the intensity of agricultural practices (Virto *et al.*, 2005).

Irrigation with wastewater raises, however, sanitary problems: risk of viral and bacterial infection both for farmers and crops as well as other problems due to the presence of toxic substances. Many studies have been conducted to point out the effects of the biological depuration process on the microbiological quality of these waters and on crop pollution by pathogens (Wolter and Kandiah, 1997).

It is very important to estimate the benefit of irrigation, based on appropriate soil analysis, before building an irrigation system, so as to be able to monitor the effects of irrigation on soil properties, as long as irrigation is used. For adequate water management, the proper use of these irrigation systems is important as well to avoid soil degradation and water contamination. The irrigation water should be regularly monitored chemically and biologically. The sanitary aspect of irrigation water in food safety should not be underestimated: there might be a possibility of crop pollution via irrigating water. Recent environmental investigations indicated pollution of surface or underground irrigation water with several faecal pathogens, which can also contaminate the apple harvest and have potential effects on public health. To analyse irrigation water quality on presence of pesticide, metals, salts or even pathogens, is rather an exception than a rule in Slovenia. It also has to be kept in mind that improper irrigation can cause severe damages to the environment (Miller and Donahue, 1995; Tedeschi and Dell'Aquilla, 2005).

In general, irrigation systems in Slovenia are technically more or less well prepared (Slovenian Irrigation Project, 1998), but not enough attention is devoted to soil conditions (before, during and after irrigation). Fruit or vegetable producers should not only focus on yields (here the benefit of irrigation is already well known), but also be aware of the irrigation consequences in soil conditions. In northern-east Slovenia, many orchards lay on hilly terrains, so technical specificity on different slopes and possibility of erosion should be take into consideration. Under the same weather conditions and very heterogeneous parent material, different soils will not react in the same way, either chemically or physically. According to specific physical, chemical and biological properties of each soil type, irrigation can have many advantages and also disadvantages. So far, in Slovenia there is still no serious study on irrigation effects on soil properties, especially soil microflora and soil structure.

III.2. Organic amendments

Application of organic fertilizers is linked to soil organic carbon and nitrogen pools, which consequently change microbial parameters and plant nutrition.

Maintenance of soil organic matter is important for the long-term productivity of agroecosystems. Soil application of organic amendments is a management strategy to counteract the progressive loss of organic matter (Marinari *et al*., 2000; Tejada *et al*., 2008). The addition of organic amendments may improve soil physico-chemical, biochemical and microbiological properties involved in biogeochemical cycles and thus positively influences plant productivity parameters. The organic amendments are a source of slow-releasing nutrients and available energy for soil microorganisms (Gomez *et al*., 2006). Among the main benefits attributed to the use of organic amendments are an improved soil aggregation and reduced bulk density, a greater water holding capacity, stabilization of pH, an increased CEC (Sasal *et al*., 2000; Tejada *et al*., 2008). Less nutrient potential loss and particularly a reduction in the loss of nitrate are also quoted as positive effects of organic farming. As this could also promote plant health, it seems possible to obtain equivalent or even higher yields in organic production than with conventional farming (Bulluck *et al*., 2002; Courtney and Mullen, 2008).

Swiss researchers (Fließbach *et al*., 2007) concluded that organic farming with composted manure is the only agricultural practice that limits the decrease of carbon content in the soil. They show that organic farming is the best agricultural practice for sustainable land management, in particular through the enhancement of soil microbial activity, leading to increased mineral exchange between plants and soil. The use of amendments has been reported previously to increase soil organic matter, provide nutrients and improve microbial activity (Lee *et al*., 2004). The results are conditioned by the composition of amendments, the rate of application and the soil type (Albiach *et al*., 2001; Tejada and Gonzalez, 2003). Furthermore, as soils are the basis of food production, preserving their quality with manure and low chemical use is essential for sustainable land management, even if these farming systems are not the most productive.

Less understood, however, are the effects of organic amendments on soil food webs, which contain the biotic assemblages responsible for decomposition and generation of soluble nutrients for plant uptake. Soil food webs also contain parasitic organisms, such as plant-parasitic nematodes, whose densities are influenced by the presence of host plants, the soil environment, and regulation by predators and pathogens (all factors that are potentially influenced by organic amendments). Maximizing the efficacy of organic amendments towards improving soil health requires an understanding of how this practice affects the entire soil food web (beneficial and pathogenic/parasitic components), and how these effects are mitigated by other agricultural practices, such as tillage. However, tillage can also be used to incorporate amendments into the soil, and therefore should expand their effect into deeper soil layers (Treonis *et al*., 2010).

Organic fruit production in Slovenia has steadily increased in recent years due to the excellent returns for growers. In 2011, almost 3 % of Slovene farmers were registered as organic

producers. Slovenia still has convenient natural conditions for organic farming; so small farmers in this trend see a good or even the only solution for surviving (mostly in combination with so called “eco-tourism”). Organic farming in Slovenia is a part of Agro-environmental programme. Slovenian farmers are supported by The Institute for Sustainable Development, they are well organised in their own union and they have their own trademark ("Biodar").

Offer of organic products in Slovenia still does not follow the demand, consequently resulting in prices which are 20-40 % higher, on average, compared with conventional agricultural products. Organic products are mostly sold on special organic markets or on farms, as a part of tourist offer. Most people see organic food as something pure and healthy, but there is also another point of view, which is less known: some consumers have doubts concerning about food safety in organic production, because soils which have received material (like organic fertilisers and irrigation water, as in our case), can be potential pathogen sources. This view of food safety linked with organic production is very interesting and requires further studies, because it can also help people to be acquainted with healthy nourishment.

III.3 Liming

Liming is a traditional agricultural practice to counteract soil acidification and improving calcium and magnesium supply, with effects on crops and soil quality (including physical and biological aspects). In spite of the extended lime application, the investigation of liming effects on organic matter remained restricted to C content, mass ratio of carbon to nitrogen (C/N ratio) and carbon storage (Derome *et al*., 1986; Persson *et al*., 1995). The following observations gave rise to the expectation that liming may also influence the chemical composition of soil organic matter (SOM). The C/N ratio of the organic surface layer material usually decrease (Belkacem and Nys, 1995; Marschner and Wilczynski, 1991) after lime application. Tree growth may be inhibited (Derome *et al*., 1986) by liming, presumably as a consequence of the growth stimulation of ground (weeds) vegetation (Rodenkirchen, 1998). The soil fauna populations are usually influenced by liming and, in general, resemble that of nutrient-rich soils (Persson, 1988). Soil microbial biomass (Smolander and Mälkönen, 1994; Badalucco *et al*., 1992), microbial activity (Anderson, 1998) and the potential for nitrification and nitrate leaching (Neale *et al*., 1997; De Boer *et al*., 1993) may increase after liming. Root growth in the organic layer may be stimulated (Raspe and Haug, 1998) or inhibited (Helmisaari and Hallbäcken, 1999) by liming.

IV. Research aims

Our research is dealing with two aims:

(1) the effect of irrigation on soil quality in hilly terrain, where soil water characteristics, structural stability, total soil organic content and microbial biomass are used as the main indicators for irrigation and erosion prognosis;

(2) the effect of different organic products (fertilizers) and liming on soil quality with the goal to enhance soil microbial biomass and its activity towards better plant nutrition.

In our studies, microbes play a very important role regardless of the plant production. Microbial biomass is a measure of the size of microbial population, and a large microbial population is more likely to exhibit biodiversity and pathogen suppressiveness than a reduced population. In intensive agricultural production with irrigation, microbial biomass and diversity might be threatened due to improper irrigation or even due to intensive pesticide application, what could lead to soil degradation in long term. In organic farming, the main goal with different agricultural techniques with low pesticide input is to enhance microbial activity, thus improve element recycling and soil fertility. As microorganisms respond very quickly to changes in land management (Entry *et al*., 2008), microbial biomass and basal respiration were used as potential indicators in orchard soils. In case of using irrigation water of bad quality or improper organic fertilizers, there is also a possible risk of yield pollution with pathogens via irrigation water or organic fertilizers. For this reason, irrigation and organic farming should be controlled also from microbiological point of view.

Activity of microorganisms and structural stability are directly affected by soil moisture content and there is also a close link between activity of soil micro-organisms and structural stability (Lynch & Bragg, 1985; Kostopoulou and Zotos, 2005). For this reason, water dynamics was observed during two seasons under different water regimes. Aggregate stability of the surface soil is critical for determining the rate of infiltration of rainfall or irrigation, thereby minimising potentially erosive runoff. These investigations where accompanied by the determination of some basic physical and chemical soil characteristics such as pH, CEC, SOM and water content, and some additional parameters like structural stability. These abiotic characteristics are complementary for a better understanding of the biological and biochemical properties and support the final evaluation of soil quality (Mäder *et al*., 1997; Filip, 1998).

The aim of this research was to develop an efficient methodology that combines physical, chemical, and microbiological soil parameters to evaluate the effects of agricultural practices (irrigation, organic fertilization, liming) on soil quality and the sustainability of crop production in Slovenian orchards.

Chapter 2

Materials and methods

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I Study sites

I.1 General presentation

Both experimental fields are located in northeastern Slovenia, 10 km from Maribor (Fig. 2.1a & b). The first location named Gačnik is settled in the north of Maribor and the second experimental area named Pohorski dvor is settled on the foothill of Pohorje, in south of Maribor (Fig. 2.2).

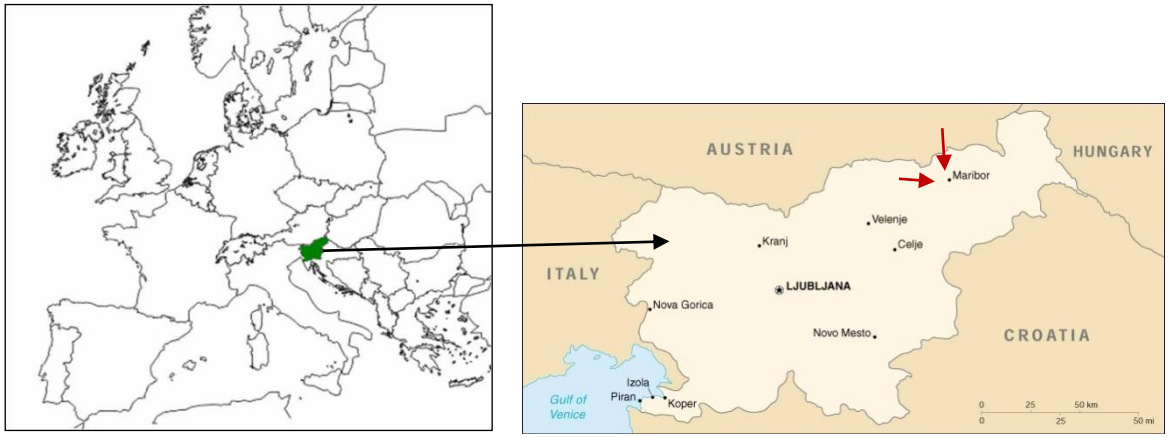


Fig. 2.1a & b: Location of Slovenia in Europe and position of the experimental fields in Slovenia.

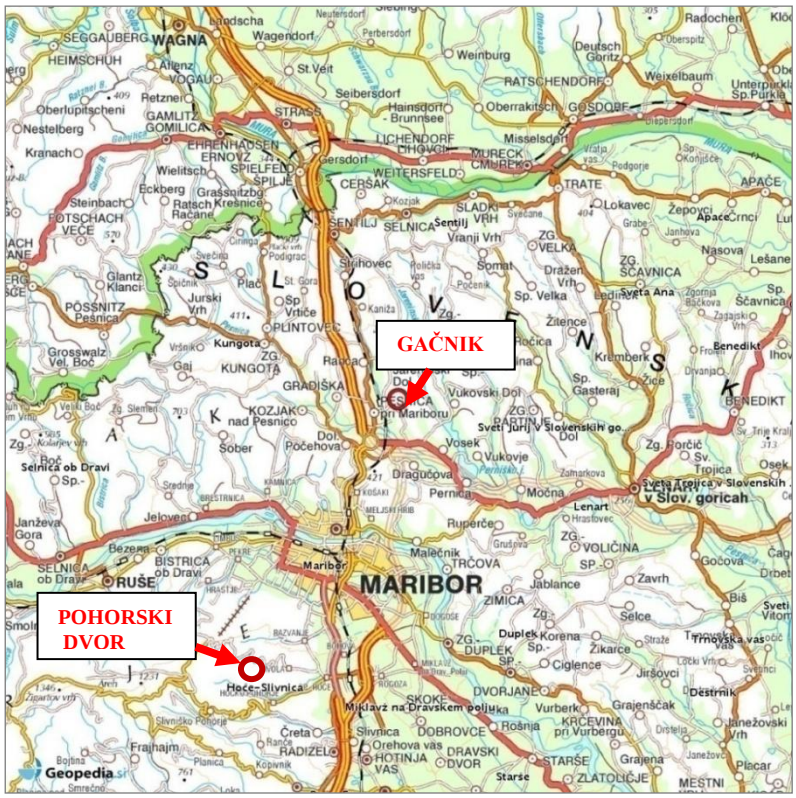
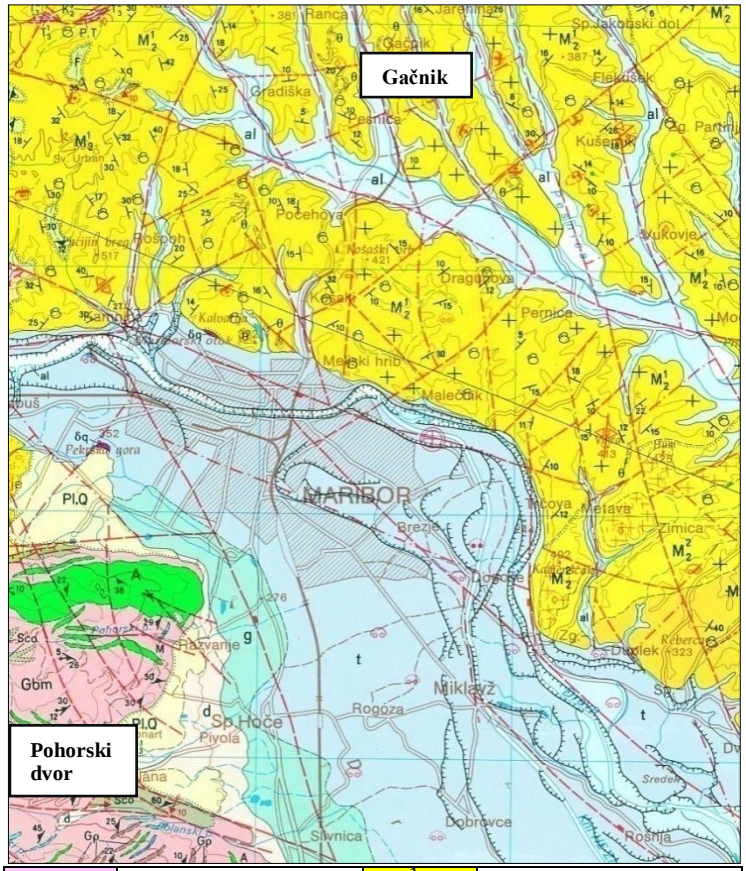


Fig. 2.2: Locations of experimental fields in Slovenia.

I.1.1 *Geology*

Both experimental locations are geologically very different according to the geological map of Maribor (Buser, 1987): Gačnik is settled on young sedimentary material (Pliocene marls) and Pohorski dvor is settled on metamorphic basement rock (micaschist) (Fig. 2.3). According to different bedrock, two very different soils are developed on each location: Calcaric Cambisol on marls and Dystric Cambisol on metamorphic micaschist.



Gbm	Muscovite-biotite gneiss	T_3^1	Clay-marl chist, slate limestone
a1	Alluvium	δq	Tonalite
Pl, Q	Sand, sandy clay, clayey gravel, conglomerate	A	Amphibolite
M_2^2	Gravel, sand, clay, sand marl	t	River terraces
M_2^1	Sand marl, sandstone	g	Sandy clay with gravel
P, T	Quarz sandstone	d	Deluvium

Fig. 2.3: Geological map of exposed bedrock in the surrounding of Maribor; insert from Geological map of Maribor 1:100 000 (Buser, 1987).

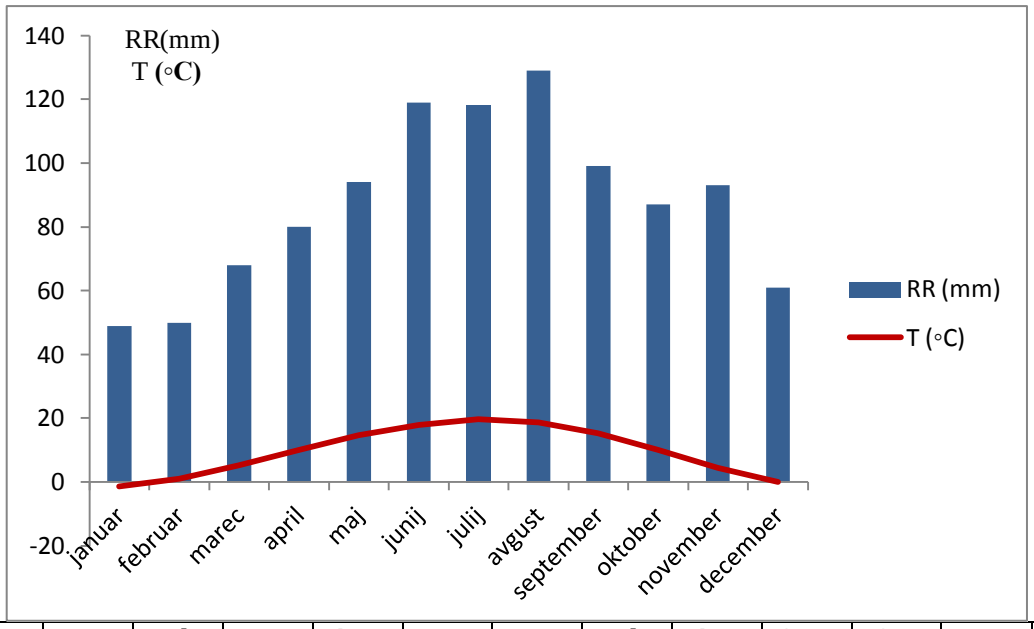
The first location, Gačnik, belongs to the Eastern Alps. The bedrock exposed at Gačnik, belongs to a sedimentary basin, which took place during the period from Miocene to Pliocene. Sedimentation started at Helvetian, with clastic sediments (conglomerate). Later gravels, marls, sands and clays were deposited until Pliocene. During the Pliocene-Quaternary some deposits of gravels, sands and clays were observed, but the main sediments are marshy sediments, sandy clays, gravels and alluvial material. In Gačnik, the bedrock is Helvetian marls with remains of foraminifers, fish teeth and bones and urchins. The Helvetian period is developed on 750 m thickness.

The Pohorski dvor settle belongs to the geological unit of the Eastern Alps. It is the upper part of Eastern Alps exposing gneiss, micaschists, eclogites, amphibolites, marbles, quartzites and diaphtorites of the Pohorian metamorphic series. The basic rocks in Pohorski dvor consist mostly of biotite-muscovite micaschist, covered by diluvium of transported and already weathered rock material, coming from Pohorje hill.

I.1.2 *Climate*

Both studied sites were close surroundings of Maribor, so data according to the climate and weather are measured at Maribor meteorological station.

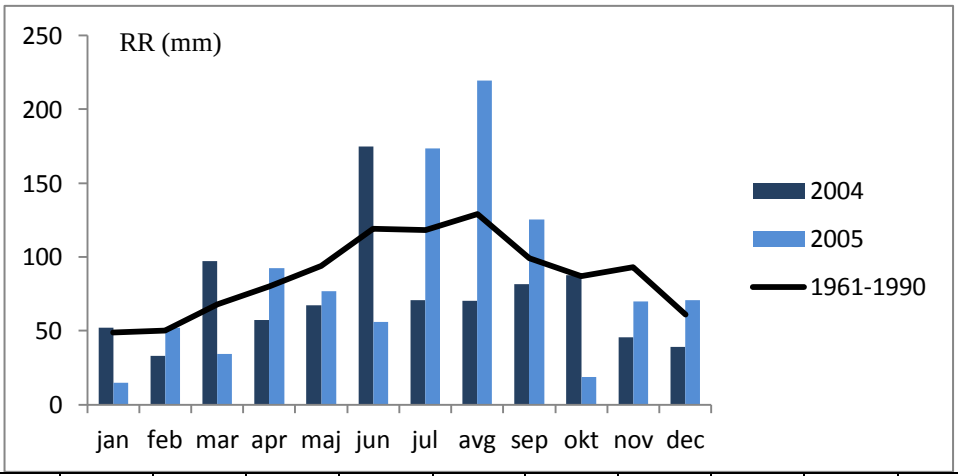
The climate in this region is moderately continental, with mean annual rainfall of 1045 mm and mean annual temperature of 9.7˚C (National Meteorological Service of Slovenia).
In the northeast of Slovenia, where the influence of the continental climate is the strongest, the greatest differences between maximal and minimal air temperatures occur. The maximum value of air temperature is usually reached in July, while the minimum value is observed in January.
Annual precipitation changes with years, but in Maribor most of the rainfall occurs in the summer time (June, July and especially in August). January and February represent months with the lowest rainfall amount. Such distribution is the consequence of the area's geomorphology and the fact that most of the precipitation is brought by southwest winds. Snow cover is quite frequent in winter, in spite of the ever more frequent green winters.
Monthly distribution of rainfall and air temperatures (1961-1990) is presented in Fig. 2.4.



1961-1990	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.	Oct.	Nov.	Dec.
RR (mm)	49	50	68	80	94	119	118	129	99	87	93	61
T (◦C)	-1.3	1.1	5.2	10	14.7	17.9	19.6	18.7	15.2	10.1	4.5	0.1

Fig. 2.4: Monthly distribution of precipitations and average temperature in Maribor (1961-1990).

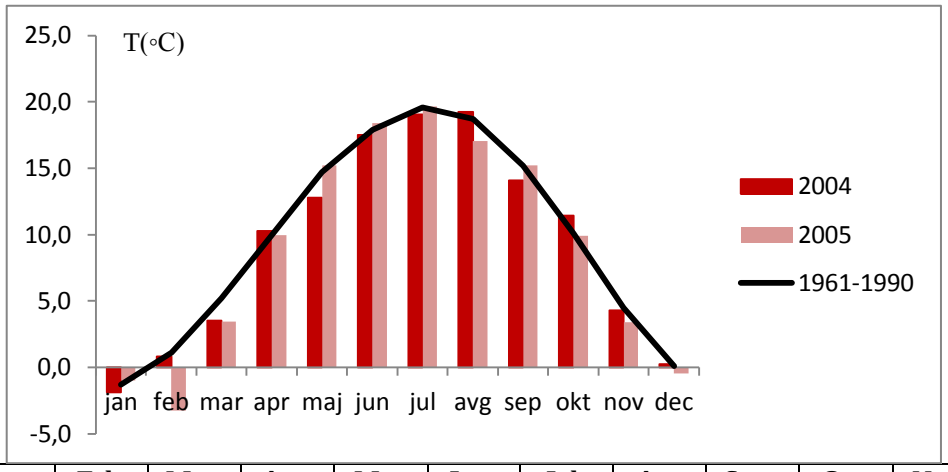
Most of field investigations were done during 2004 and 2005. For this period monthly rainfall precipitation and air temperatures for both locations are presented in Fig. 2.4, 2.5 & 2.6, comparing to 30-year (1961-1990) average climate conditions.



(mm)	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.	Oct.	Nov.	Dec.
2004	52.3	33	97.2	57.4	67.5	174.7	70.8	70.3	81.7	87.7	45.5	39.2
2005	14.7	52.3	34.5	92.5	77	56.2	173.4	219.5	125.5	18.7	70.1	70.7
1961-1990	49	50	68	80	94	119	118	129	99	87	93	61

Fig. 2.5: Monthly distribution of precipitations through the years 2004 and 2005 at Maribor meteorological station, compared to 30-year (1961-1990) average monthly precipitations.

When comparing the precipitations in 2004 and 2005, a large difference is observed: the summer time (July, August) in 2004 was extremely dry, while in 2005 extremely wet. June 2004 is the wettest month in the whole year, with much higher value compared to the long-lasting (1961-1990) mean value. Conversely, 2005 was an exceptional year with a rainy summer (518.4 mm rainfall during July, August and September) with maximal precipitation in August, so no irrigation was needed in this season.



(◦C)	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.	Oct.	Nov.	Dec.
2004	-1.9	0.8	3.5	10.3	12.8	17.5	19	19.3	14.1	11.4	4.3	0.2
2005	-1.0	-3.2	3.4	10.0	15.2	18.4	19.7	17.1	15.2	9.9	3.4	-0.4
1961-1991	-1.3	1.1	5.2	10.0	14.7	17.9	19.6	18.7	15.2	10.1	4.5	0.1

Fig. 2.6: Monthly distribution of average air temperatures through the years 2004 and 2005 in Maribor experimental station, compared to 30-years (1961-1990) average monthly air temperatures.

According to the monthly temperatures in both experimental years, there are no big deviations compared to long-lasting climatic conditions (Fig. 2.6). In 2004, August had the highest average temperature, while the rest of summer the temperatures in 2004 were lower than in 2005.

I.2 Detailed presentation of studied sites

I.2.1 *Location Gačnik*

Gačnik is an experimental orchard station for fruit growing, which belongs to Agriculture and Forest Chamber of Slovenia. It is situated on a hilly terrain (10-15 % slope), 290 m above sea level. It occupies the head of an elementary watershed (10 ha) which is ended by an artificial pond used for irrigation (Fig. 2.8 & 2.9). Many fruit species are cultivated there, but apple trees (*Malus Domestica* Borkh.) cv. 'Breaburn' were chosen for of an irrigation study. The system of apple production here is integrated with intensive production and dense plantation system, grafted on a weak rootstock (M9). The system of water supply is drip irrigation.

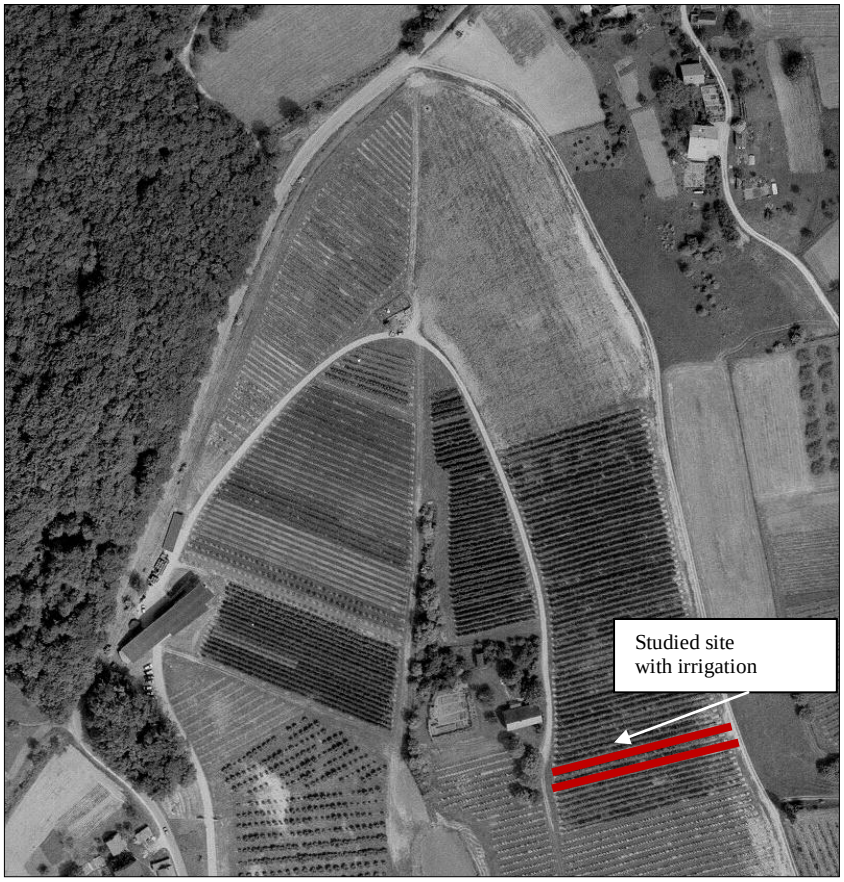


Fig. 2.7: Aerophoto picture of experimental orchard in Gačnik

It is important to emphasize that the soils of this study are strongly anthropogenic, as slope modelling were performed before the present plantation, due to change from terraces (one meter wide) to vertical plantation system, where the soils were moved from bottom to top of the slope (Fig. 2.7 & 2.10). Previous agricultural interventions were the following:

- 1990 to 1991: starting the installation of the experimental station of Gačnik. Previous orchard (cherry trees) on the terraces was removed into a vertical type of orchard on the slope. Soils were deeply ploughed (70 cm in depth) and flatted by bulldozer.
- Five years of soil resting.
- 1997: shallow ploughing (20 cm in depth) before planting.
- Spring 1998: planting of apple trees and installation of irrigation system.

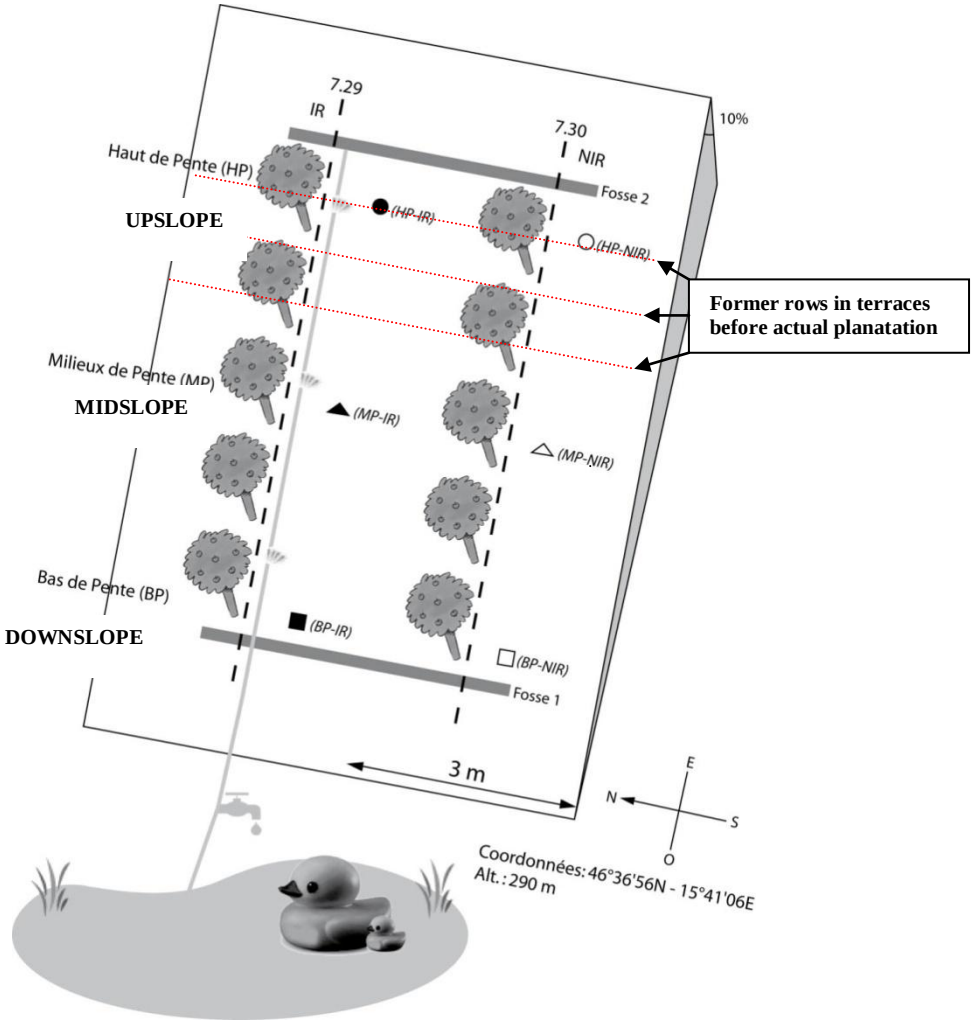


Fig. 2.8: Sketch of the studied site

In the study site, strong erosion processes are expected due to an important slope (15 %), trees plantation in the slope, irrigation system and bedrock (crumby marls). For these reasons, the soils are covered with natural grass. Covering soils with plants on the hilly terrain can function as anti-erosive practice, increase soil organic matter content (Andreux, 1996) and improve soil physical characteristics (CIVC, 2001).

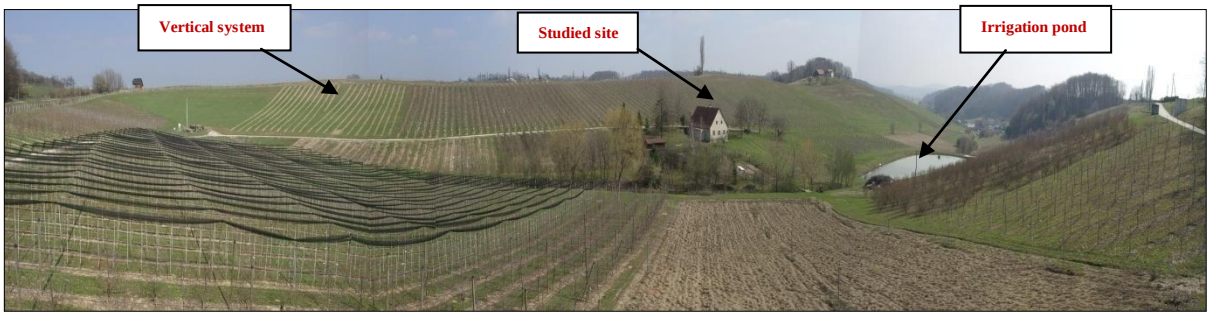


Fig. 2.9: A panorama view on experimental orchard in Gačnik

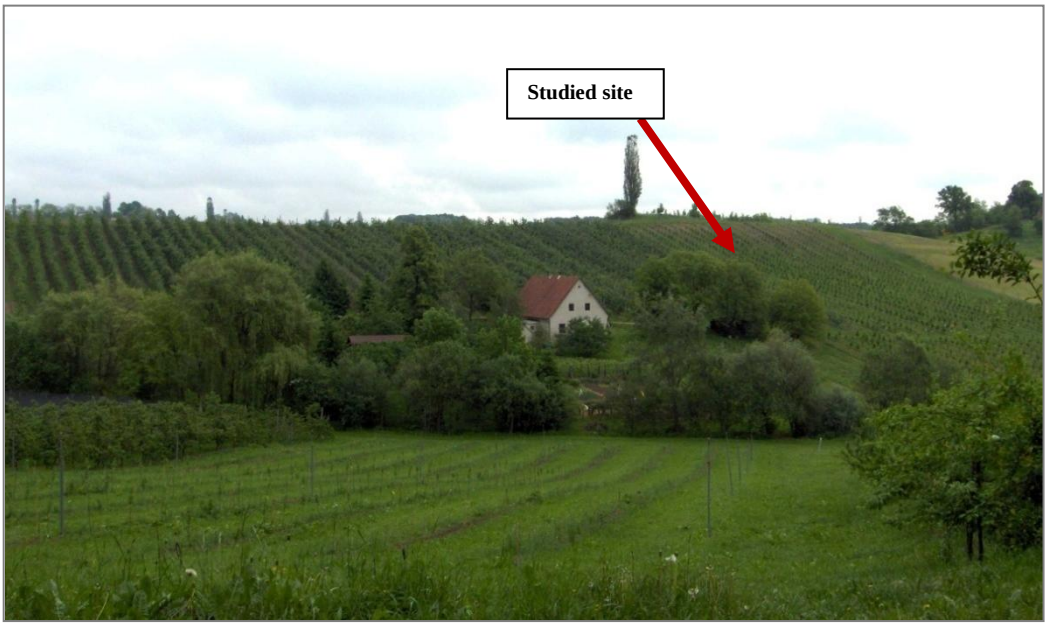


Fig. 2.10: General picture of experimental orchard in Gačnik

The study site consists of two parallel 100 m-length rows: the first row is irrigated (7.29) and the second one is not irrigated (7.30), each of them containing 120 trees of "Breaburn" apple cultivar. The distance between trees in each row is 0.8 m, the distance between two rows is 3 m. The surface of the studied site is about 400 m^2. Between the rows (in inter-row), soil is covered with natural grass 2 m wide, while bare soil takes place under the tree lines as a result of herbicide treatment (in the row) (Fig. 2.11). Soil properties were observed at 3 different slope positions (upslope, midslope and downslope) on irrigated and non-irrigated rows. Altogether 6 plots were studied according to water treatment and slope. On each plot, three different soil depths (0-5, 5-15 and 15-30 cm) were investigated.

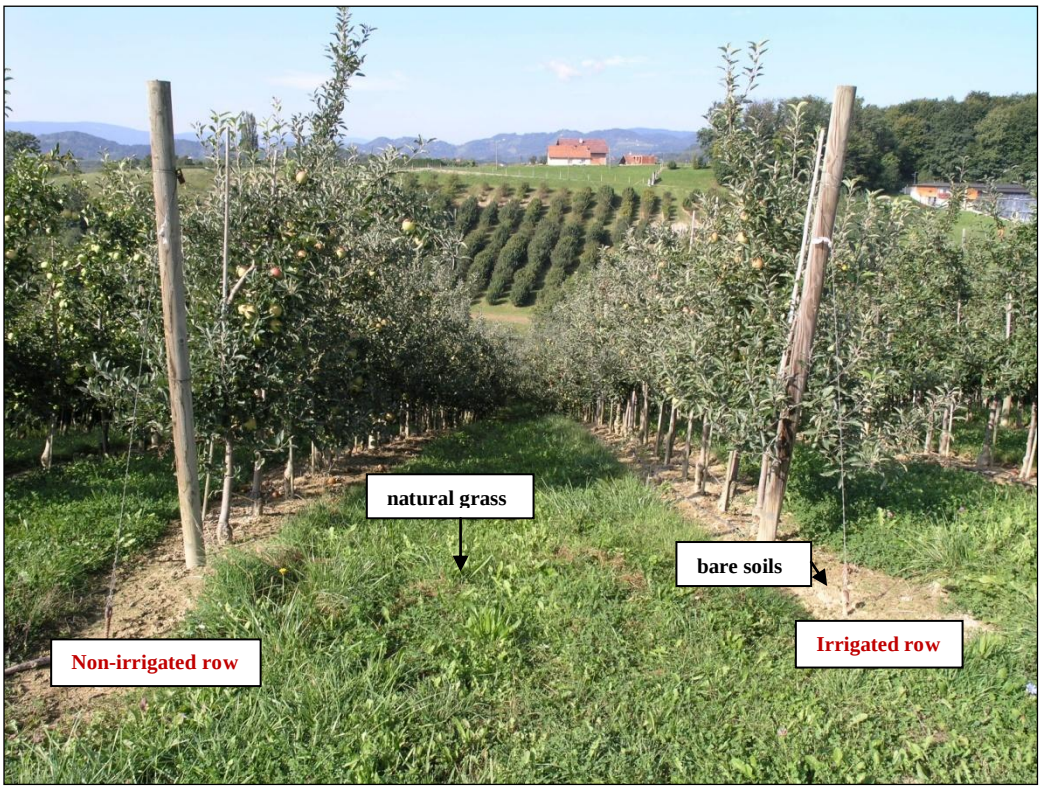


Fig. 2.11: Studied site with irrigated and non-irrigated rows (picture was taken from the upslope position)

I.2.1.1 Soils

The soil type is an Eutric Calcaric Cambisol (FAO, 1998), developed on a marl bedrock. Soils are altered by human activities like deep ploughing. The soil texture is silty clayey.

I.2.1.1 Application of treatments

Irrigation

Irrigation water source is an artificial water pond, located at the bottom of the experimental station, surrounded by orchards (pond area: 1 ha, pond depth: 3.75 m). The irrigation pump is settled one meter below the water level in the middle of the pond. The distance between irrigation pond and our studied site is around 60 m. Water is collected by runoff and precipitation in this small basin; waters coming from springs and streams are excluded. The pond is alive with fish, frogs and other living organisms.

Irrigation has been practiced since 1998, when this orchard was planted. The system of irrigation is drip irrigation with individual apertures by the trees, which is able to supply a volume of water up to 2 $L^{-1}tree^{-1}day$ (under 2-4 bar pressure). The irrigation was adjusted to precipitation, measured at the station. If the local rainfall > 2 mm per day, there is no irrigation during the next day; if the local rainfall > 5 mm per day, there is no irrigation during the next 3 days. Irrigation has usually been performed from blooming till ripping: from 0 to 70 days per year, depending on climatic conditions.

Tab. 2.1: Number of irrigation days per month in the period 1998 – 2005, in Gačnik.

Month/year	1998	1999	2000	2001	2002	2003	2004	2005
May	10	0	0	10	15	15	5	0
June	0	0	15	0	10	20	0	0
July	0	0	10	20	10	20	15	0
August	0	0	15	10	0	15	10	0
Together	**10**	**0**	**40**	**40**	**35**	**70**	**30**	**0**

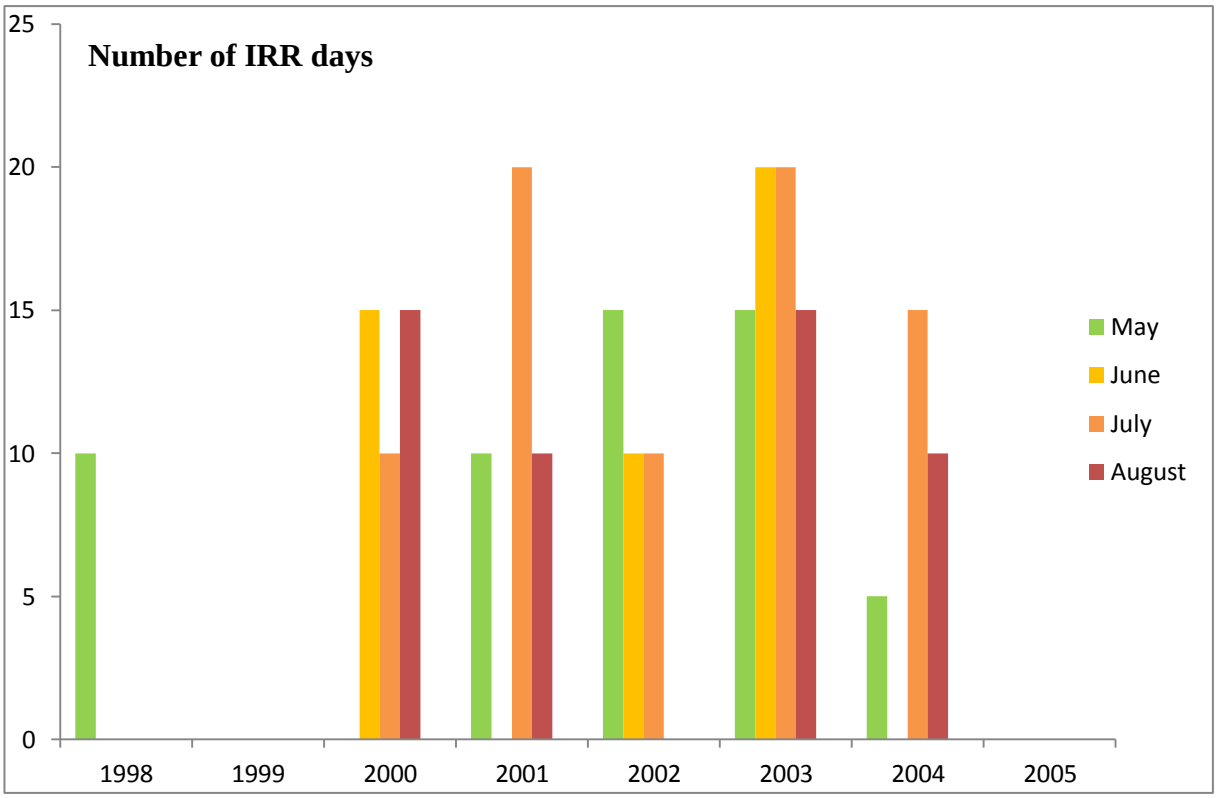


Fig. 2.12: Dynamics of irrigation in the period 1998 - 2005 (in days of irrigation per month).

No irrigation was done in 1999 and 2005 (Tab. 2.1 and Fig. 2.12). Irrigation was the most important in the year 2003, when the number of total irrigation days strongly deviated from other years. As irrigation was the common practice in this experimental orchard before 2004 (especially in 2003), we decided to choose it for our study.

There were no accurate measurements of soil water content till 2004. In 2004 and 2005, soil water potential was followed with tensiometers almost every week in the growing season (from June to September).

Fertilising and mulching

Stock fertilising with K and P was carried out in the 1999 and 2000 (according to the result of soil chemical analysis): 100 kg.ha^{-1} P_2O_5 and 60 kg.ha^{-1} K_2O. After the year 2000, 60 kg.ha^{-1} P_2O_5 and 60 kg.ha^{-1} K_2O were applied every second year.

Every year since planting, 60-70 kg.ha^{-1} of N was applied, only under the tree rows. Nitrogen fertilisation has been applied twice in two equivalent parts: at the end of April and at the beginning of June. The used nitrogen fertiliser was urea (granulated form). Every fifth year

extra nitrogen fertilising (30-40 kg.ha^{-1} of N) was performed on all the area including on grass in the inter-row, to preserve the grass cover, thus to prevent erosion.

In spring, all plant residues (leaves, fruits, branches), which are collected during the season in the rows, are mechanically raked in the inter-row and mulched together with grass. Mulching between the rows was performed seven to eight times per season, usually once in March (mostly branches), twice in May and July, once in August (mostly grass) and once in October (grass and fruits). Approximate quantity of organic litter (the whole of leaves, branches and fruits) per tree is 1-1.5 kg.

Application of pesticides

Application of pesticides was done about 15 times per year, according to rainfall: in dry years 11-12 times per season and in wet years 16-17 times per season (Tab. 2.2 & 2.3).
The number of pesticides applications was fourteen in 2004 and sixteen in 2005.
Herbicides which were used in strips (active chemical substances) were: oxyfluorfen, glyphosate and gluphosinate. Pesticides were used for preventing the invasion of insects (Oleodiazinon, Phosalone etc.) and fungi (Dithianon, Dichlofluanid etc.).

Tab. 2.2: Overview of applied pesticides during 2004.

Date	**Name and active substance**	**Type of pesticide**	**Dose per hectare**
9th April	Oleodiazinon (oil + diazinon) White oil (paraffinic oil) Champion (cupper hydroxide 50%)	insecticide insecticide fungicide	10.0 l (800 l water) 10.0 l (800 l water) 2.5 kg (800 l water)
19th April	Chorus (ciprodinil 75%) Champion (cupper hydroxide 50%)	fungicide fungicide	0.25 kg (200 l water) 0.75 kg (200 l water)
26th April	Chorus (ciprodinil 75%)		0.3 kg (200 l water)
3rd May	Delan (ditianon 70%) Karathane (dinokap 35%)	fungicide fungicide	0.7 l (200 l water) 0.6 l (200 l water)
7th May	Delan (ditianon 70%)	Fungicide	0.7 l (200 l water)
14th May	Zato (trifloksistrobin 50%) Polyram comby (metiram)	fungicide fungicide	0.15 kg (200 l water) 2.5 kg (200 l water)
24th May	Captan (kaptan) Score (difenokonazol 25%)	fungicide fungicide	2,0 kg (200 l water) 0.25 l (200 l water)
31th May	Captan (kaptan)	Fungicide	2.5 kg (200 l water)
Date	**Name and active substance**	**Type of pesticide**	**Dose per hectare**
7th June	Polyram comby (metiram) Indar 5 WG (fenbukonazol 5%) Basudin 600 EW (diazinon)	fungicide fungicide insecticide	2.5 kg (200 l water) 0.9 kg (200 l water) 1.2 l (200 l water)
17th June	Dithane M45 (mankozeb 80%)	Fungicide	2.5 kg (200 l water)
28th June	Delan (ditianon 70%) Karathane (dinokap 35%)	Fungicide Fungicide	0.7 l (200 l water) 0.6 l (200 l water)
12th July	Captan (kaptan)	Fungicide	2.0 kg (200 l water)
23rd July	Dithane M45 (mankozeb 80%) Zato (trifloksistrobin 50%) Pepelin (sulphur 80%) Zolone liquid (fosalon)	Fungicide fungicide fungicide insecticide	2.0 kg (200 l water) 0.15 kg (200 l water) 2.5 kg (200 l water) 2.5 l (200 l water)
6th August	Euparen (diklofluanid) Basudin 600 EW (diazinon)	Fungicide Insecticide	2.0 kg (200 l water) 1.2 l (200 l water)

Tab. 2.3: Overview of applied pesticides during 2005.

Date	Name and active substance	Type of pesticide	Dose per hectar
15th April	Frutapon (parrafinic oil 98%)	Insecticide	15.0 l (600 l water)
	Sylit (dodin)	fungicide	1.6 l (600 l water)
	Cupro 190 SC (cupper sulphate 19%)	fungicide	4.0 l (600 l water)
	Basudin 600 EW (diazinon)	insecticide	1.2 l (600 l water)
22nd April	Chorus (ciprodinil 75%)	Fungicide	0.3 kg (200 l water)
	Karathane (dinokap 35%)	Fungicide	0.6 l (200 l water)
28th April	Chorus (ciprodinil 75%)	Fungicide	0.3 kg (200 l water)
	Bayleton spec (triadimefon)	fungicide	0.7 kg (200 l water)
6th May	Delan 750 SC (ditianon 70%)	Fungicide	0.6 l (200 l water)
	Score (difenokonazol 25%)	fungicide	0.2 l (200 l water)
	Calypso (tiakloprid 48%)	fungicide	0.2 l (200 l water)
16th May	Delan 750 SC (ditianon 70%)	Fungicide	0.7 l (200 l water)
20th May	Captan 50 WP (kaptan)	Fungicide	2.0 kg (200 l water)
	Stroby (krezoksim metal 50%)	Fungicide	0.2 kg (200 l water)
30th May	Delan 750 SC (ditianon 70%)	Fungicide	0.8 l (200 l water)
	Tilt (propikonazol)	Fungicide	0.6 l (200 l water)
7th June	Merpan (kaptan 50%)	Fungicide	1.2 kg (200 l water)
	Rubigan (fenarimol)	fungicide	0.6 l (200 l water)
	Mospilan (acetamiprid 20%)	insecticide	0.4 kg (200 l water)
14th June	Merpan DWG 80 (kaptan 50%)	Fungicide	1.2 kg (200 l water)
	Indar 5 WG (fenbukonazol 5%)	Fungicide	0.9 kg (200 l water)
23rd June	Dithane DG (mankozeb 75%)	Fungicide	2.5 kg (200 l water)
27th June	Chorus (ciprodinil 75%)	Fungicide	0.3 kg (200 l water)
2nd July	Sylit (dodin)	Fungicide	1.0 l (200 l water)
	Score (difenokonazol 25%)	Fungicide	0.25 l (200 l water)
7th July	Chorus (ciprodinil 75%)	Fungicide	0.3 kg (200 l water)
14th July	Polyram combi (metiram)	Fungicide	2.5 kg (200 l water)
	Karathane (dinokap 35%)	Fungicide	0.6 l (200 l water)
21st July	Euparen multi WG 50 (diklofluanid)	Fungicide	2.0 kg (200 l water)
	Zolone liquid (fosalon)	Insecticide	2.0 l (200 l water)
6th August	Euparen multi (tolilfluanid)	Fungicide	2.0 kg (200 l water)

Because of intensive use of different types of pesticides in the experimental station, irrigation water could be contaminated by pesticide residues.

I.2.2 *Location Pohorski dvor*

The experimental centre of Pohorski dvor belongs to Agriculture Faculty of Maribor. The experimental site is situated on a hilly terrain (5 % slope), 340 m above sea level (Fig. 2.13). In this experimental centre many fruit species are present, but for organic farming study apple trees (*Malus Domestica* Borkh.) cv. 'Topaz' was chosen. The system of apple production here was organic production with dense plantation system on a weak rootstock (M9).

For a better understanding of the results, the history of agricultural interventions on this experimental field should be taken in consideration.
Short history of preparing experimental field of the orchard with 'Topaz' apples:

- Untill 1995, this field was cultivated with cereals (maize).
- After 3 years of resting (until 1998), the soil was shallow ploughed in 1997 before planting.
- The planting of trees was done in the spring 1998.

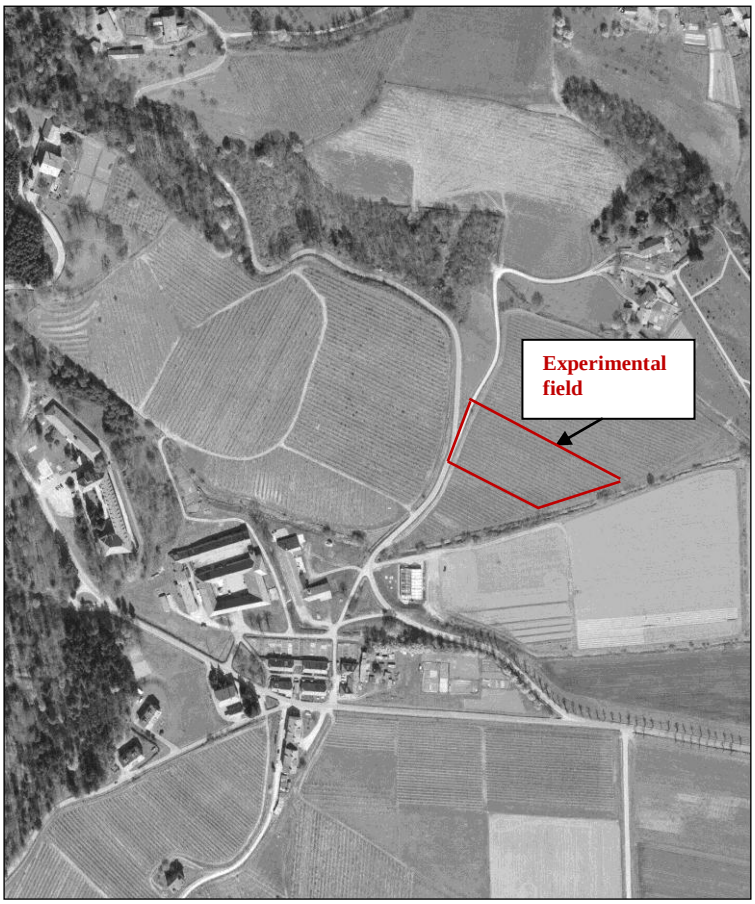


Fig. 2.13: Aerophoto of the experimental orchard in Pohorski dvor

Fig. 2.14: A panoramic view of experimental orchard in Pohorski dvor.

The experimental site is made of five parallel rows, each of them contains 240 trees of 'Topaz' apple cultivar. The distance between trees in each row is 0.7 m, the distance between two rows being 3.5 m. The surface of the experimental plot is about 3000 m^2 (when 4 blocks are included) or 1600 m^2 (when only three blocks are included). The soil between the tree rows (inter-row) is covered with natural grass, weeds on the row being mechanically removed periodically (Fig. 2.15). This part of the orchard lies on a 5 % slope, in eastern-western exposition. Soils are heterogeneous along the slight slope (rows), thus for sampling three blocks were investigated at two depths (5-15 and 15-30 cm). Block I lies at downslope position of this gently slope (Fig. 2.14). In our experiment, two factors were considered: organic fertilizer (4 different organic products + control) and liming. Ten different treatments are performed: each organic fertiliser (+ control) with and without liming. One plot presents each combination of treatments in each block (30 plots together). Each plot includes ten trees with isolating trees between the plots. Fertiliser and lime were applied to the soil surface (10 cm).

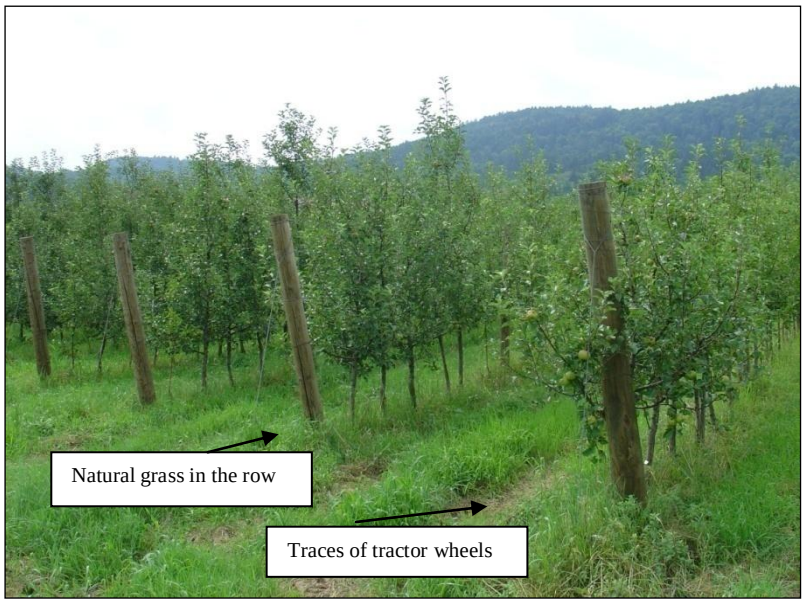


Fig. 2.15: Experimental site in organic apple production (picture was taken from downslope position)

	Treatments				
Block 3 (top)	**1**	9	3	**10**	**5**
	8	4	7	**6**	2
Block 2	7	2	8	**1**	3
	4	**6**	**5**	9	**10**
Block 1 (depression)	**5**	**10**	**6**	4	2
	7	9	3	**1**	8

Fig. 2.16: Schema of the experimental field in Pohorski dvor

On the schema (Fig. 2.16) the treatment numbers (from 1 to 10) represent combinations of four different organic fertilizers and a control, without and with liming (1=Compo Guano without liming, 6= Compo Guano with liming, 5='absolute control' without organic fertilizer and without liming and 10=control without organic fertilizer and with liming).

I.2.2.1 Soils

The soil type is 'Dystric Cambisol' (FAO, 1998), developed on schist. The soil texture is silty loam. The main soil characteristics before the experiment are reported in Table 2.4 and 2.5.

These soil analyses were used for general presentation of the soil characteristics before starting the experiment.

Tab. 2.4: Basic soil analysis (0-30 cm) in apple orchard cv. 'Topaz' in Pohorski dvor in the year 2002.

Soil depth (cm)	SOM (%)	pH in KCl	Phosphorus (mg/100g)	Potassium (mg/100g)	Magnesium (mg/100g)	Boron (ppm)	Total nitrogen (%)
0-30	5.13	4.8	2.4	9.7	8.8	0.11	0.22

Because of low soil pH (4.8), availability of nutrients and microbial activity might be affected. The level of SOM is very good. Available phosphorus (Olsen method, 1982) is very low, but potassium is in good level.

Tab. 2.5: Initial situation of soil mineral nitrogen before starting the experiment (April, 2002).

Soil depth (cm)	NH_4 – N (mg/kg)	NO_3 – N (mg/kg)	w (%)	Gravel (%)
0 – 30	2.05	0.74	11.1	10.1
30 – 60	0.80	1.63	14.4	12.3
60 – 90	0.45	0.68	14.6	12.0

Considering a bulk density of 1.3, the stock of N-NH_4 or N-NH_3 till 90 cm are very similar (1.3 and 1.2 kg.m^{-2} respectively). But the vertical concentrations in profile are very different. Ammonium nitrogen is the highest in the surface layer and it decreases with depth, while nitrate is the highest in the sub-surface layer (30-60 cm), presumable because of the leaching process.

I.2.2.2 Application of treatments

Organic fertilising

Since 2002, different organic fertilisers were applied in this field experiment. Chemical characteristics of different organic fertilisers are presented in Table 2.6. In this study, 3 fertilisers with different amount of nitrogen were included: Compo Guano (11 % N), Agrovit (5 %) and Biosol (8 %). Organic fertilisers were applied every spring (in April) from 2002 to 2005, according to nutritional requirements of apple trees (60 kg N^{-1} ha^{-1} year).

From Table 2.6, Compo guano contained the highest percentage of total and mineral (ammonium) N and the other nutrients, comparing to the other products. Ammonium presented one third of total nitrogen, which could be readily nitrified by soil microbes in forms available to plants. Amounts of total phosphorous, potassium, calcium, magnesium and sulphur were also the highest. In Compo guano, soluble organic matter presented the major part of total organic matter (97.4 %), which is very important for plant nutrition (Fig. 2.17). This non-stable organic matter could be easily mineralised in the soil and enable bigger concentration of available nutrients. The other two products (Biosol and Agrovit) included much more stable organic matter, which is harder to decompose. For these reasons, Compo

guano was assumed to be the most efficient organic fertiliser. In our experiment with microbial properties, only one organic fertiliser was chosen: Compo guano.

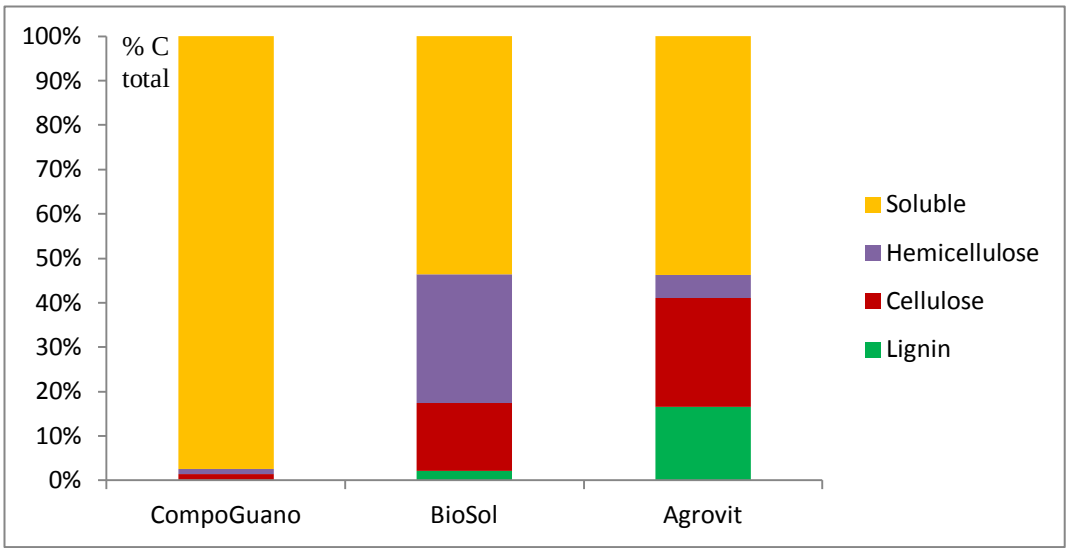


Fig. 2.17: Biochemical composition of different organic products

Tab. 2.6: Composition of the three different organic fertilizers

Name of the product→	**unit**	**Compo Guano**	**BioSol**	**Agrovit**
parameter↓				
w (105°C)	%	13.1	8.2	4.1
Dry matter	% of fresh weight	86.9	91.8	85.9
Organic matter	% of fresh weight	45.0	85.8	11.8
C org	% of fresh weight	22.5	42.9	5.9
N total	% of fresh weight	12.3	6.9	1.0
$N\text{-}NH_4$	% of fresh weight	3.7	0.32	0.75
C / N		1.8	6.3	5.8
Phosphorus total (P_2O_5)	% of fresh weight	7.3	1.1	3.8
Potassium total (K_2O)	% of fresh weight	4.8	1.0	6.3
Calcium (CaO)	% of fresh weight	12.6	0.49	12.5
Magnesium (MgO)	% of fresh weight	3.9	0.1	1.6
Sulphur (SO_3)	% of fresh weight	3.3	4.8	0.57
Organic fractions				
SOL (soluble)	% OM	97.4	53.6	53.7
HEM (hemicellulose)	% OM	1.1	29.1	5.2
CEL (cellulose)	% OM	1.3	15.3	24.5
LIC (lignin-cutin)	% OM	0.2	2.1	16.6
CEW (cellulose Wende)	% OM	1.9	9.6	38.7
ISB	(calculated on OM)	0.09	0.15	0.19
Tr	% OM	17.8	10.2	10.0
Organic Matter (OM)	kg	450	858	118
Stable OM	kg	40	129	22
Total Nitrogen (N)	kg	123	69	10
Ammonium N ($N\text{-}NH_4$)	kg	37	3.2	7.5
Total Phosphorus (P_2O_5)	kg	73	11	38
Total Potassium (K_2O)	kg	48	10	63

Liming

Liming was performed to allow better conditions for microbial decomposition of organic matter and nitrification in this acidic soil. Without suitable rising of soil pH, we could not expect a positive effect of organic fertilizers. Rising soil pH was performed slowly (by half pH unit) twice a year to avoid stress for soil microbes.
Lime material was 98 % natural grinded (120 µm) limestone ($CaCO_3$) with 1.5 % $MgCO_3$, 0.02 % FeO_3 and 0.05 % Al_2O_3. The commercial name is Calcivit VP.
The lime requirement was calculated by an old internal system, based on potential (in 0.1 N KCl solution) and hydrolytic (in 0.5 M Ca-acetate) soil acidity. With these two data, tables with the aimed pH values allowed calculation of lime addition, expressed as t/ha CaO. Needs were properly calculated on the basis of molecular mass of CaO and $CaCO_3$, considering also the purity of lime material and area of individual plots.

During our study, liming was performed twice a year, in the spring (beginning of May) and in autumn (November), three weeks before organic fertilizers application. For each period, 2 kg lime per plot (7 m^2) was added. Lime was mechanically incorporated within the first 10 cm of the soil.
Soil sampling was always done just before lime and fertilizers application.

Application of pesticides

Pesticides were used for preventing the invasion of insects (Oleodiazinon etc.) and fungi, according to the list of permitted substances in organic farming policy (Tab. 2.7 & 2.8).

Tab. 2.7: Overview of all applied pesticides in the year 2004.

Date	Name and active substance	Type of pesticide	Dose per hectare
9th April	Lime-sulphur broth (calcium polysulphide; 60% sulphur)	fungicide	30.0 L
21st April	Lime-sulphur broth (calcium polysulphide; 60% sulphur)	Fungicide	12.0 L
	Neem Azal (azadirahtin 1%)	Insecticide	3.0 L
	Cuprablau (30 % copper in copper hydroxide form+ 2% zinc)	Fungicide	1.0 kg (200 l water)
7thMay	Cosan (80% sulphur)	Fungicide	2.5 kg (200 l water)
	Cuprablau (30 % copper in copper hydroxide form+ 2% zinc)	Fungicide	1.0 kg (200 l water)
18th May	Cosan (80% sulphur)	Fungicide	2.5 kg (200 l water)
	Cupropin (50% copper hydroxide)	Fungicide	0.5 kg (200 l water)
29th May	Madex (1% granular virus *Cydia pomonella*)	Insecticide	0.15 L
11th June	Cosan (80% sulphur)	Fungicide	1.5 kg (200 l water)
13th July	Cocana soap (cocos fat)	Fungicide	20.0 kg (200 l water)
	Cosan (80% sulphur)	Fungicide	2.0 kg (200 l water)
25th July	Cocana soap (cocos fat)	Fungicide	20.0 kg (200 l water)

Tab. 2.8: Overview of all applied pesticides in the year 2005.

Date	Name and active substance	Type of pesticide	Dose per hectare
10th April	Lime-sulphur broth (calcium polysulphide; 60% sulphur)	Fungicide	35.0 L
22nd April	Cuprablau z-ultra (30 % copper in copper hydroxide form+ 2% zinc)	Fungicide	15.0 L
3rdMay	Neem Azal (azadirahtin 1%) Cosan (80% sulphur)	Insecticide Fungicide	3.0 L 2.5 kg (200 l water)
14th May	Cosan (80% sulphur)	Fungicide	2.5 kg (200 l water)
31st May	Cosan (80% sulphur) Cuprablau (30 % copper in copper hydroxide form+ 2% zinc) Madex (1% granular virus *Cydia pomonella*)	Fungicide Fungicide Insecticide	2.5 kg (200 l water) 1.5 kg (200 l water) 0.15 l
20th June	Cosan Cuprablau (30 % copper in copper hydroxide form+ 2% zinc) Madex (1% granular virus *Cydia pomonella*)	Fungicide Fungicide Insecticide	2.5 kg (200 l water) 0.8 kg (200 l water) 0.1 L
14th July	Cosan (80% sulphur) Madex (1% granular virus *Cydia pomonella*)	Fungicide Insecticide	2.5 kg (200 l water) 0.1 L
2nd August	Cosan (80% sulphur) Cuprablau (30 % copper in copper hydroxide form+ 2% zinc) Madex (1% granular virus *Cydia pomonella*)	Fungicide Fungicide Insecticide	2.5 kg (200 l water) 0.8 kg (200 l water) 0.1 L

Mechanical applications

In each growing season, 2-3 mechanical grass cuttings and mulching were done.

I.2.3 *Pohorski dvor and Gačnik: similarities and differences*

Both study sites have some similarities and differences. Gačnik and Pohorski dvor locations lie in the surroundings of Maribor, so the same climate was considered. This region is well known for apple production and this was reason for choosing apple culture (on the same rootstock) with high density tree planting.

On the other hand, very obvious differences were evidenced in both sites. Sites were completely different in topography, geology and soil types. The first difference is shown in topography: at Gačnik location the apple orchard lies on a hilly terrain (15 %), while at Pohorski dvor the experimental site is settled only on a light slope gradient (5 %). Another important factor, which affects soil development, is bedrock, which strongly differs from one location to another: in Gačnik marl is the basic rock, while at Pohorski dvor the micaschist was observed. Therefore, two different soil types are developed: Calcaric cambisol on marl (Gačnik location) with high soil pH, and Dystric cambisol on mica-schist with very low soil pH (Pohorski dvor location). Considering agricultural practices, there are also some important differences, which might impact soil properties. At Gačnik location, integrated apple production is performed with intensive use of pesticides, compared to organic apple production at Pohorski dvor, where application of pesticides (and other chemical substances) is strictly limited and defined. Mechanical and herbicidal treatment of soils under the trees at Gačnik location lead to bare soil (herbicide strip), while at Pohorski dvor there is a natural grass.

Finally, investigations were focused on the effect of irrigation and slope at Gačnik and the effect of organic fertilising and liming at Pohorski dvor - on soil quality. Even if microbial biomass is a common parameter measured in both locations, these two studies are quite different. According to different kinds of treatment, water supply also differs in the two experimental fields. Processing of statistical data was also different: at Gačnik only two rows of apple orchard were involved in the trial, so no real statistical significantly differences can

be expected; while at Pohorski dvor the results are repeated in three blocks (statistical block system with two factors: organic fertiliser and lime).
The main idea was to study two different treatments with two completely different soil types to find the most appropriate solution of applied agrotechnical techniques on soil quality. At Gačnik location the main target was studying the effect of slope and irrigation on soil erosion (structural stability according to soil organic matter). At Pohorski dvor, the possibility of enhancing microbial biomass (and indirectly mineralization process) with different combinations of organic fertiliser and liming was focused on.

II. Methods

II.1 Soil and water sampling

Soil sampling was performed in different ways, according to our purpose.
The list of analysed soil and water parameters is presented below.

1. **Physical analysis:**
- Total bulk density (γ_d)
- Clod bulk density (γ_{dc})
- Gravimetric water content (w)
- Water potential (ψ)
- Water structural stability after 2h, 6h
- Particle size distribution – texture

2. **Chemical analysis:**
- pH in H_2O
- Total carbonates ($CaCO_3$ total)
- Active $CaCO_3$ ($CaCO_3$ act.)
- Organic carbon (Corg)
- Organic nitrogen (Norg)
- Available phosphorus (P_2O_5)
- Cation exchange capacity (CEC)
- Exchangeable basic cations (Ca^{2+}, Mg^{2+}, K^+, Na^+)
- Mineral nitrogen (Nmin)
- Exchangeable copper (Cu^{2+})
- Exchangeable iron (Fe^{2+})
- Exchangeable hydrogen and aluminium (H^+ and Al^{3+})
- Sulphates (SO_4^-)
- Hydrogen carbonates (HCO_3^-)
- Sodium adsorption ratio (SAR)
- Ammonium (NH_4^+)
- Nitrites (NO_2^-)
- Nitrates (NO_3^-)
- Electrical conductivity (EC)

3. **Biochemical characterisations:**
- Chemical oxygen demand (COD)
- Biochemical oxygen demand (BOD)

4. **Organic matter fractionation (**in three size fractions**:** 200–2000 μm, 50–200 μm, 0–50 μm.

- Organic carbon (Corg)
- Organic nitrogen (Norg)
- Carbon isotope (δC^{13})

5. Microbiological characterisations:

- gravimetric water content (w), pH
- Microbial biomass (MB)
- Mineral carbon (Cmin)
- Labile organic matter pool (LOM)
- Enumeration of aerobic/anaerobic bacteria (No◦ aer./anaer. bacteria)
- Enumeration of fungi (N◦ fungi)
- Enumeration of coliform bacteria (N◦coliforms)
- Presence of HEV and Rota viruses (HEV, Rota)

II.1.1 *Location Gačnik*

For better review, a full list of completed soil samples in Gačnik, collected from 2004 to 2006 was established (Tab. 2.9).

Tab. 2.9: Schedule of soil sampling and analysis in Gačnik by soil parameters during 2004-2006.

	IRR			**NIR**		
	Upslope	**Mid-slope**	**Down-slope**	**Upslope**	**Mid-slope**	**Down-slope**
Physical analysis:						
Total bulk density	May 05	May 05	May 04, 05	May 05	May 05	May 04, 05
Clod' bulk density	-	-	May 04	-	-	May 04
grav. H_2O %	May, Sept. 04	May, Sept. 04	May, Sept. 04	May, Sept. 04	May, Sept. 04	May, Sept. 04
Water potential	-	-	Season 04, 05	-	-	Season 04, 05
Struct.stab. in water-2h, 6h	Sept. 04	Sept. 04	Sept. 04	Sept. 04	Sept. 04	Sept. 04
Struct.stab. in water -2h	May 05	May 05	May 05	May 05	May 05	May 05
Particle size distribution	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Chemical analysis:						
pH in H_2O	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Total $CaCO_3$	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Active $CaCO_3$	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Corg	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Norg	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
P_2O_5	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
CEC	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Exch. Ca^{2+}	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Exch. Mg^{2+}	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Exch. K^+	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Exch. Na^+	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Nmin	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05	Sept. 05
Organic matter fractionation:						
Corg	Oct. 04	Oct. 04	Oct. 04	Oct. 04	Oct. 04	Oct. 04
Norg						
δC^{13}						
Microbiological characterisations:						
(Humidity, pH)	May, Sept. 05	Sept. 04	May, Sept. 04	May, Sept. 05	Sept. 04	May, Sept. 04
Biomass		May, Sept. 05	May, Sept. 05		May, Sept. 05	May, Sept. 05
Cmin						
LOM						
N◦ aer./anaer. bacteria						
N◦ fungi	July, Oct. 06	July, Oct. 06	July, Oct. 06	July, Oct. 06	July, Oct. 06	July, Oct. 06
N◦coliforms						
HEV, Rota						

More details about sampling are found in Annexes 1, 2 & 3.

Methods of soil sampling depend on the type of further analysis. For some physical analyses we need to preserve total soil volume and soil samples have to be undisturbed, while for chemical analysis and most of microbial analyses classical sampling with auger or shovel is recommended.

In the same period we took soil samples for special microbiological analysis (especially pathogens) we also sampled irrigation water from different positions of transporting (Tab. 2.10). Water samples were taken from the middle of the pond (where the fixed pump is settled one meter bellow the water level) and from the pipes at different points along the slope. We excluded the upslope, because we found that in pipes there was not enough pressure for pushing irrigation water to this point and overcome slope effect. Water samples were carried in plastic containers or plastic bottles, and stored in cooling box at 4 °C till analysis.

Tab. 2.10: Schedule of water sampling and analysis in Gačnik in 2007.

	Irrigation water source			
	Pond	**Pipe-bottom**	**Pipe-middle**	**Pipe-top**
Chemical analysis:				
pH in H_2O	Oct.07	-	-	-
Ca^{2+}	Oct.07			
Mg^{2+}	Oct.07			
K^{+}	Oct.07			
Na^{+}	Oct.07			
Cl^{-}	Oct.07			
SO_4^{-}	Oct.07			
HCO_3^{-}	Oct.07			
SAR	Oct.07			
NH_4^{+}	Oct.07			
NO_2^{-}	Oct.07			
NO_3^{-}	Oct.07			
EC	Oct.07			
Biochemical analysis:				
COD	Oct.07	-	-	-
BOD	Oct.07			
Microbiological characterisations:				
N◦ aer./anaer. bacteria	July, Oct. 06	July, 06	July, 06	July, 06
N◦ fungi	July, Oct. 06	July, 06	July, 06	July, 06
N◦ coliforms	July, Oct. 06	July, 06	July, 06	July, 06
HEV, Rota	July, Oct. 06	July, 06	July, 06	July, 06

II.1.2 *Location Pohorski dvor*

For better review, the full list of completed soil samples in Pohorski dvor, collected from 2004 to 2006 was established (Tab. 2.11).

Tab. 2.11: Schedule of soil sampling and analysis in Pohorski dvor by soil parameters during 2004-2006.

	Organic fertiliser x lime			
	no Compo-guano, no lime	**Compo-guano, no lime**	**no Compo-guano, lime**	**Compo-guano, Lime**
Physical analysis:				
Particle size distribution	May 04	May 04	May 04	May 04
Chemical analysis:				
pH in H_2O, KCl	May 04	May 04	May 04	May 04
Total $CaCO_3$	May 04	May 04	May 04	May 04
Active $CaCO_3$	May 04	May 04	May 04	May 04
Corg	May 04	May 04	May 04	May 04
Norg	May 04	May 04	May 04	May 04
P_2O_5	May 04	May 04	May 04	May 04
CEC	May 04	May 04	May 04	May 04
Exch. Ca^{2+}	May 04	May 04	May 04	May 04
Exch. Mg^{2+}	May 04	May 04	May 04	May 04
Exch. K^{+}	May 04	May 04	May 04	May 04
Exch. Na^{+}	May 04	May 04	May 04	May 04
Microbiological characterisations:				
(Humidity, pH)	May, Sept. 04, 05	May, Sept. 04, 05	May, Sept. 04, 05	May, Sept. 04, 05
Biomass	May, Sept. 04, 05	May, Sept. 04, 05	May, Sept. 04, 05	May, Sept. 04, 05
Cmin	May, Sept. 04, 05	May, Sept. 04, 05	May, Sept. 04, 05	May, Sept. 04, 05
LOM	May, Sept. 04, 05	May, Sept. 04	May, Sept. 04, 05	May, Sept. 04
N◦ aer./anaer. bacteria	July, Oct. 06	July, Oct. 06	July, Oct. 06	July, Oct. 06
N◦ fungi	July, Oct. 06	July, Oct. 06	July, Oct. 06	July, Oct. 06
N◦coliforms	July, Oct. 06	July, Oct. 06	July, Oct. 06	July, Oct. 06
HEV, Rota	July, Oct. 06	July, Oct. 06	July, Oct. 06	July, Oct. 06

More details about sampling in this location are explained in Annex 4.

Samples were taken with an auger from different soil depths to perform chemical and some basic soil microbial analysis (i.e. microbial biomass, carbon mineralization). For certain physical analyses (like structural stability, clod porosity and organic matter size fractionation), soil samples were taken by using small shovel, trying not to disturb soil structure or aggregates too much. Mostly, the soil samples were taken in the middle position of each row, in the line between trees (except for comparing the sunny and shady sides of each row, then soils were sampled from both sides of the herbicide strip, not in the middle). Usually almost ten replicates were carefully mixed to obtain one representative sample (0.5-1.0 kg) (except samples taken from the soil profiles). Soil samples were kept in plastic bags or plastic boxes and stored at 4 °C. Fresh soil samples were also collected for gravimetric water content analysis.

Prior to structural stability analysis and microbial analysis, fresh samples were sieved at 4 mm. Samples for basic microbial analysis were always taken at the beginning and at the end of vegetation season
For chemical analyses dry soils were sieved at 2 mm.
For some special microbiological analyses (i.e. fungi, bacteria and viruses), sampling was done with a small shovel from soil surface (0-5 cm) only and kept stored at 4°C.

Bulk density was measured using 100 cm^3 metal cylinder and soil samples were taken with a hammer-driven core sampler. Sampling for bulk density was done at field capacity conditions, a few days after rainfall. For this analysis, three replications were made for each sample.

II.2 Soil and water regime characterization

II.2.1 *Soil characterization*

Three profiles were dug at Gačnik location in order to describe the morphological properties of irrigated to non-irrigated soils. Along the profiles and down to the bedrock, the following soil features were described: structure, texture, colour, humidity, presence of stones and earthworms, root depth and distribution, presence of carbonates and hydromorphic features. Soil samples were taken at different depths for further investigation.

In Pohorski dvor,two soil pits with auger were dug in inter-row space in blocks I and III (where tensiometers were settled). Simple description of some morphological soil properties was done like humidity, texture, structure, consistence, colour and presence of hydromorphyc features.

II.2.2 *Water regime characterization*

II.2.2.1 Water retention curves

Water retention curves were established at Biotechnical Faculty (Centre for Soil and Environmental Science), in Ljubljana on undisturbed soil cores sampled in the non irrigated row at 20, 40 and 80 cm depth for Gačnik location and in treatment 5 (pure control) in two blocks (I and III) at 20, 40 and 80 cm depth for Pohorski dvor location.

II.2.2.2 Tensiometers

Nine electronic tensiometers (SDEC SMS 2500 S) were located downslope at three different depths: 20, 40 and 80 cm (Fig. 2.18). Total water potential was measured in irrigated row, non-irrigated row and in the inter-row in Gačnik. In Pohorski dvor , measurements of water potential (ψ) in soils from treatment 5 (pure control), treatment 10 (lime only) and inter-row in blocks I and III were compared, .

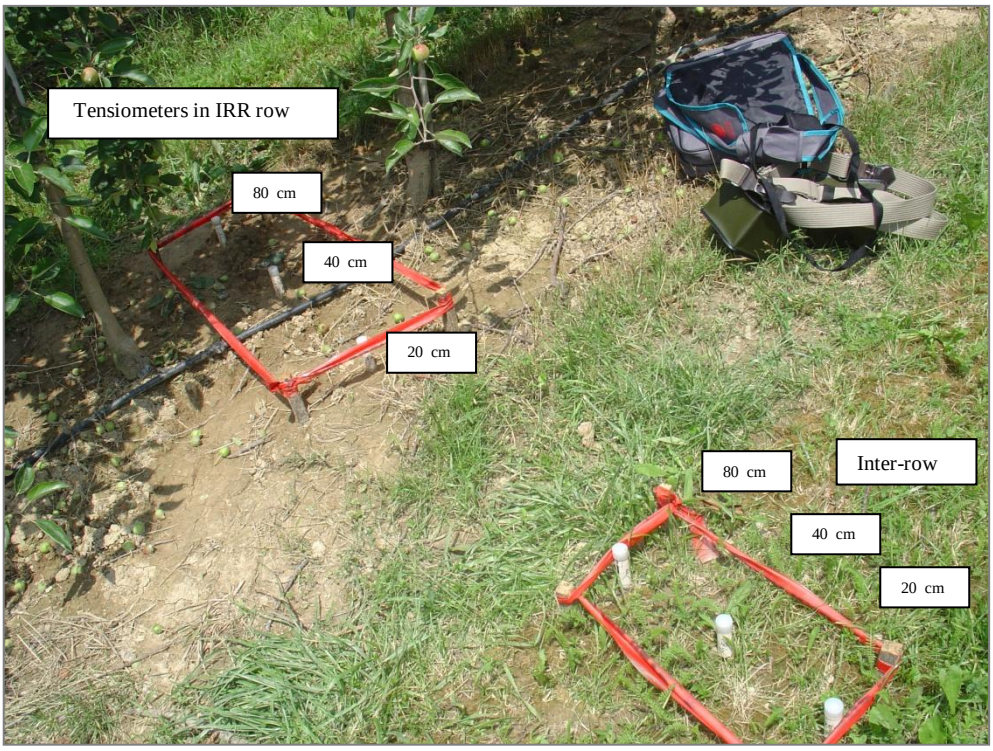


Fig. 2.18: Photograph showing the groups of three tensiometers located in the irrigated row and in the inter-row in Gačnik apple orchard.

II.3 Analytical methods for soils and water characterization

II.3.1 *Chemical methods*

Soil samples were air dried, sieved at 2 mm and homogenised before analyses.
Chemical soil and water analyses were performed in different laboratories, in France and Slovenia. In France, soils were analysed at the Soil Analysis Laboratory of INRA-Arras, at ENESAD, Dijon, Équipe Milieu Physique et Environnement, and at Université de Bourgogne, Dijon, UFR des Sciences de la Terre et de l'Environnement, Laboratory GéoSol. In Slovenia, some chemical analyses were done at University of Maribor, Faculty of Agriculture, Department of Chemistry, Agrochemistry and Soil Science, and Department of Microbiology, Biochemistry, Molecular Biology and Biotechnology, Faculty for Chemistry and Chemical Engineering.

Preliminary chemical soil analyses were done at the Soil Analysis Laboratory of INRA-Arras, on samples collected in spring 2004:

- total (volumetric method, according to ISO 10693) $CaCO_3$ and active $CaCO_3$ (Drouineau-Galet's method, according to NF X31-106)
- EDTA-extractable Cu (NF X31-120)
- organic carbon (ISO 10694)
- organic nitrogen (ISO 13878)

Complete chemical analyses of soils were done at ENESAD, Dijon, Équipe Milieu Physique & Environnement, on samples collected in Autumn 2005:

- Particle size distribution (pipet method, according to X 31-107)
- pH (electrometric method, in water according to ISO 10390)

- total (volumetric method, according to ISO 10693) and active $CaCO_3$ (Drouineau-Galet's method, according to NF X31-106)
- organic carbon (Anne's method, according to NF X31-109)
- organic nitrogen (Kjeldahl's method, according to ISO 11261)
- C/N, by calculation, from above organic C and N contents.
- P_2O_5 (Joret-Hebert method, according to NF X31-161)
- exchangeable cation capacity (T) (according to NF X 31-130)
- exch. Ca, Mg, K, Na (ammonium acetate method, according to NF X 31-108)

At the Faculty of Agriculture, Department of Chemistry, Agrochemistry and Soil Science, Maribor, the following chemical analyses were performed:

- mineral nitrogen (NH_4^+ and NO_3^-) (ÖNORM L 1091)
- pH (electrometric method, in water according to ISO 10390)
- total and active $CaCO_3$ (volumetric method, according to ISO 10693 and Drouineau-Galet's method, according to NF X31-106)
- soil organic matter content (Walkey Black method, by Nelson and Sommers, 1982)

At the Faculty of Chemistry and Chemical Engineering, Maribor, the following special chemical analyses of water were performed:

- chemical oxygen demand (COD) (SIST ISO 6060)
- biochemical oxygen demand (BOD) (SIST ISO 5815)

II.3.1.1 Analytical methods for determining C, N and $\delta^{13}C$ in each grain size fraction

These analysis were done after physical organic matter fractionation at the University of Burgundy, UFR des Sciences de la Terre et de l'Environnement, Laboratoire GéoSol.
The analytical method for determining carbon (C) and nitrogen (N) was made by dry combustion method in a 'Carlo Erba CNS 1500' CHN analyser. Soil organic samples were mineralised in a pure oxygen atmosphere at 1032 ◦C in the presence of CuO as catalyst. The released CO_2 was than purified and injected in a 'VG-Isochrom Micromass' spectrometer fitted with triple-ion collector and dual inlet system. The natural abundance of stable isotope ^{13}C was determined on each sample and expressed in δ units (‰), by reference to the international standard PDB (Craig, 1957), according to the following equation:

$$\delta^{13}C‰ = 10^3 \times [(^{13}C/^{12}C)_{sp} - (^{13}C/^{12}C)_{PDB}] / (^{13}C/^{12}C)_{PDB}$$

C and N contents were determined by using sulphanilamide standard (41.84 % of C and 16.27 % of N). Calibration for $\delta^{13}C$ was made by using the international graphite standard USGS24 ($\delta^{13}C = -16.0 \pm 0.1‰$).
Duplicates or triplicates were done to get a precision better than ± 0.15 ‰ δ units.

II.3.2 *Analytical methods of physical soil properties characterization*

Analyses were performed at different sites.
At the Faculty of Agriculture, Maribor, Department of Chemistry, Agrochemistry and Soil Science:

- bulk density and total porosity of soil (Blake and Hartge, 1986)
- soil humidity (gravimetric water content) (Gardner, 1965)

At AgroSup Dijon (ex ENESAD), Équipe Milieu Physique et Environnement:

- clods porosity (AFNOR, 1993)

At the University of Burgundy, UFR des Sciences de la Terre et de l'Environnement, Laboratoire GéoSol:

- structural stability (Bartoli *et al*., 1991)
- organic matter size fractionation (Bartoli *et al*., 1991)
- analysing of Corg, Norg and $\delta^{13}C$

At the Biochemical Faculty of Ljubljana, Centre for Soil and Environmental Science:

- water retention curves (Klute, 1986)

II.3.2.1 Bulk density (γ_b)

Soil bulk density was determined by using the core method (Blake, 1965). Metal cores with known volume (112.3 cm^3; 53 mm diameter and 51 mm height) were used for collecting soil samples at different depths. For one representative sample three replication samples were taken. The soil was weighted and dried at 105 °C for 24 h. Bulk density was calculated from the ratio between mass of oven dry soil and total soil volume:

Bulk density (γ_b) [g cm^{-3}] = M (dry soil) / V (total soil volume)

II.3.2.2 Gravimetric water content (W)

Gravimetric soil moisture content (W) was calculated from soil sample weights, before and after drying at 105 °C for 24 hours (Gardner, 1965). It is calculated as the ratio between mass of water and mass of oven dry soil:

Gravimetric soil moisture content (W) [%] = (mass of water / mass of oven dry soil) x 100

II.3.2.3 Clods porosity

Clod' porosity was determined by using the 'petroleum method' (AFNOR, 1993). In this method, Archimede principle is used for determining the total volume of the clod (pores + solid part). Archimede force is proportional to the volume of displaced liquid (petroleum in our case).

For calculation of total clod' volume, information of petroleum density (γ_{dp}) at defined temperature is needed, as well.

γ_{dp} [g cm^{-3}] = ($M\gamma_p$ / V_p)

γ_{dp} = density of petroleum
M_p = mass of petroleum
V_P = volume of petroleum
$P_{Arch.} = m_p$
$P_{Arch.}$ = Archimedes force
M_p = mass of displaced petroleum

$P_{Arch.} = \gamma_{dp} * V_{total\ clod} \rightarrow V_{total\ clod} = P_{Arch.} / \gamma_{dp}$

γ_{dc} [g cm^{-3}] = $M_{dry\ clod}$ / $V_{total\ clod}$

Dry clod's samples ($M_{dry\ clod}$) were saturated with petroleum by immersion during 24 hours.

After saturation, clods were gently wiped with Kleenex paper to remove the excess of petroleum.
A measurement of Archimedes force was performed in a baker, filled with petroleum (Fig. 2.19). First, only Archimedes force applied to empty net was measured and after, Archimedes force of net+clod was measured again in the baker with petroleum. The difference between two measurements represented the reaction of Archimedes force on the clod.

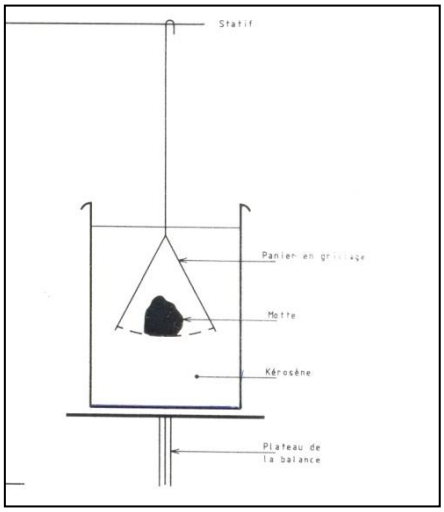


Fig. 2.19: Measuring the Archimedes force

II.3.2.4 Structural stability

Percentage of water stable soil aggregates was determined by the method described by Bartoli *et al*. (1991), based on the method of Kemper and Rosenau (1986).

A sample of 10 g of air dried soil ≤ 5 mm particle size was added to 250 ml distilled water in a wet sieving machine designed in the Centre de Pédologie Biologique, CNRS. Nine brass sieves (height 70 mm, diameter 60 mm, wire aperture 200 µm) were immersed in distilled water to 2 cm depth and oscillated horizontally in sinusoidal pattern with amplitude of 2 cm and frequency of 1.6 Hz (Fig. 2.20). Triplicate samples were shaken in this manner for periods of 2 and 6 hours. The aggregates remaining on the sieve were oven-dried (105 °C) and weighed (AS). Then the aggregates were crushed by hand, leaving only the sand fraction >200 µm particle size on the sieve. The sand was oven dried and weighed (S). The fraction of water stable >200 µm aggregates (A) was calculated as the difference between (AS) and (S), and expressed as a percentage of 105 °C oven-dried soil.

The percentage of water stable aggregates in water after desegregation time is calculated as a difference between total mass of oven-dried soil remaining on the sieves (aggregates + coarse sand) and coarse sand mass. Result is expressed in percentage of total initial soil:

$$SA\ (\%) = ((M_{total\ sieved} - M_{coarse\ sand}) / M_{sample}) \times 100$$

Where:
SA (%) = percentage of stable soil aggregates
$M_{total\ sieved}$ = soil mass remaining on the sieve after drying in oven (soil aggregates + coarse sand)
$M_{coarse\ sand}$ = particles > 200 µm, which are remaining on the sieve after drying

M_{sample} = initial soil sample mass (air dried)

To get the results as exact as possible and minimise the final error, we also have to take into consideration the gravimetric water content (w) of the air-dried soil. The soil samples after drying in oven at 105 °C for 24 hours are completely dry i.e. without humidity. It is necessary to correct these masses in relation to initial moisture of soil samples:

$$T_{otal\ sieved\ corr.} = T_{otal\ sieved} + w\ \%\ (T_{otal\ sieved})$$

where: w% = percentage of humidity of initial soil sample
Here is the final equation for calculation percentage of water stable aggregates:

$$\mathbf{SA\ (\%) = ((T_{otal\ sieved\ corr.} - M_{coarse\ sand}) / M_{sample}) \times 100}$$

Fig. 2.20: Picture of the machine for structural stability

II.3.2.5 Organic matter grain size fractionation

Soil size fractionation consists of two stages: i) a dispersion treatment and ii) wet sieving.

The fractionation was performed in water medium, advising the method of Andreux *et al.* (1980)**.** Thirty grams of dried (50 °C) and sieved (0-2 mm) soils were first dispersed by mechanical shaking in 300 ml of water, during 16 h (ratio soil/water = 1/10). Size fragmentation was done by wet sieving (200 and 50 µm mesh sizes) combined with gentle horizontal circular shaking. Three fractions were obtained: the coarse sand fraction A (200–2000 µm), fine sand fraction B (50–200 µm), and the silt and clay fraction C (0–5 µm). The finest soil fraction (C) was centrifuged for 40 minutes at 9500 rpm before further drying (Beckman Avanti J25). Each fraction was dried at 105°C for 24 h prior to analysis; all fractions were ground by hand in agate mortar.

Because the soil samples contained carbonates, HCl decarbonation was performed on 1 g of sample with 200 ml of 2.0M HCl on the horizontal shaker for 2 hours. Excess of HCl was removed by washing with distilled water, recovering initial pH 6.0 in the supernatant. Centrifugation step was performed 15 mn at 10.000 rpm. Finally, the sample was dried and crushed again manually in an agate mortar before measurement. About 0.25 mg of soil powder from each sample was weighted and tightly sealed in a tin capsule for measuring Corg, Norg, and $\delta^{13}C$.

II.3.3 *Analytical methods of microbiological soil and water properties*

Basic microbial analyses of soils were performed at UMR "Microbiologie des Sols et de l'Environnement" INRA, Dijon, while specific microbial analyses of soils and irrigation water were performed at the University of Maribor, Faculty of Agriculture and Life Sciences, Department of Microbiology, Biochemistry, Molecular Biology and Biotechnology.

At INRA Laboratory of Dijon, further basic microbial analyses were done:
- soil microbial biomass (Vance *et al*., 1987, modified by Chaussod *et al*., 1988)
- carbon mineralization (Chaussod *et al*., 1986)
- labile organic matter pool (Lemaitre *et al*., 1995).

At the Faculty of Agriculture in Maribor, specific microbial analyses in soils and irrigation water were performed as:
- enumeration of fungi (APHA, 1989)
- enumeration of total aerobic and anaerobic bacteria (APHA, 1989)
- enumeration of total Coliforms (Clesceri *et al*., 1989; APHA, 1989)
- enumeration of *E. Coli* (APHA, 1989)
- detection of Hepatitis E and Rotavirus (USEPA Manual of Methods for Virology - EPA publication EPA/600/4-84/013 (R-7).

II.3.3.1 Soil microbial biomass

Soil microbial biomass was determined by using $CHCl_3$ fumigation-extraction method (Vance *et al*., 1987), modified by Chaussod *et al*. (1988).

Fresh soil samples were stored at 4 °C and sieved at 5 mm before analysis. For each sample, three subsamples (each with three replicates) are required:
- one sub-sample for determining gravimetric soil moisture (24 hours at 105 °C)
- one non-fumigated sample (40 g oven-dry equivalent) immediately extracted with 200 ml of 0.05 N K_2SO_4, for soluble organic C and inorganic N measurement. On some occasions, pH of the extract was also measured.
- one fumigated sample (40 g oven-dry equivalent), for organic C measurement after K_2SO_4 extraction as above.

Non-fumigated samples (3 replicates per soil sample) were placed in plastic bottles with 200 ml 0.05 N K_2SO_4, than shaken with a horizontal shaker for 45 minutes at 180 rpm. After shaking, samples were centrifuged (type Sorvall RC 26 PLUS Kendro Lab. Products) at 10 °C for 10 mn at 5000 rpm. From each supernatant, two small (10 ml) sub-samples were taken: one for measuring organic C and another one for measuring inorganic N. Organic C was measured just after, but sub-samples for inorganic N were frozen (-18°C) until analysis. Before measuring organic C on the TOC analyser, two drops of orthophosphoric acid were added in each sub-sample, to remove mineral C. Defined volume (200 µl) of the sub-sample was injected with a syringe into the analyser. Organic C in the supernatant was measured by persulphate-UV oxidation, using a Dohrmann DC80 analyser.

The next step was the preparation of soil samples for fumigation. Fresh samples were placed in 100-ml glass vials and put into vacuum desiccators. After pumping all the air from the desiccators, ethanol-free chloroform (150 ml) was introduced into the desiccators. Fumigation

took 16 hours, a period of time enough to kill all the microbes. As a solvent, chloroform disrupts the cell walls of the microbes, releasing intracellular organic matter, which is then extractable with 25 mM K_2SO_4.

Further processing of fumigated samples was the same as with non-fumigated (shaking, centrifugation, pipetting, measuring Corg on TOC analyser).

After determining of total dissolved C on a TOC analyser, microbial biomass is calculated as the difference between C in the fumigated and non-fumigated samples, giving the chloroform-labile C pool (EC), which is proportional to microbial biomass C:

E C [mg C kg^{-1} soil] = [(C fumigated soil) – (C non fumigated soil)]
Biomass C [mg C kg^{-1} soil] = EC / k_{EC}

where k_{EC} means extractable fraction of biomass, assumed to be about 0.38.

II.3.3.2 Labile organic matter pool

After removing the supernatant that contained the extractable microbial biomass (see the above protocol for microbial biomass), the residual soil was used to determine the labile organic C. It corresponds to a fraction of labile soil OM, which is mainly derived from microbial biomass and the biodegradation products of organic residues. It was extracted by autoclaving (in K_2SO_4 25 mM solution) the pellet for 16 h at 120 °C (Lemaître *et al.*, 1995). Soluble organic C was measured by a persulphate-UV oxidation procedure with a TOC analyser (Dohrmann DC80).

II.3.3.3 Carbon mineralization (respiration of soil microbes)

A medium-term (28 days) aerobic incubation was used, to determine the potential of the samples to mineralise organic C. Soil samples (40 g oven-dry equivalent, with three replications) were incubated at 28 °C in 550 ml glass flasks closed with rubber stoppers. Glass vials containing 10 ml of 0.25 M NaOH were placed in the flasks, to trap the evolved CO_2. Three blanks (empty glass flasks, with only the NaOH trap) were prepared, to take into account the C-CO_3 already present in NaOH and the C-CO_2 in the atmosphere of the flasks. While respiring, the soil microbes produced CO_2, which accumulated in the NaOH traps. The flasks were opened after 10 days, to replenish the atmosphere and change the NaOH traps. C-CO_2 was measured in this first set of traps by NDIR, using the Dohrmann DC80 analyser. After 28 days of incubation, the measurement was repeated on the second set of traps. The sum of accumulated C-CO_2 in NaOH traps after 10 and 28 days of incubation represented total C mineralization or respiration rate (in mg C kg^{-1} dry mass) (Chaussod *et al.*, 1986).

II.3.3.4 Enumeration of bacteria and fungi present in soil and water samples

Using the spread-plate technique and 100 µl from the serial dilutions (up to10^{-6}) of soil and water samples, plates for the determination of aerobic (APC) and anaerobic plate counts (ANAPC), fungi, total and faecal (thermo tolerant) coliforms and *E. coli* were prepared using selective culture media according to Standard Methods (APHA, 1989; Clesceri *et al.*, 1989).

Aerobic and anaerobic bacterial plate counts were made with NA agar (Nutrient agar), and plates were incubated at 22°C for 48 hours (aerobically and anaerobically in anaerobic boxes). For fungi counting PDA (Potato Dextrose agar) was used and plates were incubated at 22 °C for 4 days aerobically. Total and thermo tolerant coliforms counts were made on EEC agar (Hi Crome Selective agar). Total coliforms plates were incubated at 37°C, while thermo tolerant (faecal) coliforms were incubated at 44°C for 24 hours aerobically. In both types of coliform plates two typical colonies were counted: blue to dark violet colour (*E. coli*) and light salmon colour (the rest of coliforms). In all serial dilutions colonies between 30 to 300 colonies were counted.
The viable titer is determined by counting colonies (CFU's) per volume plated and multiplying by the dilution factor (Madigan *et al.*, 2000).

Viable titer = (CFU/volume plated) x Dilution factor

II.3.3.5 Detection of enteroviruses in soils and water

Isolation of enteroviruses in water

Twenty litres of irrigation water were collected in the field (Gačnik) and concentrated by a conventional filter adsorption-elution method (modified by Rutjes *et al.*, 2005). 0.05 M magnesium chloride was added to the water sample to enable the formation of a virus-magnesium complex. By reducing the pH to 3.8 with 0.5 M HCl, these complexes adsorb to a negatively charged Millipore filter (1.2 µm, 0.65 µm, 0.45 µm, 0.22 µm, Millipore, Etten-Leur, The Netherlands). The finest filter (0.22 µm) was washed in 5M NaOH solution. Viruses were eluted from the filter with elution buffer (pH 9.0) containing 3 % beef extract (Difco Laboratories, Detroit, MI). The typical eluate volume of 20 L of raw sewage water is approximately 50 ml. Eluates were stored at –70 °C until further use.

Isolation of enteroviruses in soil

One hundred grams of soil were simultaneously collected. The protocol for isolation of viruses was followed by USEPA Manual of Methods for Virology - EPA publication EPA/600/4-84/013 (R-7). Briefly, 100 mL of buffered 10 % beef extract was added to 100 g of soil and vessel was placed on magnetic stirrer for 30 min. reducing the pH between 3.5 and 6.0 with 0.5 M HCl was done. Further, centrifugation of mixture was performed (2500 rpm for 30 minutes at +4°C). Decanted supernatant fluid was filtrated through 0.22 µm porous negatively charge Millipore filter. Eluate was stored until assay at -70 °C.

II.3.3.6 Qualitative analysis of HEV and Rotavirus with dotblot assay

Dot Blot was performed to determine the presence of specific enteroviruses (HEV and Rotavirus) in elutes. Elutes were loaded under gravity onto the nitro-cellulose transfer membrane (Pierce, Rockford, USA) by use of Dot Blot apparatus (BioRad).
Supernatant containing HEV and Rotavirus were applied to the membrane as the positive controls. The membrane was washed three times with 0.1 ml of 1X TBS (20 mmol l^{-1} tris and 137 $mmol^{-1}$ NaCl, pH 7.6). Non-specific binding sites were blocked with blocking solution for 16 h at 4 °C. The membranes were incubated for 1 h at room temperature with primary

antibodies: polyclonal rabbit anti HEV (ABcam) and goat polyclonal antiRota (ABcam) diluted in blocking solution. Following subsequent was washed three times with 1 x TTBS (each wash 10 mn under gentle shaking), membranes were incubated for additional hours at room temperature with secondary antibody: the anti-rabbit and anti-goat peroxidase-conjugated IgG (Sigma) diluted in blocking solution. Membranes were then washed three times with 1 x TTBS again and developed with Supersignal West Pico chemiluminescent substrate system (Pierce, Rockford, USA). After developing for 5 mn, the membranes were exposed to Biomax MR-1 film (Sigma-Kodak) for 10 mn with use of Sigma processing chemicals Kodak GBX developer and fixer system.

II.4 Statistical analysis

The data were statistically analysed as a split-plot design (main plots are irrigated and non-irrigated rows, subplots were slopes of the hill and soil depths) at Gačnik location and as a two factor trial (the first factor is organic fertiliser, the second factor is liming) at Pohorski dvor location. The second location has a stronger statistical basis compared to the first one, as three blocks were included in the experiment. Statistical procedures were carried out with the software package Statgraphics Centurion XV (Statgraphic®, 2005). The means were separated by the Duncan test, considering a significance level $p<0.05$. Analysis of variance (ANOVA) was used to find significance of effects of different factors on soil quality attributes.

Chapter 3

Results in Gačnik

Chapter 3: Results Gačnik

I. Soil morphology

For a better understanding of the results of our study, it is very important to describe the morphological properties of soils first and represent soils in general.

I.1 Soil profiles

To compare soil organization according to slope and the effects of irrigation, two soil profiles were described. Both soil profiles were opened from the irrigated row (across inter-row) to the non irrigated row at two slope positions: upslope and downslope position, perpendicularly to the rows (Fig. 3.2 & 3.3).

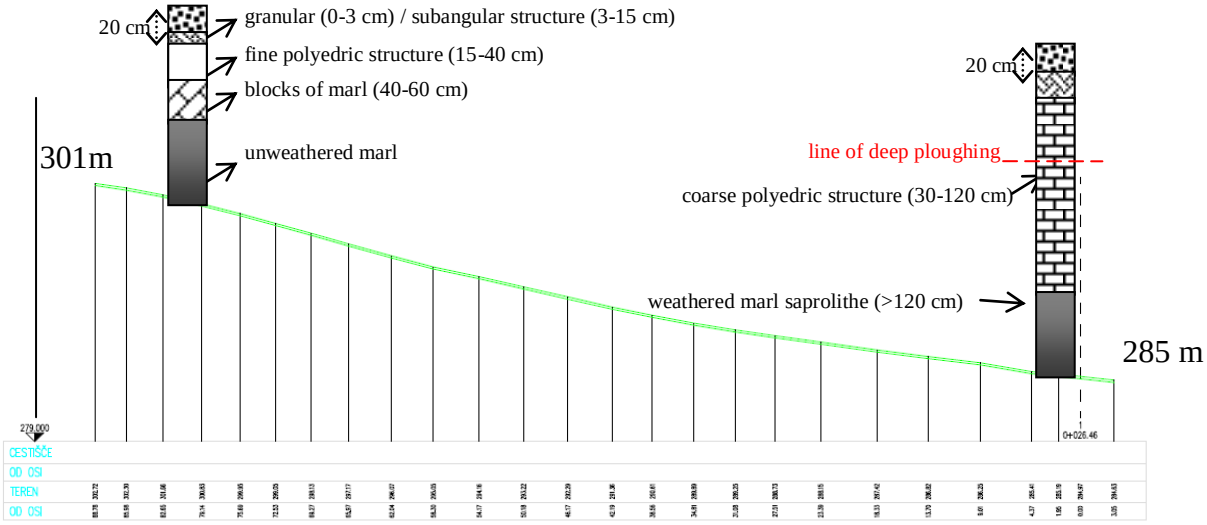


Fig. 3.1: Scheme of the slope with location of the two soil profiles

Figures. 3.1, 3.2 & 3.3, showed the soil deepened with slope. The hard unweathered marl bedrock occurred at 60 cm depth upslope while downslope the soft weathered saprolithe occurred from 100 cm depth. Macrostructure of the structural horizon also differed from fine polyhedric upslope to coarse polyhedric downslope. Upslope blocks of marl occurred from 40 to 60 cm while, downslope, evidences of deep ploughing till 60 cm occurred. Structure of the topsoil horizons varied with depth from granular (0 - 3 cm) to subangular polyhedric (3 - 15 cm) and fine polyhedric (> 15 cm) upslope to granular (0 - 3 cm) to subangular polyhedric (3 - 15 cm) and fine polyedric (15 - 30 cm) to coarse polyhedric (> 30 cm) downslope.

Fig. 3.2: Soil profile from IRR to NIR rows (upslope position)

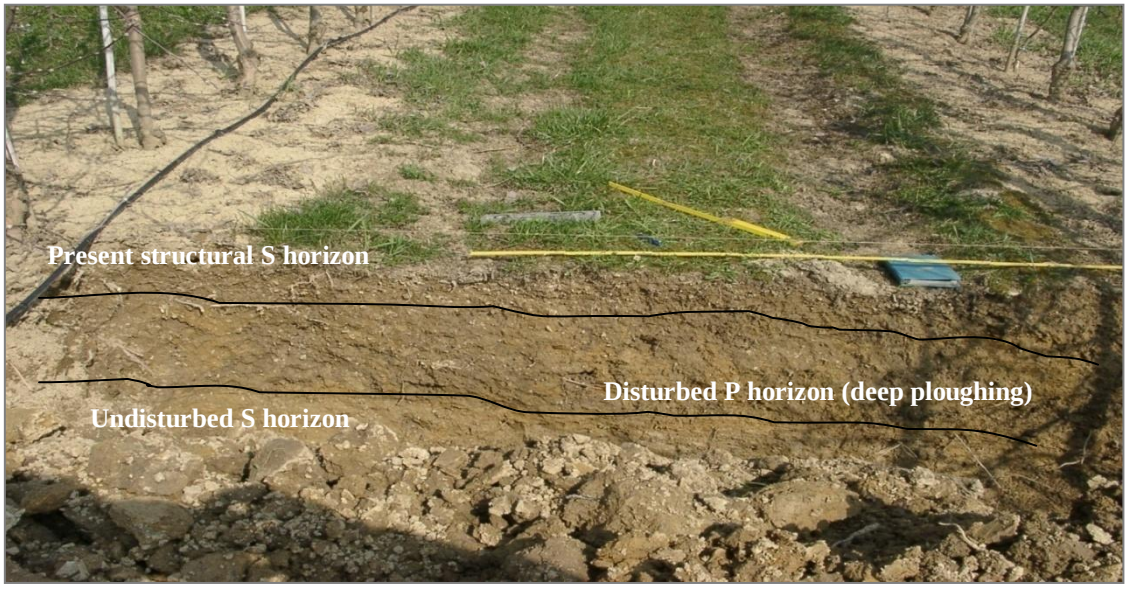


Fig. 3.3: Soil profile from IRR to NIR rows (downslope position)

Soil surface observations

Some obvious differences in the soil surface were observed according to slope and sun exposure. In the upper third of the slope, brighter soil colour and development of large cracks were noticed. Presumably, brighter colour is related to lower organic matter content and cracks to drier soil conditions. According to the sun exposure, at the sunny side of the rows, thicker structural crust (3 cm) was observed (Fig. 3.4); while at the shady side, structural crust (1 cm) covered with green algae appeared (Fig 3.5).

Fig. 3.4: Shady and sunny side in the row

Fig. 3.5: Structural crust covered by algae

I.1.1 *Upslope soil profile*

The upslope profile was dug till 60 cm, although boundary of marl as a weathering horizon was settled already at 40 cm (Fig 3.6 & Tab. 3.1). The soil was very dry and we noticed many vertical cracks all over the profile, which probably resulted from shrinking of the clayey structural horizon developed from marl (Fig. 3.7). These cracks enabled water and roots to move through the soil profile. Due to cracks development, surface water runoff should not be so strong and additionally aeration should be good enough in spite of high clay content. Some earthworms and fine roots were found through vertical cracks. Under 40 cm, roots were mostly growing horizontally due to lamellar structure of marl.
In the first 3 cm, the structure was granular to subangular polyedric, then polyedric up to 15 cm, below this line some individual blocks of marl appeared, contributing partly to lamellar structure. In the first 10 cm, biological activity was weak, while deeper roots density was higher till 40 cm and many earthworms' channels were noticed.

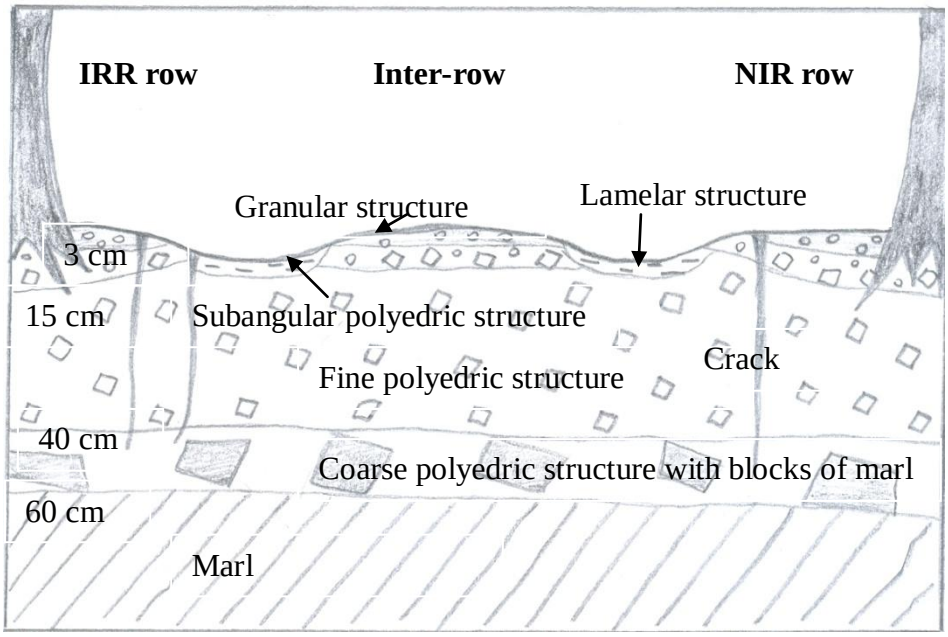


Fig. 3.6: Sketch of the upslope soil profile

Fig. 3.7: Upslope Soil profile from the in inter-row

No morphological differences were observed between IRR and NIR rows at upslope position.

In inter-row (upslope position), soils under and between the wheels were also observed. These soils were covered by grass. In all inter-row, the texture was silty clay.

In the soils between the wheels, progressive transition from fine granular structure (in the first 5 cm) via subangular polyedric structure (< 2 mm size) to fine polyedric and finally coarse polyedric structure occurred. Very abundant grass roots were observed till 5 cm. Colour: 10 YR 5/3 till 5 cm, from 5 to 15 cm 10YR 5/3, after 10YR 6/6 as a matrix with 10YR 5/3 mottles.
In all inter-row, soils were fresh till 10 cm, while they became humid deeper. Biological activity was moderate, fine roots appeared in the first 5 cm, deeper lateral tree roots appeared. Weathering marl horizon appeared at 40 cm in the whole inter-row.

In soils under the wheels, in first 3 cm a lamellar structure was noticed, very well developed; lamellas were 2-5 mm thick. Lamellar structure was a consequence of the strong compaction due to tractor wheels. From 3-10 cm, fine polyedric structure appeared (< 1 cm size). Below 10 cm, coarse polyedric structure occurred. Biological activity was reduced due to compaction; till 5 cm, fine roots of grass and few earthworms channels were observed, while tree roots showed the highest density in the 10-30 cm layer. Vertical variation of colours was interpreted in terms of organic matter content. In the first few cm dark brown (10YR 3/3) was related to high organic matter content, while in 5-20 cm less organic matter was present (10YR 5/3 as a matrix and 10YR 5/4 as mottles). From 20-30 cm 10YR 6/8 colour appeared, with mottles of 10YR 5/3. From 30-40 cm, hydromorphic features with mix grey colour (2,5YR 6/2) and yellowish brown (10YR 5/8) were noticed.

Table 3.1: Description of upslope soil profile (in the row)

UPSLOPE								
Depth (cm)	**Treat.**	**Humidity**	**Texture**	**Structure**	**Porosity**	**Colour**	**$CaCO_3$**	**Biological activity**
0-10	IRR	dry (fresh at shady side and very dry at sunny side)	clay + fine silt (micas)	0- 3 cm: granular to subangular 3-15 cm: subangular to coarse polyedric	high	surface: matrix 5Y 8/3 and mottles 5Y 7/3 0-5 cm: 10 YR 5/4 5-15 cm: matrix 10 YR 5/3 and mottles 10 YR 5/8	strong reaction with HCl	weak (few roots)
	NIR	dry (fresh at shady side and very dry at sunny side)	clay + fine silt (micas)	0- 5 cm: granular to subangular 5-10 cm: subangular	high	surface: 10 YR 7/3 (sunny side) 10 YR 4/4 (shady side) 0-10 cm: 10 YR 5/4	strong reaction with HCl	weak (few roots)
10-40	IRR	dry, more fresh deeper	clay + fine silt (micas); blocks of marl appeared	10-20 cm: polyedric 20-40 cm: some lamellar (marl)	low (compacted soil)	10-20 cm: matrix 10 YR 5/3 and mottles 10 YR 5/4, 10 YR 5/8 20-40 cm: matrix 10 YR 6/8 and mottles 10 YR 5/8	strong reaction with HCl	high (high roots density, earthworms)
	NIR	Fresh	clay + fine silt (micas)	10-15 cm: subangular 15-40 cm: polyedric + some lamellar (marl)	low (compacted soil)	matrix 10 YR 4/4 and mottles 10 YR 5/4, 10 YR 5/8	strong reaction with HCl (marl)	high (high roots density, earthworms)
40-50	IRR	Fresh	clay; more big blocks of marl	lamellar (marl)	very low (compacted soil)	matrix 10 YR 5/2	strong reaction with HCl (marl)	weak (few horizontal roots from trees)
	NIR	Fresh	clay; blocks of marl	coarse polyedric	medium	matrix 10 YR 6/8	strong reaction with HCl (marl)	weak (few horizontal roots from trees)

I.1.2 *Downslope soil profile*

At downslope position, less clay was noticed in the first 30 cm than deeper. The structure in the surface layer (till 5 cm) was very finely granulated, while deeper (till 15 cm), a mixture of granular and subangular polyedric structure was observed. Then the structure was only polyedric and size of the aggregates increased with depth, from fine between 15 to 30 cm to very coarse under 60 cm, with a correlative decrease of macroporosity as intra-aggregate pores were scarce (Fig 3.8 & Tab.3.2).
Very good biological activity was noticed till 60 cm (many channels of earthworms, ants, moles and mice; roots are placed mostly horizontally, density of roots was the biggest between 10 and 30 cm). Porosity was good. A lot of fine roots inside the clods were noticed.
Water content was higher under 60 cm. Bellow this line, hydromorphic features were observed (basic colour 10YR 5/4; brown spot 10 YR 5/6 and grey spot 2.5 YR 6/3).

Watching soil colour, soil in this profile was strongly mixed from 30 to 60 cm, what was noticed as a mixture of large volumes: dark brown volumes (10YR 5/4) surrounded by a lighter brown matrix (10 YR 5/6). Here more sand was observed than in the other layers. The weathered saprolithe occurred at 1m.
In upper layers (till 80 cm), carbonates (around 10%) were detected, no carbonates were noticed from 80 cm till 1 m.
No morphological differences were observed between IRR and NIR rows at downslope position (Fig. 3.9a &b).

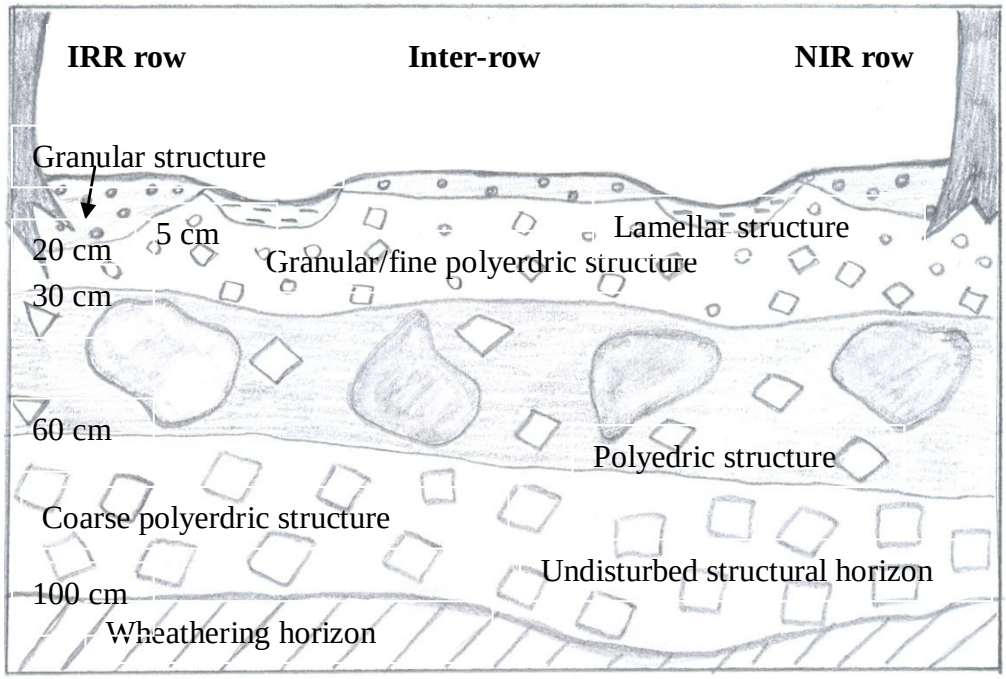


Fig. 3.8: Scheme of the soil profile at downslope position

Fig. 3.9a &b: Soil profiles from IRR and NIR soils at downslope position

Table 3.2: Description of the downslope soil profile (in the rows)

DOWNSLOPE								
Depth (cm)	**Treat.**	**Humidity**	**Texture**	**Structure**	**Porosity**	**Colour**	**$CaCO_3$**	**Biological activity**
0-15	IRR	fresh (shady side more humid that sunny side)	clay+ fine silt (micas)	0-5 cm: fine granular 5-15 cm: subangular + fine polyedric	high	surface: 10 YR 7/3 0-15 cm: 10 YR 4/4	strong reaction with HCl	till 10 cm not good, deeper better (tree roots)
	NIR	fresh (shady side more humid that sunny side)	clay+ fine silt (micas)	0-3cm: fine granular 3-5 cm: subangular 5-15: fine polyedric	high	surface: 10 YR 7/3 0-3 cm: matrix 10 YR 4/3 and mottles 10 YR 5/3 5-15 cm: matrix 10 YR 5/4 and mottles 10 YR 5/8	strong reaction with HCl	till 10 cm not good, deeper better
15-30	IRR	strong reaction with HCl	fresher than in upper layer	fine polyedric	very higher	matrix 10 YR 5/4 and mottles 2,5Y 5/3 (local hydromorphic features)	clay + fine silt (micas)	intensive (fine roots, earthworms and tree roots)
	NIR	strong reaction with HCl	fresher than in upper layer	fine polyedric	high	matrix 10 YR 5/4 and mottles 10 YR 4/4, 10 YR 5/8 (local hydromorphic features)	clay + fine silt (micas)	intensive (fine roots, earthworms and tree roots)
30-60	IRR NIR	weak reaction with HCl	humid	coarse polyedric	low (no intra-aggregates pores)	mottles 10 YR 5/6 and 2,5 YR 6/3 (local hydromorphic features)	clay	good (earthworms channels)
60-90		very week reaction with HCl	humid	very coarse polyedric	low (no intra-aggregates pores)	matrix 10 YR 5/4 and 2,5 YR 5/4 (local hydromorphic features)	clay	poor due to compaction (few fine roots)

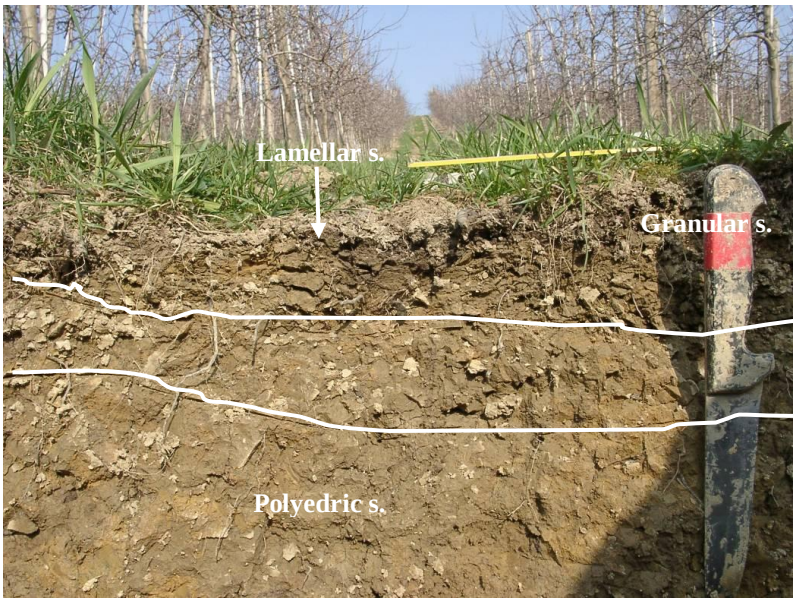


Fig. 3.10: Between wheel and under wheel soil organization downslope (the size of knife is 30 cm).

In the inter-row downslope, soil surface was completely covered with grass.

In the soils between the wheels (Fig. 3.10 right side), in the first 5 cm, fine granular to subangular structure (< 2 mm size of aggregates) was observed, with a gradually passage to fine polyedric structure lower, which progressively became coarser. Many grass roots were present till 5 cm. Colour: 10 YR 3/3 in first 3 cm, from 3-15 cm 10 YR 4/4 and deeper 10 YR 5/3. Under 10 cm, local faint hydromorphic features were noticed, while porosity was lower. In all inter-row soils were fresh till 10 cm and water content increased deeper.

In soils under the wheels (Fig. 3.10 left side), lamellar structure was noticed in the first 4 cm a, very well developed; lamellas were 2-5 mm size. From 3-10 cm, fine polyedric structure appeared (< 1 cm size). Below 10 cm, coarse polyedric structure began to appear (2-3 cm size). Till 10 cm, a lot of grass roots were observed, but just a few tree roots and earthworms channels were noticed here. From the soil colour, it was obvious that organic matter was higher in the first few cm (10YR 3/2) than lower (10 YR 4/4). Under 10 cm, 10YR 5/8 hydromorphic mottles appeared locally. Low porosity was observed due to strong compaction.

I.2 Comparison of soils from different water regime and slope positions

No morphological differences were observed between IRR and NIR rows at both slope positions.
Comparison of soils from the two slope positions showed that soils from upslope had less biological activity and less organic matter. Geological substratum appeared already at 60 cm or less (in the individual blocks). They were drier due to their thickness and runoff water through big vertical cracks and runoff along the slope. While downslope soils where much deeper, weathered marl saprolithe appeared at one meter. Soil layer with favourable granular structure was thicker (till 10 cm), while upslope it was only 3 cm thick. Downslope was also characterized by a large heterogeneous horizon between 30 and 60 cm depth composed by a

mixture of large dark brown volumes embedded in a lighter brown matrix, resulting from deep ploughing.

I.3 Conclusions

1. We can conclude that this orchard soil is very anthropogenic and heterogeneous, resulting of terracing and land levelling before planting trees, and deep ploughing (60 cm).
2. According the slope position, differences in soil depth were noticeable: shallow soil upslope (40 cm) and deep soil downslope (100 cm).
3. On the surface, structural crust (1-3 cm thickness) was observed.
4. Development of structural horizons were noticed from 0 to 15 cm, as a result of shallow ploughing and present pedogenesis from previous disturbed P horizon below. In these horizons, granular to subangular structure was noticed in upper layer, while in deeper layers compact polyedric structure occurred.
5. No differences in soil structure were noticed between IRR and NIR rows.

II. Soil water regime and quality of irrigation water

II.1 Water retention curves

Water retention curves were done on NIR soil samples (control) for three depths: 20-25, 40-45 and 80-85 cm at downslope position, where tensiometers were settled. These curves were done measuring water content at 6 pressure heads: 33, 100, 200, 300, 500, 1000 and 1500 kPa (Tab. 3.3 in Fig. 3.11).

Tab. 3.3: Volumetric water content (θ) and water ratio (ϑ) at different pressure heads.

	Pressure (kPa)											
	33		**100**		**300**		**500**		**1000**		**1500**	
Depth (cm)	**θ**	**ϑ**	**θ**	**ϑ**	**θ**	**ϑ**	**θ**	**ϑ**	**θ**	**ϑ**	**θ**	**ϑ**
20-25	0.50	1.03	0.43	0.88	0.35	0.73	0.33	0.69	0.36	0.74	0.36	0.75
40-45	0.53	1.07	0.46	0.92	0.37	0.75	0.35	0.70	0.37	0.73	0.34	0.69
80-85	0.50	0.95	0.42	0.80	0.31	0.59	0.27	0.51	0.28	0.54	0.24	0.46

θ ($cm^3.cm^{-3}$) = volumetric water content; ϑ = water ratio

Volumetric water content was calculated from gravimetric water content and bulk density of soils in each layer:

θ ($cm^3.cm^{-3}$) = W (gg^{-1}) * γ_d

where

θ ($cm^3.cm^{-3}$) = volumetric water content

W ($g.g^{-1}$) = gravimetric water content

ϑ ($cm^3.cm^{-3}$) = water ratio = V_{water} / V_{solid}

γ_d ($g.cm^{-3}$) = bulk density

Measured bulk density were 1.29, 1.32 and 1.39 $g.cm^{-3}$ for the layers 20-25, 40-45 and 80-85 cm respectively.

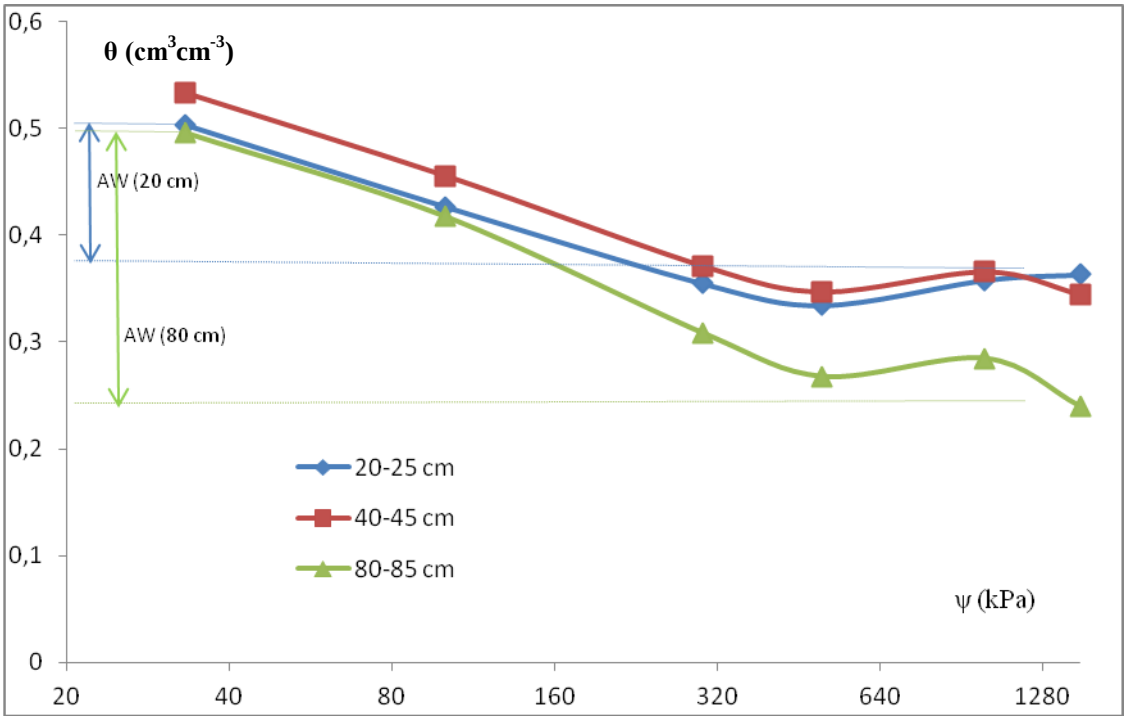


Fig. 3.11: Water retention curves in Gačnik. October 2004.

Typical soil water retention curves (SWRC) are shown in Fig. 3.11 SWRC shows how, when a soil is dried from saturation, the water content, θ, decreases as the soil water potential, ψ, becomes more negative.
Available water (AW) is defined as a difference in water content between field capacity (FC) and wilting point (WP).
AW = FC – WP
Wilting point (WP) = 4.2 pF (15 bar = 1500 kPa)
Field capacity (FC) = 2.5 pF (0.33 bar = 33 kPa)

At FC, all three layers contained more or less the same water content (the surface and the deepest soil layers 0.5 cm^3cm^{-3} and middle layer 0.53 cm^3cm^{-3}). Significant difference occurred at WP, where the deepest soil layer held 30 % less water (0.24 $cm^3.cm^{-3}$) comparing the middle (0.34 $cm3.cm^{-3}$) and the surface layer (0.36 $cm^3.cm^{-3}$).

Available water increased with depth: 0.14, 0.19 and 0.26 $cm^3.cm^{-3}$ for 20-25, 40-45 and 80-85 cm, respectively.

Tab. 3.4: Particle size distribution of the three layers at downslope position (where tensiometers were settled)

Depth (cm)	% coarse sand	% fine sand	% coarse silt	% fine silt	% clay
20-25 cm	0.7	1.9	14.6	38.7	44.1
40-45 cm	0.4	2.0	15.0	36.8	45.7
80-85 cm	0.1	1.8	11.9	45.5	40.7

As seen in Tab. 3.4, textures of the different layers were silty clay. Percentage of all particle size fractions were more or less the same in the first two layers, while deeper decreasing of clay and increasing of fine silt was noticed, which could also explain the lower amount of water retained of WP and bigger amount of available water in this deeper layer. The curves for available water in the upper soil layers (20-25 cm and 40-45 cm) had more or less a similar slope pattern. Here we are dealing with fine polyedric structure with higher clay content, which explained less available water for plants.

II.2 Soil water regime in 2004 and 2005

To follow the soil water regime, measurements of water potential (ψ) from IRR, NIR rows and soils from inter-row were compared, at three soil layers: 20-25 cm, 40-45 cm and 80-85 cm during the two seasons (2004 & 2005), at downslope position. Distributions of soil water potential according to treatment and season with rainfall distribution by weeks are presented in Fig. 3.12a, b & c and 3.13a, b & c. Measurements of water potential are presented in Annex 5.

II.2.1 *Evolution of soil water potential along 2004 summer*

Distribution of rainfall in two studied seasons is presented in Tab. 3.5. In June 2004, 175 mm of rainfall was measured, which probably enabled sufficient water for plants. In July 2004, 70 mm of rainfall was measured, while in the first two decades of August only 18 mm and in the third decade of August 52 mm of rainfall was measured. In the first decade of September 2004, only a small quantity of precipitation was measured (12 mm). Two main dry periods were noticed in the season 2004: in the middle of July and in the first half of August. If

rainfall was insufficient, irrigation was performed with a rate of 2 litres/day/tree. In the first dry period, 15 days of irrigation was performed (from 15th of July till 1st August), while in the second dry period, 10 days of irrigation was performed (from 15th till 25th of August).

Tab. 3.5: Monthly summer rainfall in mm, Maribor (from Fig. 2.5)

Time period	June	July	August	September	mean July-September
2004	174.7	70.8	70.3	81.7	74.1
2005	56.2	173.4	219.5	135.5	191.5
mean 61-90	119	118	126	99	117

Comparing water potential curves in different soil layers, the deepest soil layer (80 cm) showed bigger variation than upper layers. Soil from the deepest soil layer (80-85 cm) had the lowest water potential comparing soils from upper layers, especially in dry periods.

Some differences occurred in pattern of water potential measurements in IRR, NIR rows and in inter-row according to dry periods. Water potential very clearly followed the rainfall distribution in all situations. Till the beginning of July, when rainfall occurred regularly, water retention measurements were very similar according to treatments and depth. In the first dry period (in the middle of July), a decrease of water potential in soils from all three layers happened simultaneously. In this period, irrigation was performed and permitted a rising of water potential in IRR row in the third decade of July, associated to a moderate rainfall. Due to water deficit in first half of August, water potential strongly decreased in the middle of August - but not simultaneously in all three layers and treatments. At this minimum peak, different reactions among soil layers were noticed: soils from surface layer reacted more rapidly to the addition of water from rainfall and from irrigation comparing to deeper layers. Mid-layer (40-45 cm) responded to irrigation with a few days delay. The deepest soil layer (80-85 cm) also responded with a few more days of delay after mid-layer. Water potential measurements in this dry period were much lower comparing to dry period in July, especially in IRR row. At the end of August, different response of water potential was observed in IRR and NIR rows: additional water from irrigation helped to raise water potential in IRR row from very low values. On both minimum peaks, IRR row had lower water potential than NIR row. This systematic observed feature may be related to a more superficial root system in the irrigated row induced by the drip irrigation as shows by Isberie (1995) mentioning drier soil around the dripper.

Soil water potential pattern in soil from inter-row strongly differed from those in the rows. Till beginning of July, when rainfall occurred regularly, water potential measurements were more or less the same, but lower than in IRR and NIR rows.
In the first dry period (in the middle of July), decrease of water potential in soils from all three layers happened more or less simultaneously. In this period, only in the deepest soil layer some differences were noticed at the end of July with rising of water potential with a few days of delay. Due to rainfall deficit in the first half of August, water potential simultaneously decreased in lower layers only. In the third week of August, the strongest response to the rainfall happened in the deepest soil layer again, while in the surface layer almost no response was noticed. Delay between rainfall event and potential rising of the deepest tensiometer without any rising of the upper tensiometers means that water did not come vertically from the surface but laterally from upslope with ten or fifteen days of delay. It was hypodermic water fluxes circulating laterally above the marl saprolithe. Later, also due to lack of rainfall water, water potential in deeper soil layers began to decrease again.

The more superficial tensiometer (20-25 cm) after its decrease in the first dry period, kept the same potential at -300 hPa without rising, following the rainfall events at the end of July and the end of August. It could be related to the evapotranspiration of the grass cover in the inter-row. To compare, in the NIR the potential were higher (between 100 and 200 hPa) and responded to the precipitations which could be related to: (1) a better infiltration and a lower evaporation of the bare soil in the row and (2) rainfall interception by the trees: water flow along the trunk could represent according to species between 3 to 30% of rainfall under forest (Ulrich *et al*., 1995)
The intermediate tensiometer (40-45 cm) decreased continuously from the first dry till the mid of August (-700 hPa) due to grass evapotranspiration and water uptake from tree roots. Then the potential rose following the rainfall at the end of August while the more superficial tensiometer did not react, which could be related to preferential flow along the cracks in this dry clayey soil.

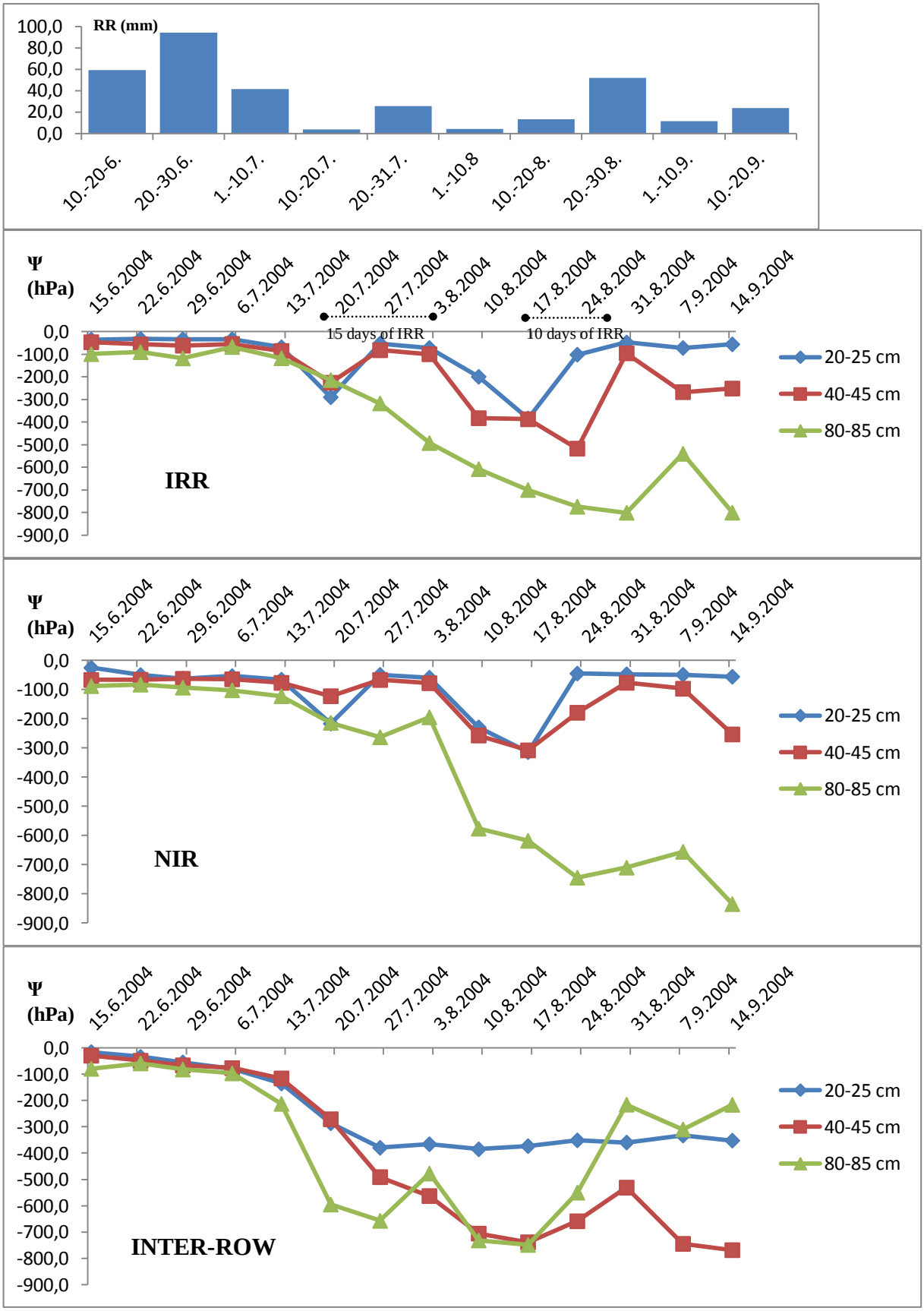


Fig. 3.12a & b & c: Measurements of water potential (ψ) in IRR row (a), in NIR row (b) and inter-row (c) with distribution of rainfall (RR) at downslope position, in Gačnik 2004.

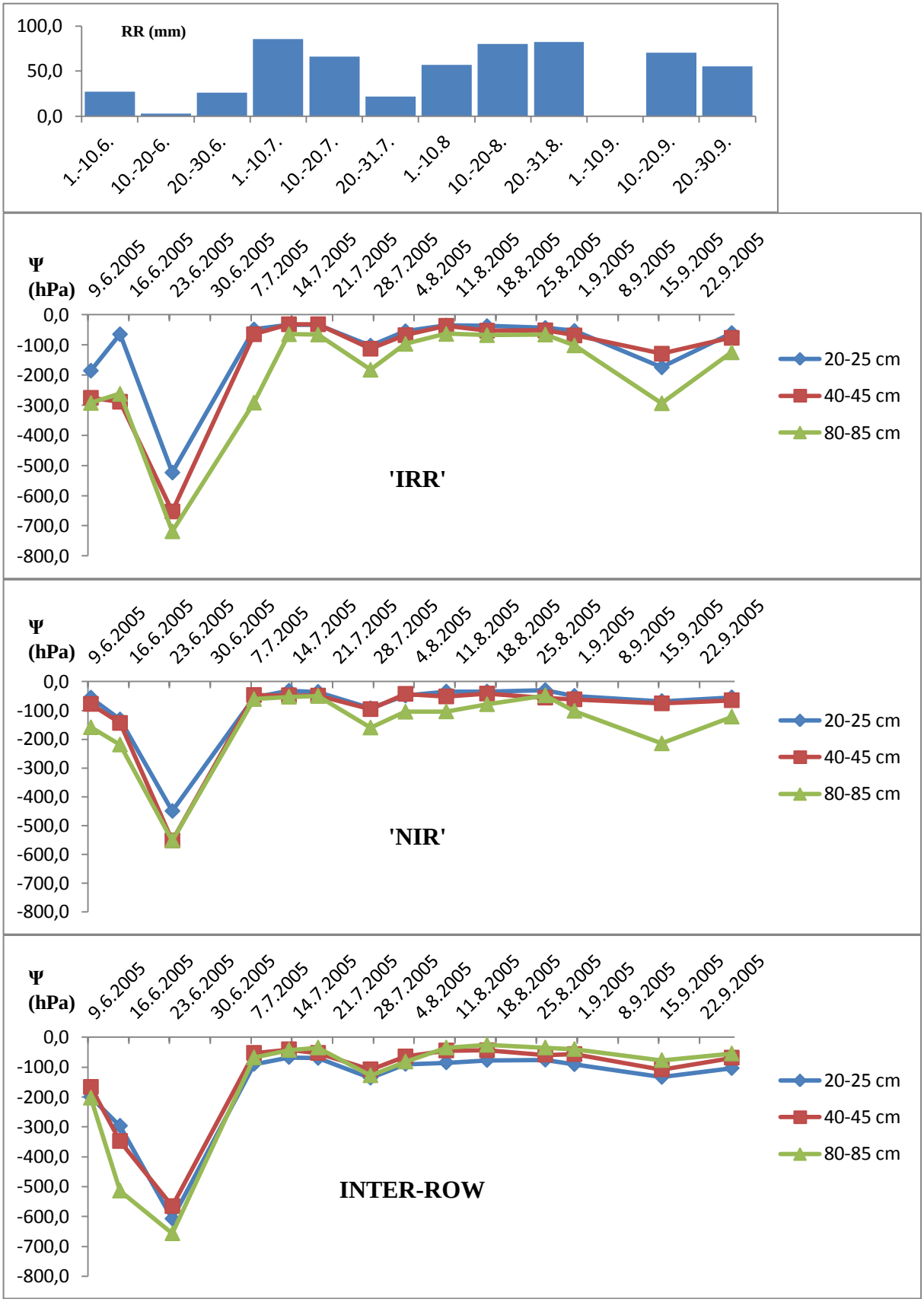


Fig. 3.13a & b & c: Measurements of water potential (ψ) in IRR row (a), NIR row (b) and inter-row (c) with distribution of rainfall (RR) in Gačnik, 2005.

II.2.2 *Evolution of soil water potential along 2005 summer*

The season 2005 was very wet (Tab. 3.5), especially July, August and September. June was the driest month in the season 2005: two small amount of rainfall (27 mm) occurred in the first and in the third decade. In July 2005, 173 mm of rainfall occurred; 220 mm in August and in 126 mm of rainfall in September was measured. The total amount of precipitation in the summer 2005 was high and this was also noticed from the measurements of water potential. Curves of all three soil layers had similar shape at each treatment, especially in inter-row.

The main dry period was noticed in the second half of June. In this minimum peak, the upper soil layer (20-25 cm) in IRR soil and in inter-row had the lowest water potential, while in NIR soils the highest. In the deepest layer (80-85 cm), water potential was the lowest in IRR soil again. At the end of June and the beginning of July, rainfall caused increasing of water potential. From the beginning of July 2005, water potential measurements were close to the field capacity. Then two (much smaller) minimum peaks occurred: in the last decade of July 2005 and in the middle of September 2005. In the first small peak (in July), the deepest layer showed the lowest water potential in all studied soils. During September, water potential was slightly smaller in the deepest layer (in NIR and in IRR rows) than in upper layers. No irrigation was needed in 2005.
General shapes of water potential measurements were related to rainfall amounts. Some differences were noticed in water potential among soil layers in the rows; while in inter-row, the measurements of water potential in all three layers were almost the same.

II.2.3 *Comparison summers 2004 and 2005*

Comparing the two seasons (2004 and 2005), summer 2004 was much drier than 2005. In 2004, two dry periods occurred (one at end of July and another at the end of August), while in 2005 one strong dry period happened earlier (in June) and two small “dry periods” followed (in July and in September).

NIR row showed higher water potential than IRR row in both years. This is surprising, but it must be pointed out that organic matter content was generally higher in NIR treatment, with positive effect on water retention. Another possible explanation could be more developed trees in the IRR row due to more favourable water conditions what could participated to more developed root system and thus more intensive root suction (personal communication, Mr. Zadravec, The Head of experimental station: the yield of irrigated rows was higher than non irrigated one). In both seasons, the lowest water potential was measured in the deepest soil layer of IRR and NIR rows. Comparing the two seasons, a big difference occurred in patterns in soils from inter-row. In 2005, the tensiometer response of soil from inter-row was much more similar to soils from rows (IRR, NIR) than the previous season. In 2004, the obvious effect of grass water uptake was observed on the behaviour of the two first tensiometers and incidence of delayed lateral hypodermic flow for the deeper tensiometer.

Measurements of water potential in all treatments and in both seasons never reached the wilting point. According that apple trees require 70-80% of water of FC and factor 0.5 for easily available water of FC (Slovenian irrigation project, 1998), some available water deficit occurred in the season 2004 (especially in the deepest layer).

Soil matrix potential (SMP), especially in non-saline areas, is considered a better criterion for characterizing crop soil water availability than gravimetric or volumetric water content. Numerous studies using tensiometers to measure SMP and schedule irrigation have been reported (Clark *et al.*, 1996; Shock *et al.*, 2000; Wilson *et al.*, 2001).
Although the rain affected the targeted soil matrix potential readings at depth of 20 cm. temporal and spatial changes in soil water in the observed profile suggest that matrix potential at 20 cm immediately under emitter can be used as an index for scheduling drip irrigation (Wang *et al.*, 2007).

II.3 Quality of irrigation water

Irrigation water was sampled from two locations: i) directly from the primary source of irrigation (i.e. irrigation pond) and ii) from irrigation pipe. The main purpose for sampling from irrigation pipe was the microbiological sanitary aspect, but it can also help to understand the chemistry of irrigated soils. For this reason, the extended chemical analysis was made for water from irrigation pond only. The results are presented in Tab. 3.6.

Tab. 3.6: Main characteristics of irrigation water, October 2007 (Faculty of Agriculture and Life Sciences - Department of Microbiology, Biochemistry, Molecular Biology and Biotehnology and Faculty for Chemistry and Chemical Engineering).

Parameter	Unit	IRR water (pond)	(IRR pipe)
pH		7.8	8.0
Ca^{++}	$Mmol.L^{-1}$	4.38	
Mg^{++}		2.64	
K^{+}		0.06	
Na^{+}		0.72	
Cl^{-}		0.56	
SO_4^{-}		0.35	
HCO_3^{-}		5.9	
SAR		0.38	
NH_4^{+}	$mg.L^{-1}$	<0.1	
NO_2^{-}		0.045	
NO_3^{-}		10	
EC	$mS.cm^{-1}$	0.61	
COD	$Mg\ O_2\ .L^{-1}$	180	240
BOD		100	120

Basic chemical parameters were measured for assessing irrigation water. Beside pH and macronutrients, electroconductivity (EC) and sodium adsorption ratio (SAR) were measured or calculated. Chemical and biochemical oxygen demand (COD and BOD) were measured for assessing pollution of irrigated water.

The pH values of irrigation water ranged between 7.8 and 8.0, lying within the normal range according to Ayers and Westcot (1985) as well as by WHO (1992), reporting that correct pH values for irrigation are between 6.4 to 8.4.

The EC value of IRR water was 0.61 $mS.cm^{-1}$. According Ayers and Westcot (1985), this value allows a classification as “no restriction for irrigation”. With such an EC value, this water is convenient for irrigation according to the Official Gazette of Republic Slovenia (limit value: 2 $mS.cm^{-1}$).

The data revealed that Ca^{2+} was the dominating cation, followed by Mg^{2+} and Na^{+}; whereas K^{+} was the lowest. On the other hand, the main soluble anions were (in descending order): $HCO^{-}_{3} > Cl^{-} > SO_4^{=}$. The predominance of soluble ions of Ca^{+2} and Mg^{+2} was positively reflected on the low SAR value, which was 0.43. Such low SAR value in the investigated water-pond gives none degree of restriction for irrigation of agricultural crops (Metcaff and Eddy (2003).

Nitrate was found in appreciably high concentration (10 $mg.L^{-1}$) compared to ammonium and nitrite (0.1 and 0.05 $mg.L^{-1}$). The amount of nitrate is on borderline by Official Gazette of Republic Slovenia (limit value is 10 $mg.L^{-1}$). According to FAO (1992) and WHO (1992) guidelines, high nitrogen concentration must be avoided not only for sensitive crops but also to avoid nitrate leaching.

The concentrations of COD and BOD (180 and 100 mgL^{-1}) exceeded the permissible limit for water reuse as recommended by Metcaff and Eddy (2003). These high values indicate the presence of organic matter, a large part of which is easily mineralized by microorganisms (Metcaff and Eddy, 2003).

Some parameters (pH, COD, BOD) were measured also in water from the irrigation pipe. The pH was roughly the same as in the pond. However, COD and BOD levels were slightly higher in irrigation pipe than in the pond.

III. Physico-chemical soil characteristics

III.1 General soil characteristics

III.1.1 *Results*

Complete chemical analyses, including particle-size distribution were done in September 2005. General soil properties for comparing irrigated (IRR) and non irrigated (NIR) soils are presented in Tab. 3.7a &b & c, according to slope position.

The soil texture is silty clay in general. In both water treatments, the percentage of clay in the surface layer (0-30 cm) is more than 40%. Clay and silt fractions represent more or less an equal percentage of soil particles, while sand fraction represents only a few percents (from 1.0-4.9%).

Particle size distribution did not vary largely according to depth, slope or treatment (Fig. 3.14).

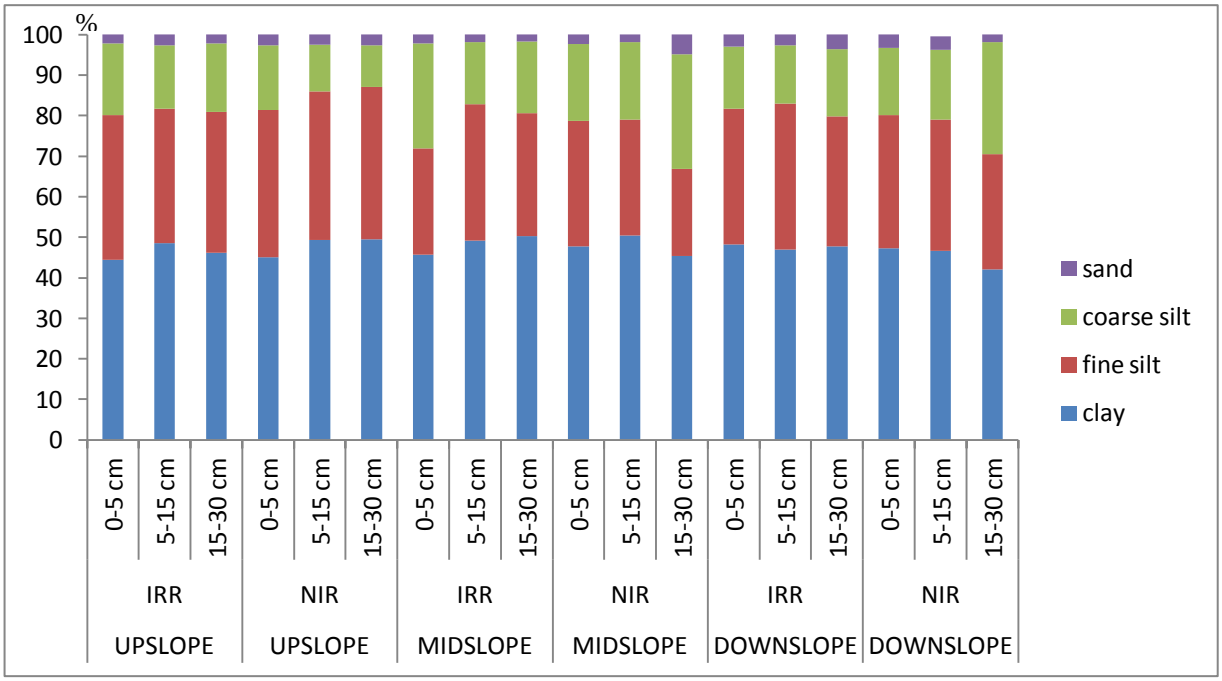


Fig. 3.14: Particle size distribution according to slope, treatment (IRR/NIR) and soil depth.

Tab. 3.7 a, b, c: Basic soil characteristics of irrigated and non irrigated rows at upslope (a), midslope (b) and downslope (c) positions , September 2005

Slope position	**UPSLOPE**					
Water treatment	**IRR**			**NIR**		
Soil depth (cm)	**0-5**	**5-15**	**15-30**	**0-5**	**5-15**	**15-30**
Gravel and cobbles (> 2000 µm)	-	-	-	-	-	-
Total sand (53-2000 µm)	2.2	2.7	2.2	2.6	2.5	2.7
Coarse silt (20-53 µm)	17.6	15.6	16.9	16.0	11.5	10.3
Fine silt (2-20 µm)	35.7	33.2	34.7	36.4	36.6	37.6
Clay (<2 µm)	44.5	48.5	46.2	45.0	49.4	49.5
pH (water)	8.1	8.25	8.4	8.0	8.1	8.3
Total $CaCO_3$ %	17.0	17.6	16.7	14.3	15.1	18.8
Active lime %	7.7	8.4	8.2	7.6	7.9	8.2
Org. Carbon %	0.79	0.64	0.52	1.28	1.07	0.71
Total Nitrogen ‰	1.55	1.80	1.15	2.15	1.75	1.15
C/N	5.1	3.5	4.5	6.0	6.1	6.2
Organic matter %	1.36	1.10	0.89	2.20	1.84	1.22
Phosphorus (P_2O_5)	0.05	0.01	0.02	0.12	0.015	Traces
CEC ($cmol^+.kg^{-1}$)	11.7	12.0	12.3	15.7	15.7	15.3
Exch. Na ($cmol^+.kg^{-1}$)	0.21	0.23	0.43	0.14	0.17	0.17

Slope position	**MIDSLOPE**					
Water treatment	**IRR**			**NIR**		
Soil depth (cm)	**0-5**	**5-15**	**15-30**	**0-5**	**5-15**	**15-30**
Gravel and cobbles (> 2000 µm)	-	-	-	-	-	-
Total sand (53-2000 µm)	2.1	1.8	1.8	2.3	2.0	4.9
Coarse silt (20-53 µm)	26.0	15.4	17.7	19.0	19.0	28.2
Fine silt (2-20 µm)	26.2	33.7	30.3	30.9	28.7	21.6
Clay (<2 µm)	45.7	49.1	50.3	47.8	50.4	45.3
pH (water)	8.2	8.2	8.2	8.1	8.1	8.1
Total $CaCO_3$ %	5.7	5.7	6.5	4.1	5.1	4.7
Active lime %	3.6	4.6	4.2	2.5	3.2	3.1
Org. Carbon %	1.45	1.23	1.13	1.56	1.50	1.30
Total Nitrogen ‰	2.10	1.75	1.90	2.2	1.7	1.6
C/N	6.9	7.0	5.9	7.1	8.8	8.1
Organic matter %	2.49	2.12	1.94	2.68	2.58	2.24
Phosphorus (P_2O_5) ‰	0.26	0.095	0.01	0.18	0.11	0.06
CEC ($cmol^+.kg^{-1}$)	14.7	15	16.3	21.9	19.8	22.2
Exch. Na ($cmol^+.kg^{-1}$)	0.19	0.20	0.22	0.16	0.17	0.17

Slope position	**DOWNSLOPE**					
Water treatment	**IRR**			**NIR**		
Soil depth (cm)	**0-5**	**5-15**	**15-30**	**0-5**	**5-15**	**15-30**
Gravel and cobbles (> 2000 µm)	-	1.2	10.1	1.0	-	-
Total sand (53-2000 µm)	3.0	2.6	3.7	3.3	3.2	1.8
Coarse silt (20-53 µm)	15.4	14.4	16.6	16.5	17.7	27.7
Fine silt (2-20 µm)	33.4	36.1	32.1	32.9	32.3	28.4
Clay (<2 µm)	48.2	46.9	47.7	47.3	46.7	42.1
pH (water)	8.2	8.2	8.0	8.0	8.1	8.1
Total $CaCO_3$ %	4.3	5.0	4.9	3.6	4.3	4.6
Active lime %	2.7	3.1	3.2	2.4	2.8	3.1
Org. Carbon %	1.89	1.61	1.46	1.96	1.64	1.51
Total Nitrogen ‰	2.4	2.2	2.3	3.0	2.3	2.1
C/N	7.9	7.3	6.3	6.5	7.1	7.2
Organic matter %	3.25	2.77	2.51	3.37	2.82	2.60
Phosphorus (P_2O_5) ‰	0.18	0.095	0.69	0.22	0.12	0.05
CEC ($cmol^+.kg^{-1}$)	14.9	15.9	16.3	21.2	20	20.5
Exch. Na ($cmol^+.kg^{-1}$)	0.24	0.26	0.27	0.18	0.17	0.18

In Fig. 3.15, general presentation of soil organic matter (SOM), C/N, total carbonates and cation exchange capacity (CEC) distributions and relations among them are described. Further, each of these parameters is more precisely described in Fig. 3.16a & b and 3.17a & b.

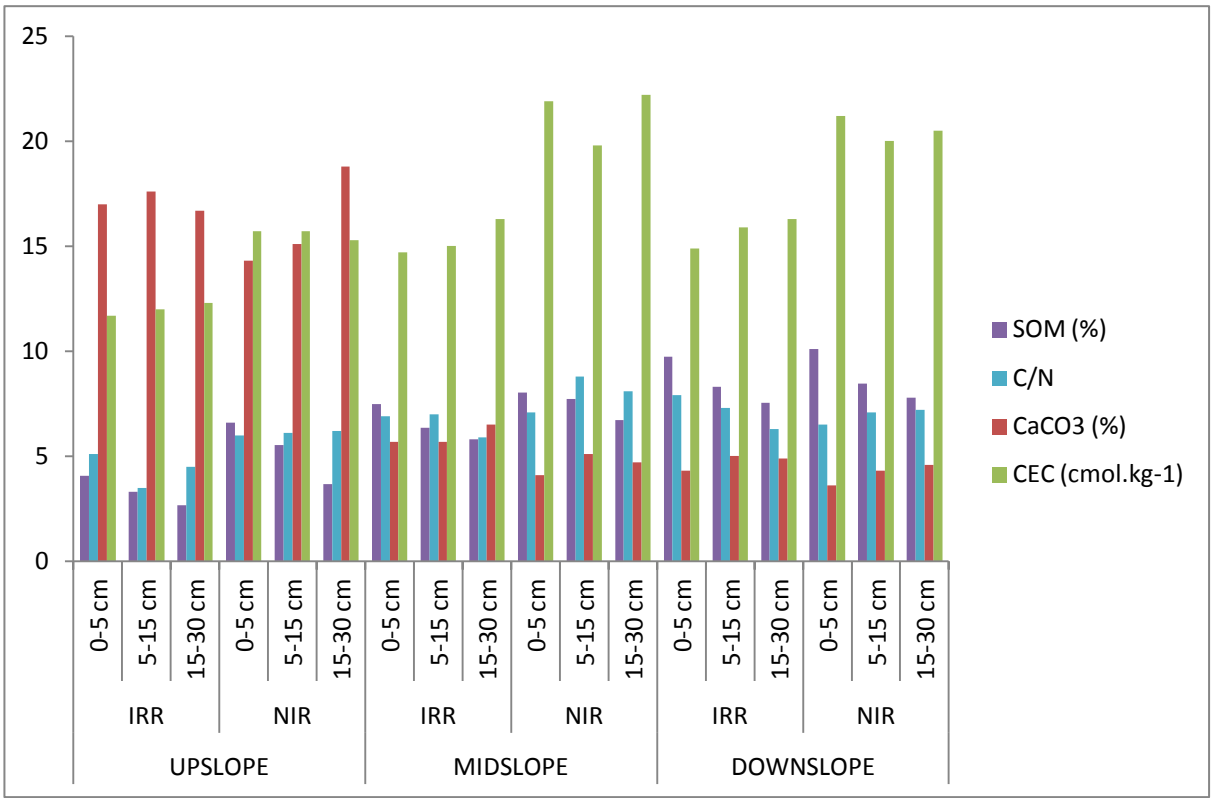


Fig. 3.15: Distribution of SOM (values are multiplied by 3), C/N, CEC and $CaCO_3$ according to slope, treatment (IRR/NIR) and soil depth.

pH in water was at least 8 in all situations (from 8.0 to 8.4 %) as a result of the presence of calcium carbonates. pH increased with depth at upslope position only, while at lower positions this pattern was not noticed. No evident difference according to slope position was observed for soil pH. pH value was more or less the same in both water treatments.

Levels of total carbonates (%$CaCO_3$) and active lime are presented in Fig. 3.16a & b. The maximum value of total $CaCO_3$ was recorded in NIR soils at upslope position in the deepest layer (18.8 %), while the minimum occurred in NIR soils at downslope in surface soil layer (3.6 %).
From the surface to 15-30 cm, total carbonate was slightly increasing. Upslope total carbonates were even three times higher compared to lower slope positions**.** A higher amount was noticed in IRR soils, especially at upslope position, while at midslope and downslope positions values were almost the same for both treatments (IRR, NIR): 5-7 % of total $CaCO_3$.

In the studied soil samples, active lime represented approximately a half of the total carbonates, on average. The maximum value (8.4%) was determined at upslope position in the 5-15 cm layer of IRR treatment, while the minimum value (2.4%) was found at downslope position in the surface soil layer (0-5 cm) of NIR treatment.

Similarly to the total carbonates content, active lime slightly increased with depth (till 15 cm) and towards upslope: the observed values were higher at upslope position than at midslope and downslope positions. A higher amount of active lime was noticed in IRR row.

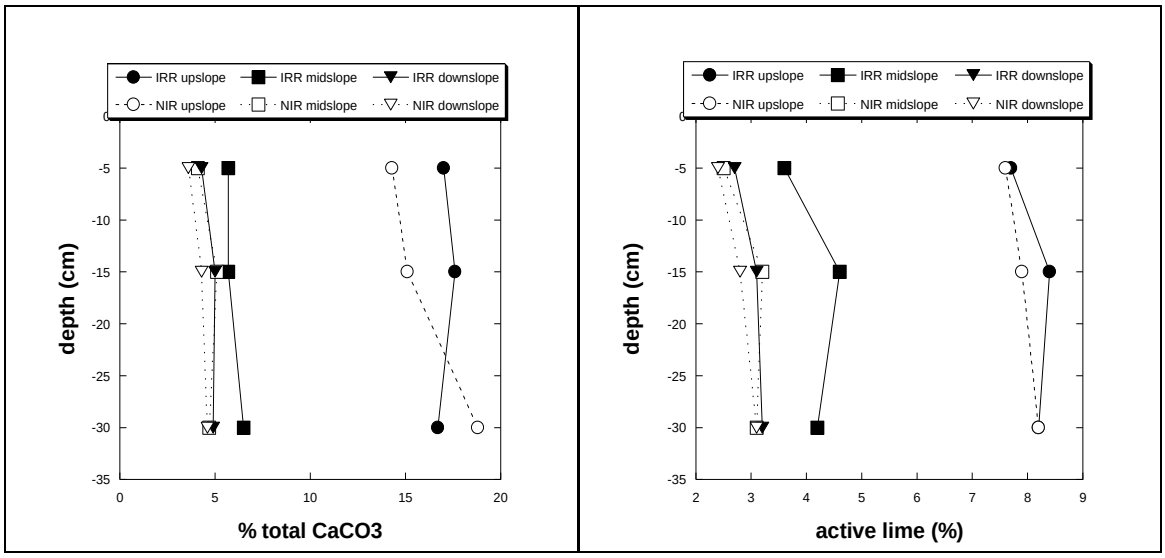


Fig. 3.16a & b: Distribution of total and active $CaCO_3$ according to a depth, slope and treatment.

Values of soil organic matter (SOM) are presented in Fig. 3.17a. Maximum Corg values were determined at downslope in the surface soil layer (0-5 cm): 1.89 % Corg in IRR and 1.96% Corg in NIR (values in IRR and NIR rows were more or less the same). Minimum Corg value was found at upslope position in the 15-30 cm layer of the IRR treatment: 0.52 %.
In most cases, a slight decrease of Corg was observed with depth (10-20 % of variation). Soil organic matter (SOM) increased towards downslope: at upslope position, SOM content was low compared to midslope and downslope positions. When Corg content was compared between the two studied rows (IRR, NIR), a higher amount of C org was noticed in the NIR row at upslope position (+ 40%). The difference was smaller at the midslope position, while downslope no difference was noticed.
Two different patterns were noticed for C/N ratio according to depth (Fig. 3.17b): in IRR row C/N ratio decreased with soil depth while it increased in NIR row. At upslope position C/N ratios was very low especially in IRR row (3.5 – 5.1) while at lower slope positions C/N ratio varied between 6.5 and 8.8. NIR row showed higher C/N ratio than IRR row, except at downslope position.

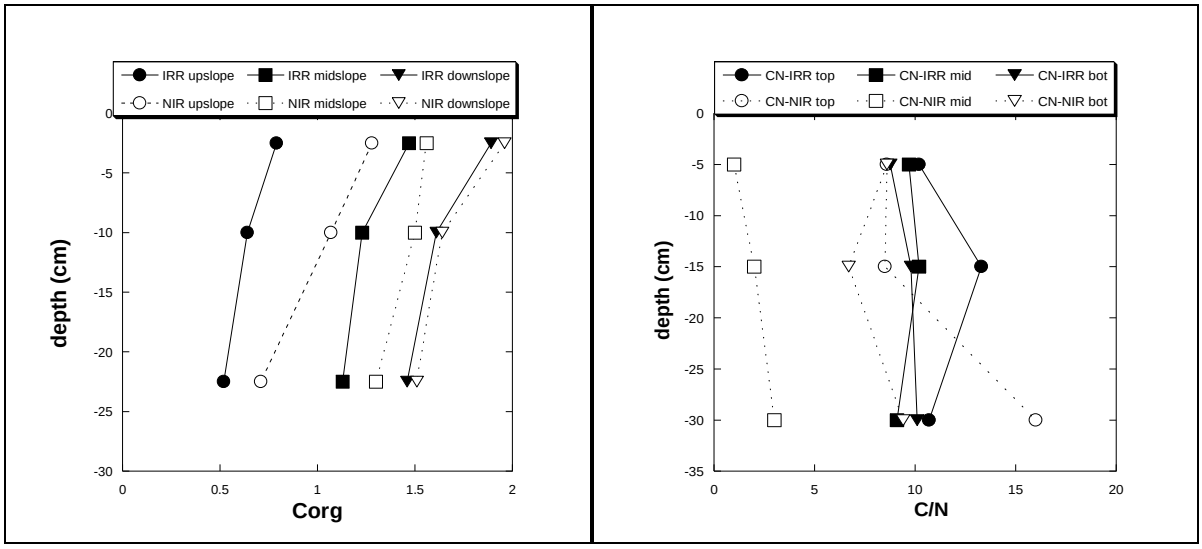


Fig. 3.17a & b: Distribution of Corg and C/N according to a depth, slope and treatment.

According to soil depth, generally no differences were found in cation exchange capacity (CEC). CEC values were higher at lower slope positions compared to upslope. Higher CEC (including exch. Na) was recorded in NIR row.

Available phosphorus was slightly higher in the surface layer compared to deeper layers, especially in NIR row. The level of available phosphorus increased towards downslope. No evident difference in available phosphorus was noticed between IRR and NIR rows.

Analyses of mineral nitrogen forms in soils were done in July 2007. The results are presented in Tab. 3.8 and Fig. 3.18a & b.

Tab. 3.8: Chemical analyses of mineral nitrogen forms in Gačnik, July 2007 (Faculty of Agriculture and Life Sciences, Department of Chemistry, Agrochemistry and Soil Science).

	IRR						**NIR**					
Soil depth (cm)	**0-5**		**5-15**		**15-30**		**0-5**		**5-15**		**15-30**	
(mg.kg^{-1})	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
UPSLOPE	2.9	51.6	4.5	64.9	4.7	38.8	3.3	38.4	2.3	25.1	2.7	35.9
MIDSLOPE	1.2	29.9	2.1	28.8	2.9	42.0	1.8	11.9	2.2	14.1	2.1	14.9
DOWNSLOPE	1.2	12.5	1.5	18.3	4.3	23.2	2.0	15.6	4.1	21.2	4.1	9.6

The major part of total mineral nitrogen was present as nitrates (about 90 %), while ammonium contributed to a minor part (about 10 %).
In general, there was more total mineral nitrogen upslope. Moreover, a slight trend of decreasing Nmin down the slope was observed. Nitrates and ammonium had the same pattern as total Nmin in IRR row (the highest values upslope), while in NIR row this pattern corresponded to nitrates only. Here, the highest ammonium value was measured at downslope position.
Comparing irrigation treatments, higher amounts of total Nmin were recorded in IRR row, with a slight trend of increasing nitrate and ammonium concentrations with soil depth.

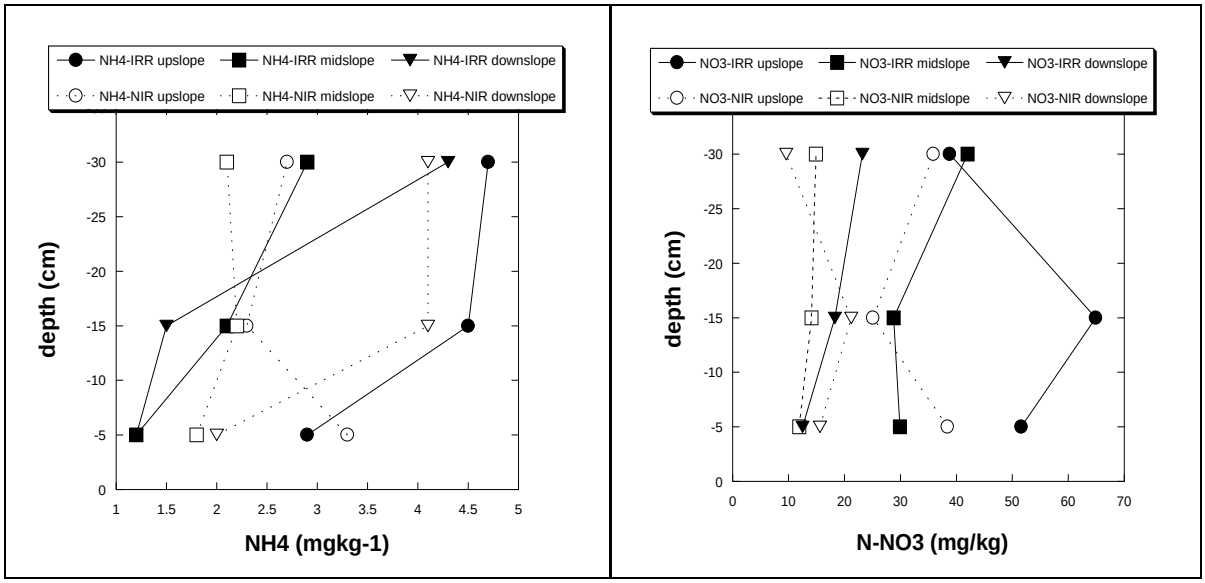


Fig. 3.18a & b: Distribution of NH_4^+ and NO_3^- according to depth, slope and treatment.

III.1.2 *Discussion*

According to physico-chemical parameters, the soils along the slope were very heterogeneous. A slightly higher percentage of clay at downslope position is interpreted as the result of erosion of fine particles. This was expected because such fine particles are easily removed either horizontally by water erosion (surface water runoff) or migration by lateral downward (subsurface) movement throughout soil profile. The expected trend over a landscape is for increased fine particles on lower slope position (Morgan, 1996).
However, it is important to point out that, comparing IRR and NIR rows, no difference was noticed between these two treatments.

No significant differences were observed in soil pH between IRR and NIR rows, which was in agreement with Nunes *et al.* (2007), Henry and Hogg, 2003 and Zhao *et al.*, (2006). Some authors reported on soil pH increase due to irrigation water (Murray *et al*., 2006). Irrigated agriculture sometimes uses large quantities of fertilizers, inducing secondary salinization (Corwin *et al*., 2006).

Carbonate increase with depth and higher carbonate content upslope was related to soil thickness and appearance of marl (only 60 cm below soil surface upslope), while downslope weathered saprolithe of marl did not appear before 1 m. More carbonates were observed in IRR row (0-5 cm).

Soil organic matter is considered the single most important indicator of soil quality and a major component in the assessment of soil quality (Larson and Pierce, 1991; Acton and Gregorich, 1995; Sikora *et al.*, 1996). Our data exhibited great variations in soil organic carbon along the slope. At upslope position, soil was poor in SOM and it was increasing towards downslope, which could be explained partly by downward transport of organic fragments, either free or associated with fine mineral particles. These findings are in agreement with those reported by Hao *et al.* (2002) who found a significant difference in soil organic carbon between upper and lower slope positions.

IRR row contained less SOM than NIR row, thus in our study irrigation was responsible for a decrease in soil organic matter. One possible hypothesis could explain this observation: due to more favourable water conditions in summer, microbial mineralization of soil OM in IRR treatment was favoured, thus decreasing SOM content. Credibility of this hypothesis will be discussed later with microbiological soil parameters. Some other authors also reported SOM increase due to IRR water (Zhao *et al*., 2006), while others did not find any effect of IRR on SOM content (Henry and Hogg, 2003). Irrigated production has also been shown to increase total nitrogen and carbon in cases where these values were low in the native condition (Lueking and Schepers, 1985).

Two different patterns in C/N ratio occurred according to soil depth: in IRR row it decreased with depth, while being the opposite for NIR row. A possible explanation is that trees under irrigated treatment grow better and give more input of fresh OM (with low C/N ratio) to the soil. C/N ratio was higher in NIR row, which is in agreement with higher SOM content and lower biological activity in this treatment. The C/N value of 10 is considered optimal for a good incorporation rate of the organic matter into the soil profile. At lower slope positions, C/N ratio was around 7 and 8, indicating an intensive mineralization process. The velocity of mineralization processes in our soil samples may result from the presence of easily mineralizable decaying debris, which may cause a faster incorporation of this organic debris into the soil profile. This will be discussed in relation with microbial biomass and activity in treatments
C/N ratios in the range 6-8:1 are usual from marine source, whereas higher ratios are likely to come from a terrestrial source (Dahlhem, 1988). Very low C/N ratio at upslope position could be explained by the hypothesis that here we are dealing mostly with SOM originating from the marl bedrock. Chemical analysis of upslope marl confirmed this statement, C/N of marl being as low as 4. Otherwise, such a low C/N is typical of the microorganisms (fungi and bacteria) containing more proteins than carbohydrates (Miller, 2000).

CEC depends mainly on SOM and clay content of the soil (Rowel, 1994; Sposito, 2000). In IRR row, CEC decreased with depth due to decrease of SOM, while in NIR row CEC increased with depth due to clay increase. Higher CEC values at downslope position occurred due to higher SOM. This result was expected and agrees with Hanafi *et al*. (1992) and Rowel (1994). Cation Exchange Capacity (CEC) was found to be slightly lower for the irrigated soils presumably due to the relatively lower OM content (Murray, 2006).
Available phosphorus was slightly higher in the surface layer probably as the result of fertilization. More available phosphorus was found downslope. Phosphorus distribution showed a similar pattern as SOM content due to their association. Available phosphorus was roughly the same in the two treatments (IRR & NIR).

Slight increase of nitrates and ammonium with depth (especially in IRR row) occurred probably due to leaching with irrigation water. No difference in fertilisation occurred which could explain the difference between the treatments, while irrigation water might have participated to higher values of nitrates in IRR row. The highest values of mineral nitrogen were noticed at upslope position, what is not clear.

III.2 Spatial distribution of organic carbon and calcium carbonates

III.2.1 *Distribution of Corg and $CaCO_3$ through soil profiles*

In July 2006, two soil profiles were opened along the slope: at upslope and downslope positions. A dense sampling of the vertical profiles has been done from surface to bedrock. Analysis of organic carbon (Corg), total carbonates (total $CaCO_3$) and active lime are presented in Tab. 3.9.

From the Tab. III.5, some new information is noticed:

1. High level of organic carbon content was measured to the marl in both soil profiles, even if there is an important decrease from the surface to 25 cm.
2. The carbon content at downslope position between 56 and 66 cm depth was twice as higher than adjacent samples and confirmed the morphological observation of deep ploughing.
3. Organic carbon was still abundant in the marl bedrock.
4. Downslope profile showed that decarbonation occurred to the base of the deep ploughing (66 cm), which is related to dissolution of carbonates due to rain water in disturbed soil layer.

Tab. 3.9: Distribution of Corg, total $CaCO_3$ and active lime through soil profiles at upslope and downslope positions in Gačnik, July 2006.

	Active lime %	**Total $CaCO_3$** %	**Corg** %
Soil depth (cm)	**UPSLOPE**		
0-3	9.0	18.1	3.13
3-8	9.1	18.8	1.87
8-14	9.6	18.8	1.68
14-19	9.1	18.8	1.48
19-24	8.9	18.9	1.16
24-30	9.4	18.9	1.50
30-38	9.9	21.3	1.01
38-45	10.5	20.7	1.25
45-56	10.9	24.7	0.96
56-62	11.0	23.9	1.13
Marl 1	10.9	23.7	**1.13**
	DOWNSLOPE		
0-2	3.1	5.8	3.86
2-7	3.8	6.7	2.01
7-12	4.3	8.3	2.37
18-23	4.0	7.3	1.74
23-32	4.3	7	1.57
32-37	4.0	6.8	1.51
37-42	3.5	6.5	1.46
42-47	4.5	9.5	1.17
47-56	4.1	9.0	1.46
56-62	4.5	8.9	**2.20**
62-66	5.8	9.9	**3.25**
66-72	7.3	14.4	1.80
72-78	11.5	23.3	1.11
78-84	11.3	22.4	0.91
84-92	10.0	21.4	0.70
92-98	11.5	22.6	0.78
Marl 2	7.3	18.3	**0.90**

Marl sediment from upslope (60-65 cm) was additionally analysed on Corg (0.66 %), total carbonates (23.7 %), active lime (10.9 %) and C/N ratio (≈ 4).

III.2.2 *Distribution of Corg along the slope in soil surface*

In July 2006, carbon content was measured on the surface soil layer (0-5 cm) in order to appreciate its distribution along the slope, with samples taken every 6 m along the slope from irrigated and non-irrigated rows and from inter-row. Data are presented in Tab. 3.10 and Fig. 3.19.

A significant variation was observed in the Corg distribution along the slope. Maximum values were found in the inter-row: 2 ± 0.1%. Minimum values occurred at upslope position in IRR and NIR rows (0.76 and 0.8 % respectively)

Almost the same pattern in Corg distribution was noticed along the slope in both treatments (IRR, NIR) and in inter-row: Corg increased towards downslope. Corg values in IRR and NIR rows were very similar, while Corg content was significantly higher in the inter-row.

Tab. 3.10: Distribution of Corg along the slope (Faculty of Agriculture and Life Sciences, Department of Chemistry, Agrochemistry and Soil Science). July 2006

Height above sea level (m)	Corg (%) IRR	Corg (%) NIR	Corg (%) Inter-row
300	1.08	0.97	1.45
299	0.8	0.76	1.34
298	1.03	1.04	1.51
297	1.47	1.29	1.81
296	1.43	1.55	2.12
295	1.58	1.35	1.92
294	1.5	1.35	1.72
293	1.33	1.25	1.87
292	1.36	1.55	2.32
291	1.73	1.6	1.95
290	1.61	1.45	2.12
289	1.4	1.67	1.89
288	1.64	1.48	1.85
287	1.84	1.49	2.25
286	1.58	1.64	1.95
285	1.78	1.37	1.85
284	1.8	1.76	1.85
283	1.22	1.68	

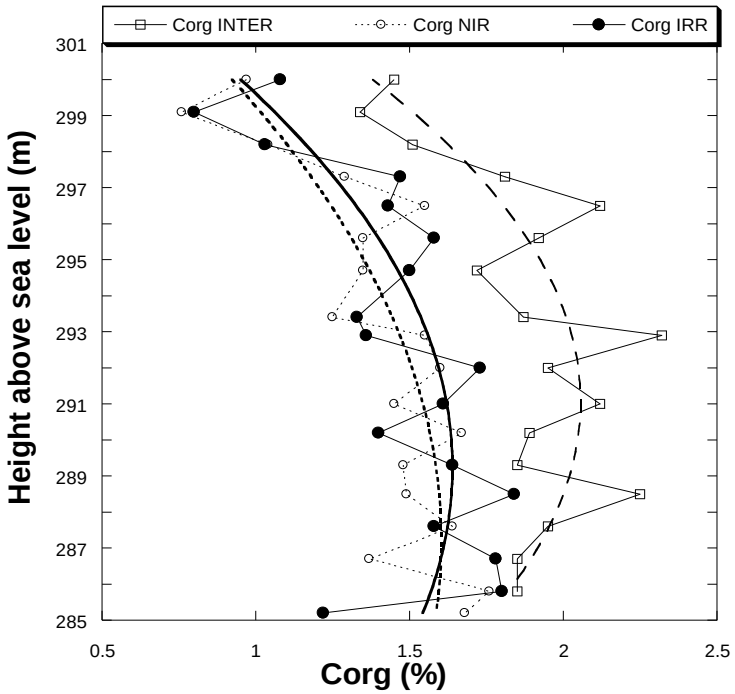


Fig. 3.19: Distribution of Corg in surface soil layer (0-5) cm along the slope.

III.2.3 *Discussion*

Corg in the surface soil layer was increasing towards downslope, which initially be interpreted as a result of fine particle erosion accumulating organic matter downslope. A second fact is also important: Corg content was higher in the inter-row compared to the rows (NIR and IRR) and was also increasing towards downslope. The occurrence of a natural grass cover explained the higher amount of total carbon, but did not explain the increasing of Corg downslope. Indeed, in case of a covered soil surface, downslope erosion of fine particles is decreased. The second hypothesis is a higher input of organic matter from trees, coming from mechanical work and mulching in the inter-row (organic debris was removed from the tree line and spread in inter-row).

Further discussion is suggested when looking at the Corg distribution in the inter-row (Fig. III.16). A "zigzag" pattern can be seen when data are interpreted as: minimum values are corresponding to a normal increasing function and 3 maximum are observed at 296, 292 and 228 m height. It can be suggested that the shape is possibly related to the former planting system with terraces or resulting from soil engineering. High peaks of organic matter content can therefore be interpreted as remaining from previous surface layers of the terraces.

Focusing on the Corg results from September 2005 (in the surface layer only), a large difference was noticed at upslope position between IRR and NIR rows (0.79% and 1.28% respectively). When soils were sampled in July 2006, such a difference was not observed. We interpreted this as a result of different soil sampling: in September 2005, composite sample was analysed (mixed from ten subsamples); while in July 2006, punctual sampling with unique samples was made. For this reason, the results from September 2005 are more representative for comparison of the results according to slope, depth and irrigation. Sampling and analysing at both sampling dates was done by the same operator in the same laboratory

with the same analytical method, indicating strong soil heterogeneity, especially at upper positions of slope.

Soil surface evolution of organic carbon and $CaCO_3$ contents (Tab. III.3a,b,c) according to slope showed two inverse patterns: Corg increased toward downslope while $CaCO_3$ decreased (Fig 3.20). Moreover, upslope surface content in Corg and $CaCO_3$ drew nearer marl composition taken from Tab. 3.9. The trends were similar according treatments.

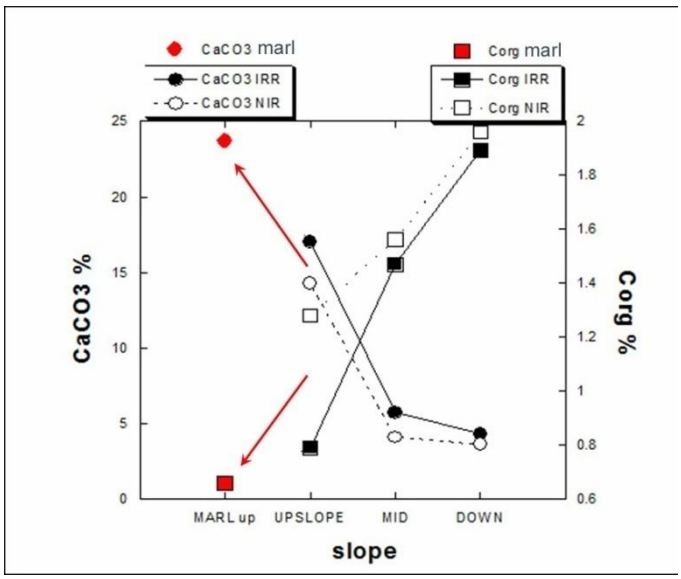


Fig. 3.20: Distribution of total $CaCO_3$ and Corg according to slope and treatment in the surface soil layer from September 2005.

IV. Pore space characterisation

Certain physical soil parameters - bulk density and clod porosity corresponding to pore space characterisation, were measured in May 2004 and in May 2006.

In May 2004, soil samples for bulk density measurement were taken from downslope position: irrigated row (IRR), non-irrigated row (NIR), inter-row and under wheel. Three repetitions by depth were performed. In the surface layer (3-7 cm), only irrigated and non-irrigated rows were compared, while in the deeper layers (20-25 cm and 40-45 cm), a comparison among all four situations was done. Additional sample from 80-85 cm layer in inter-row was taken for characterisation of the undisturbed structural horizon.
Clod samples for determination of clods void ratio (e_c) were taken from IRR and NIR rows, inter-row and under wheel at different depths (20-30 cm, 40-50 cm and 80-90 cm).

In May 2006, soil samples for bulk density measurement were taken from the three slope positions in the irrigated and non irrigated rows at three depths: 0-5 cm, 10-15 cm and 20-25 cm with two repetitions by depth.

IV.1 Downslope position

IV.1.1 *Bulk density*

As shown in Tab. 3.11 and Fig. 3.21a & b, bulk density increased, while porosity decreased with depth, in IRR and NIR soils. Total void ratio decreased with depth in both water treatments, but significantly only in IRR row (average void ratio was 1.63 in the surface layer while 1.06 and 1.02 in deeper layers). Water saturation ratio increased with depth in both rows (IRR, NIR). In the Tab. 3.11 and Fig. 3.21a & b it can be noticed that voids from upper layer are less saturated with water than in deeper layers, especially in IRR soil. In inter-row, data referring to pore space characterisation and humidity were more or less the same according to depth.

In the surface layer (3-7 cm), significant difference in soil bulk density and total void ratio occurred between irrigated and non-irrigated soils: higher total void ratio was noticed in IRR (1.63) than in NIR (1.12) rows. In the surface soil layers, water saturation ratio was higher in NIR soil. Similarly, in inter-row and under wheels, water saturation ratio was higher comparing to rows (Fig. 3.21c &d). No significant differences among situations occurred in lower layers.

Tab. 3.11: Bulk density, porosity, volumetric water content, gravimetric water content, void ratio, water ratio and water saturation ratio in IRR and in NIR rows, inter-row and under wheel at downslope position May 2004.

Treatment	Depth cm	Bulk density $g.cm^{-3}$	Porosity $cm^3.cm^{-3}$	Vol. water content $cm^3.cm^{-3}$	Grav. water content $g.g^{-1}$	Void ratio	Water ratio	Water satur. ratio	Mean water satur. Ratio
IRR	3-7	0.92	0.65	0.28	0.30	1.88	0.80	0.42	
		1.03	0.61	0.29	0.28	1.57	0.75	0.48	
		1.09	0.59	0.31	0.29	1.43	0.76	0.53	0.48
	20 - 25	1.27	0.52	0.40	0.31	1.09	0.83	0.76	
		1.2	0.55	0.37	0.31	1.21	0.81	0.67	
		1.39	0.48	0.41	0.29	0.91	0.78	0.86	0.76
	40 - 45	1.41	0.47	0.45	0.32	0.88	0.84	0.96	
		1.27	0.52	0.41	0.32	1.09	0.85	0.78	
		1.28	0.52	0.40	0.32	1.07	0.84	0.78	0.84
NIR	3-7	1.16	0.56	0.37	0.32	1.28	0.84	0.66	
		1.32	0.50	0.40	0.31	1.01	0.81	0.80	
		1.27	0.52	0.37	0.29	1.09	0.77	0.71	0.72
	20 - 25	1.4	0.47	0.42	0.30	0.89	0.80	0.90	
		1.29	0.51	0.42	0.33	1.05	0.86	0.82	
		1.25	0.53	0.39	0.31	1.12	0.82	0.73	0.82
	40 - 45	1.43	0.46	0.48	0.33	0.85	0.89	1.04*	
		1.3	0.51	0.43	0.33	1.04	0.88	0.85	0.95
		1.47	0.45	0.43	0.29	0.80	0.78	0.97	(0.91)**
Inter-row	20 - 25	1.17	0.56	0.41	0.35	1.26	0.93	0.73	
		1.51	0.43	0.42	0.28	0.75	0.73	0.97	0.94
		1.52	0.43	0.48	0.31	0.74	0.83	1.12*	(0.85)**
	40 - 45	1.41	0.47	0.43	0.30	0.88	0.81	0.92	
		1.41	0.47	0.46	0.33	0.88	0.86	0.98	0.98
		1.44	0.46	0.47	0.33	0.84	0.87	1.03*	(0.95)**
	80-85	1.38	0.48	0.47	0.34	0.92	0.90	0.98	
		1.43	0.46	0.27	0.19	0.85	0.50	0.58	0.83
		1.36	0.49	0.45	0.33	0.95	0.88	0.93	(0.95)**
Under wheel	20 - 25	1.4	0.47	0.43	0.31	0.89	0.81	0.91	
		1.57	0.41	0.46	0.29	0.69	0.78	1.13*	1.0
		1.44	0.46	0.43	0.30	0.84	0.80	0.95	(0.93)**

* Water saturation Ratio>1 => measurements errors either on bulk density or on gravimetric water content.

**Mean water saturation ratio, calculated on two data only (without *).

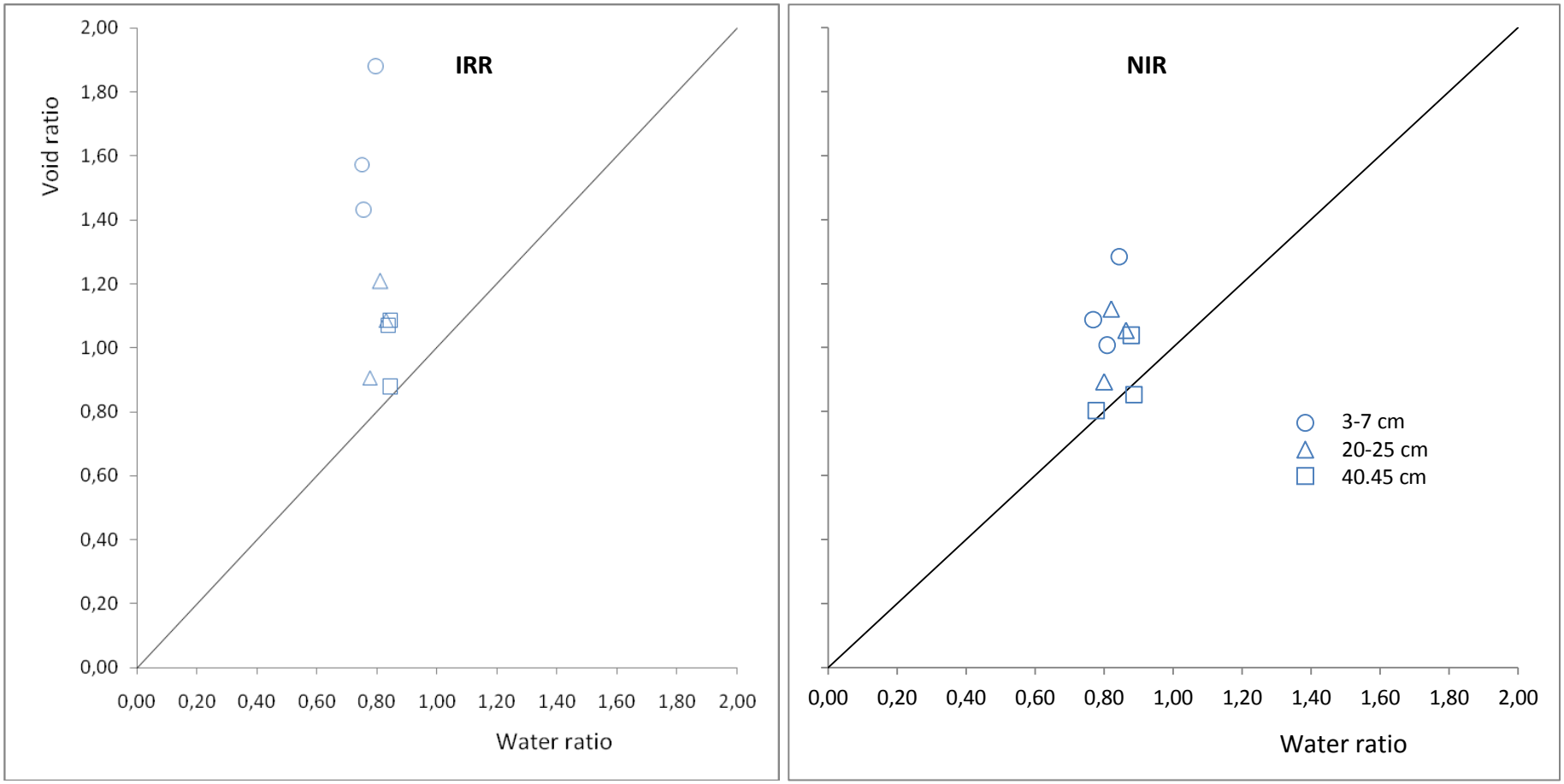


Fig. 3.21a & b: Void ratio versus water ratio relationships according to different situation (IRR and NIR rows) and depth at downslope position (bisector line = water saturation line)

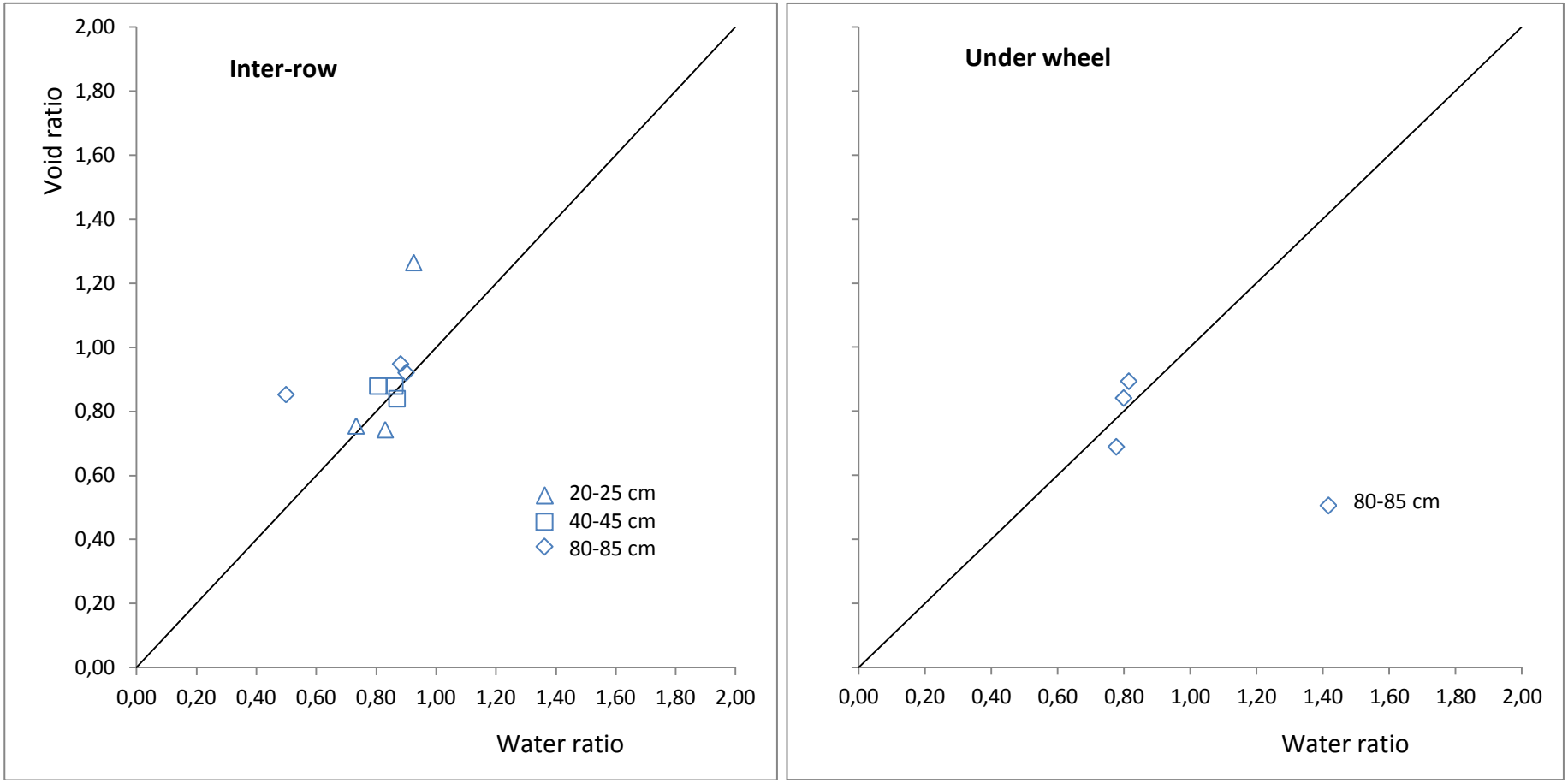


Fig. 3.21c & d: Void ratio versus water ratio relationships according to different situation (inter-row, under wheel) and depth at downslope position (bisector line = water saturation line)

IV.1.2 *Clods void ratio*

In IRR and NIR rows, clods void ratio was more or less the same through the soil profile (Tab. 3.12). In inter-row, values of clods' void ratio slightly increased from the upper (20-30 cm) to deeper soil layers (40-50 cm & 80-85 cm) (Fig. 3.22). Due to large standard deviation of clods void ratio in the in mid-layer, the difference through the soil profile is not assured. In upper soil layers, no statistical significant differences in void ratio among situations occurred.

Tab. 3.12: Clods void ratio (e_c) and inter-clods void ratio in Gačnik, May 2004.

	Depth (cm)	**n**	**e_c**	**σ (e_c)**	**$e_{T\_}$ e_c**
IRR	20-30 40-50	10 10	0.43 0.44	0.017 0.024	0.63 0.58
NIR	20-30 40-50	10 10	0.43 0.44	0.011 0.063	0.59 0.46
Inter-row	20-30 40-50 80-90	10 10 10	0.46 0.60 0.56	0.110 0.422 0.025	0.46 0.27 0.35
Under wheel	20-30	10	0.46	0.029	0.35

n = number of repetitions
e_c = clods void ratio
e_T = total void ratio from Table III.9
σ = standard deviation

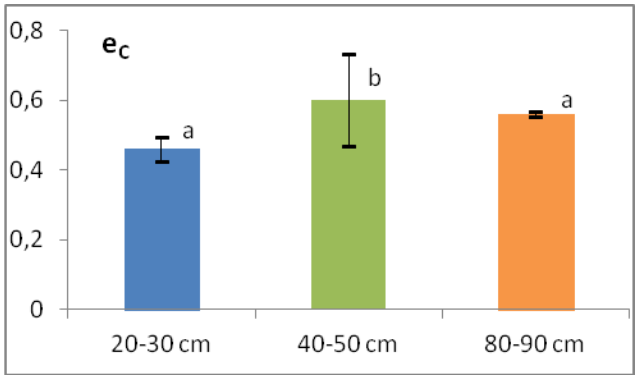


Fig. 3.22: Comparison of clods void ratio in soils from inter-row according to depth.

The difference between total void ratio and clods void ratio presents inter-clods void ratio (e_{ic}). Inter-clods void ratio decreased with depth. In the sub-surface soil layer (20-30 cm), rows e_{ic} was more or less the same and higher comparing inter-row $e_{ic,}$ while under wheels e_{ic} was almost the half than in the rows.

IV.2 Variation along the slope

For a better understanding of soil physics along the slope, the study was continued in May 2006. Irrigated and non-irrigated soils were investigated at three slope positions: upslope, midslope and downslope. At each slope position, pore space characterisation from three soil depths was studied: 0-5, 5-15 and 15-30 cm. Data from May 2006 are presented in Tab. 3.13a & b, and in Fig. 23a & b.

Tab. 3.13a: Bulk density, porosity, volumetric water content, gravimetric water content, void ratio, water ratio and water saturation ratio in IRR row according to depth at three slope positions, May 2006.

Slope position	Depth cm	Bulk density $g.cm^{-3}$	Porosity $cm^3.cm^{-3}$	Volumetric water content $cm^3.cm^{-3}$	Gravimetric water content $g.g^{-1}$	Void ratio	Water ratio	Water saturation ratio	Mean water saturation ratio
Upslope	0 - 5	1.03 0.97	0.61 0.63	0.05 0.06	0.05 0.07	1.56 1.72	0.13 0.17	0.08 0.10	0.09
	10 - 15	1.19 1.20	0.55 0.55	0.15 0.15	0.13 0.13	1.23 1.21	0.33 0.33	0.27 0.28	0.27
	20 - 25	1.45 1.27	0.45 0.52	0.21 0.18	0.14 0.15	0.83 1.09	0.38 0.39	0.46 0.35	0.41
Midslope	0 - 5	1.33 1.30	0.50 0.51	0.27 0.25	0.21 0.19	1.00 1.04	0.55 0.51	0.55 0.49	0.52
	10 - 15	1.19 1.16	0.55 0.56	0.31 0.32	0.26 0.27	1.23 1.28	0.70 0.73	0.57 0.57	0.57
	20 - 25	1.37 1.15	0.48 0.57	0.36 0.35	0.26 0.30	0.94 1.30	0.69 0.80	0.74 0.61	0.67
Downslope	0 - 5	1.26 1.17	0.53 0.56	0.15 0.13	0.12 0.11	1.11 1.26	0.32 0.29	0.29 0.23	0.26
	10 - 15	1.22 1.21	0.54 0.54	0.23 0.22	0.19 0.18	1.17 1.18	0.50 0.49	0.42 0.41	0.42
	20 - 25	1.30 1.33	0.51 0.50	0.30 0.22	0.23 0.17	1.05 0.99	0.62 0.44	0.59 0.45	0.52

Tab. 3.13b: Bulk density, porosity, volumetric water content, gravimetric water content, void ratio, water ratio and water saturation ratio in NIR row according to depth at three slope positions, May 2006.

Slope position	Depth cm	Bulk density $g.cm^{-3}$	Porosity $cm^3.cm^{-3}$	Volumetric water content $cm^3.cm^{-3}$	Gravimetric water content $g.g^{-1}$	Void ratio	Water ratio	Water saturation ratio	Mean water saturation ratio
Upslope	0 - 5	1.16 1.10	0.56 0.58	0.10 0.09	0.09 0.08	1.29 1.41	0.24 0.22	0.18 0.15	0.17
	10 - 15	1.30 1.38	0.51 0.48	0.19 0.19	0.14 0.14	1.03 0.91	0.38 0.36	0.37 0.39	0.38
	20 - 25	1.22 1.19	0.54 0.55	0.18 0.18	0.14 0.15	1.16 1.22	0.38 0.39	0.33 0.32	0.32
Midslope	0 - 5	1.76 1.27	0.33 0.52	0.09 0.14	0.05 0.11	0.50 1.09	0.14 0.29	0.27 0.26	0.27
	10 - 15	1.33 1.19	0.50 0.55	0.28 0.21	0.21 0.18	0.99 1.22	0.55 0.47	0.55 0.38	0.47
	20 - 25	1.21 1.43	0.54 0.46	0.26 0.28	0.22 0.19	1.18 0.86	0.58 0.52	0.49 0.60	0.54
Downslope	0 - 5	1.25 1.27	0.53 0.52	0.12 0.14	0.10 0.11	1.12 1.09	0.26 0.29	0.23 0.26	0.25
	10 - 15	1.13 1.19	0.57 0.55	0.22 0.25	0.20 0.21	1.34 1.24	0.53 0.57	0.39 0.46	0.43
	20 - 25	1.33 1.38	0.50 0.48	0.28 0.29	0.21 0.21	0.99 0.92	0.55 0.56	0.56 0.62	0.59

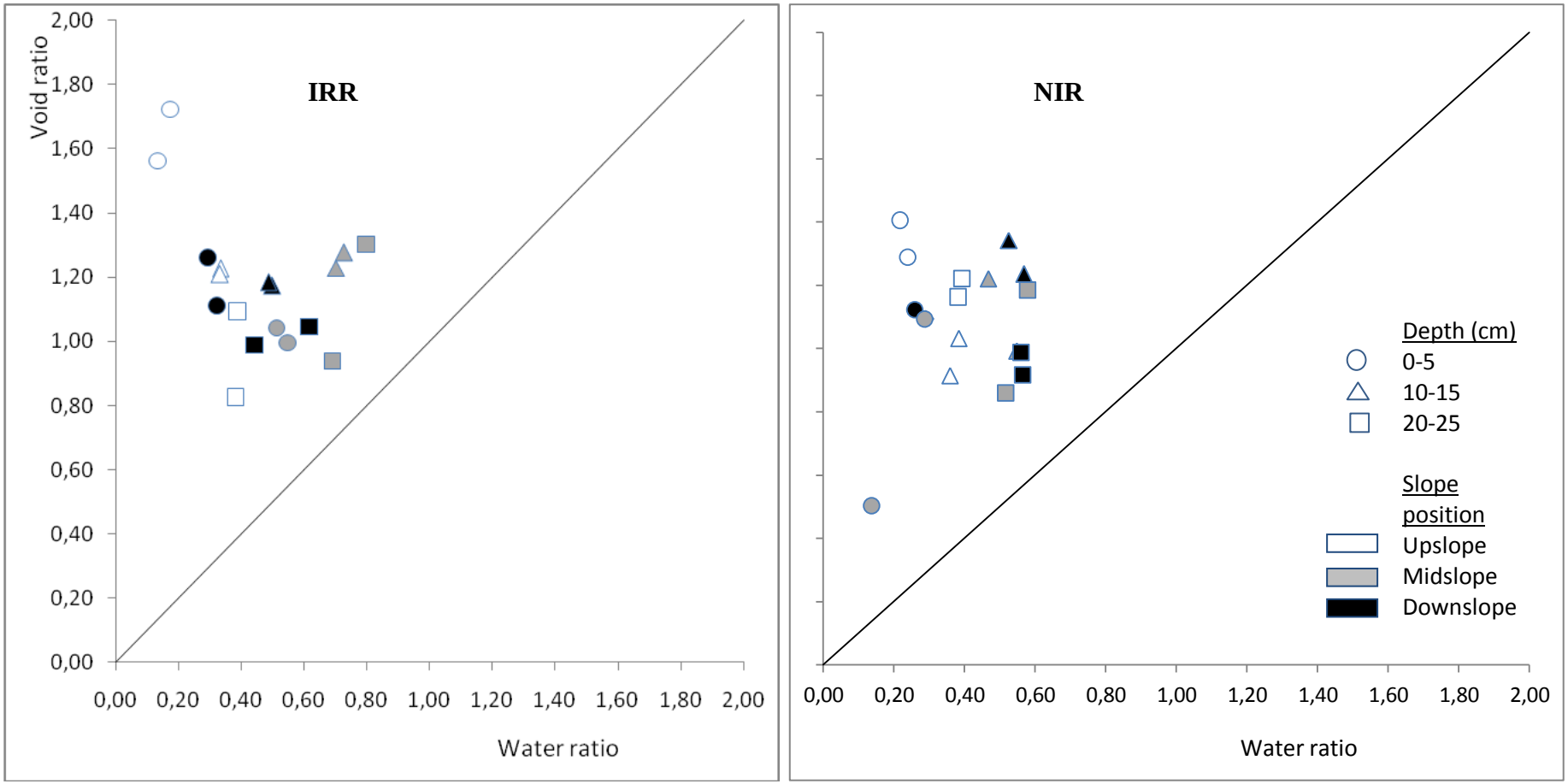


Fig. 3.23a & b: Void ratio versus water ratio relationships according to treatment (IRR. NIR), depth and slope position (bisecting line = water saturation line).

In May 2006, significant differences in total void ratio were obtained by depth, slope and treatment. According to depth, difference occurred between the first two (0-5 and 10-15 cm) and the third (20-25 cm) soil layers. Total void ratio was lower in the deepest studied layer (1.0 on average) comparing to the upper layers (on average 1.18 in the surface layer and 1.10 in the subsurface layer).

According to slope, soils from downslope position obtained the highest total void ratio (1.16 in average) and it differed from midslope position (1.03 in average). There was no significant difference in total void ratio between irrigated and non-irrigated rows, while higher values were observed in IRR row.

In the surface soil layer (0-5 cm), total void ratio at downslope position significantly differed from upper slope positions: at downslope position the total void ratio was the highest (1.44). Significant difference in total void ratio in the surface layer according to treatment was noticed at midslope position only, where total void ratio was higher in IRR soil.

In the mid-layer (10-15 cm), no statistical significant differences were noticed in total void ratio according to slope. In this layer, total void ratio in inter-row was the lowest and significantly differed from IRR soil at all slope positions. Significantly higher total void ratio in IRR soil occurred at down-slope only.

In the deepest soil layer (20-25 cm), no significant differences in total void ratio according to slope and treatment were noticed.

According to depth, gravimetric water content and water saturation ratio increased with depth: the surface layer was significant drier than lower soil layers. According to slope, water saturation ratio was the lowest upslope and the highest midslope. Comparing IRR and NIR rows, upslope NIR row was higher while IRR row was higher midslope. Downslope values were more or less the same.

IV.3 Discussion

Total void ratio decreased with depth, which could be explained with soil structure: till 5 cm. fine granular structure was observed, continued with subangular polyedric structure (5-15 cm), fine polyedric structure (till 30 cm) and from 60 cm coarse polyedric structure appeared. Comparing to granular and subangular polyedric structures, compact polyedric structure with low macroporosity is related to lower total void ratio and lower interclods void ratio. Evolution of soil structure could be related to SOM, clay content and climate. In the surface soil layer, where fine granular structure was observed, the content of SOM and percentage of clay were the highest and they decreased with soil depth. Our results were in agreement with Bachmann and Hartge (2006), who also reported of void ratio decreasing through the soil profiles and with Challa (1987), who recorded the highest bulk density generally in the lower part of the profiles where more carbonate accumulation occurred. The degree of water saturation in pores significantly increased with depth, while gravimetric water content increased slowly. In deeper layers, pores inside clods were mostly filled with water and this could explain the appearance of hydromorphic features in lower layers.

Another pattern was observed in clods void ratio (at downslope position) according to depth, values increased with depth. In the subsurface layer (20-30 cm), fine polyedric structure was noticed and deeper, coarse polyedric structure appeared, which probably contributed to higher

clods voids ratio. Close to 60 cm deepness, dark brown soil volumes with high SOM content (3.25 % Corg) and massive structure were observed. Presumably, this could be a part of the previous surface layer buried by deep ploughing (increased variability of the pore space measurements), which is shown in Fig. 3.22. On the field, large variation in soil profile in inter-row was noticed with a mixture of soil volumes of different sizes. Large standard deviations of clods' void ratio till 50 cm deepness could improve this statement.
A decrease of clod void ratio from the base undisturbed structural horizon to the surface horizons may be due to combination of natural compaction due to shrinkage and anthropogenic mechanical compaction.

In the surface layer (0-5 cm), soils from downslope indicated the highest total void ratio, which was expected due to higher SOM content. Structure in this layer was the same according to slope positions (fine granular). In the deeper layer (10-15 cm), total void ratio was higher at upslope position (in NIR row and inter-row). A possible explanation for this pattern could lie in the heterogeneity of soil in this study filed, or organisation of particles inside structure aggregates. Gravimetric water content and degree of saturation was the highest at mid-slope position and the lowest at upslope.

Higher total void ratio in IRR row comparing to NIR row is difficult to interpret. Particle size distributions in both treatments were very similar, organic matter content in IRR soil was even lower and the structure (shape and size of aggregates) in both treatments was the same (fine granular structure). The reason for differences might result from arrangement of structure and manner of organisation inside the aggregates: looser fine granular structure in IRR soil comparing to more compact in NIR rows. Soils under wheels obtained significantly lower total void ratio than inter-row, IRR and NIR rows (at 20 cm) due to compaction with heavy machinery (tractor) and water saturation ratio was higher (Tab. 3.14).

Tab. 3.14: Comparison different situations at 20-25 cm (data from Tab. 3.11)

	void ratio	water saturation ratio
IRR 20-25	1.07	0.76
NIR 20-25	1.02	0.82
inter-row 20-25	1.01	0.85
under wheel 20-25	0.87	0.93

Several authors have reported some notable effects of irrigation on soil physical conditions.
Our data showed a positive effect for the applied drip irrigation system on some soil physical properties. These findings are in harmony with the fact that the permanent irrigation system is associated with a cyclic of wetness followed by dryness. *i.e.*, swelling and shrinkage cyclic formation, which enhances reorientation soil elements and impact on soil bulk density and total porosity that represent the most important factors of soil structure criteria (Lawrence, 1977).
Zhao *et al.* (2006) stated out irrigation did not result in any changes in soil particle distribution and in bulk density. Rajaram and Erbach (1998) a similar situation discussed; the effect of drying stress on bulk density was not significant on clay-loam soils. Rickard and Cossens (1966) and Currie *et al*. (2006) found a decreasing porosity and a concomitant increase of bulk density in irrigated soils.

V. Organic matter characterisation and origin

To understand organic matter composition and origin better, a physical fractionation according to size in three fractions named A (> 200 μm), B (200-50 μm) and C (< 50 μm) was performed on soil samples from October 2004. On each fraction total carbonate content was determined and after decarbonatation, Corg, Norg and $\delta^{13}C$ were measured (detailed measurements are presented in Annex 7).

V.1 Characterization of the grain size fractions

V.1.1 *Grain size fractions abundance*

Grain size fraction abundance is shown in Tab.3.15. Fraction A was the smallest and varies between 1.2 to 4.3 %, fraction B varies between 6 to 11 %, while C fraction was the largest and varied between 86 and 90 %. The three fractions were almost constant with depth, only fraction A was slightly higher in the surface layer than below. According to slope, fractions A and B in the surface layer slightly increased toward downslope. No differences in fraction distribution was noticed between IRR and NIR rows.

Tab. 3.15: A, B and C fraction size (%) according to depth and slope position, in irrigated and in non-irrigated rows.

Irrigated row (IRR)									
depth	**Upslope**			**Midslope**			**Downslope**		
(cm)	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0-5	2.4	6.0	91.6	3.5	7.0	89.5	3.9	8.7	87.4
5-15	1.2	7.1	91.8	0.8	7.2	92.0	3.4	9.0	87.6
15-30	1.2	6.5	92.2	1.3	8.8	89.9	1.7	7.1	91.2
Non-irrigated row (NIR)									
depth	**Upslope**			**Midslope**			**Downslope**		
(cm)	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0-5	2.2	7.9	89.9	2.5	7.9	89.7	4.3	10.2	85.6
5-15	2.3	9.0	88.8	2.1	8.5	89.5	2.2	7.3	90.5
15-30	1.5	11.5	87.1	2.2	7.6	90.2	3.4	7.2	89.4

V.1.2 *Total carbonates content*

Total $CaCO_3$ content by fraction (g.100 g^{-1}) and in proportion to the total amount by sample is shown in Tab. 3.16. Total carbonates dominated in fraction C at all slope positions and depths, while amounts of carbonates in fraction A were negligible. According to depth, no significant differences occurred in total carbonate content, while a slight increase with depth in fraction C was noticed. According to slope, upslope position contained significantly higher amounts of total carbonates than lower positions, where amounts were very similar. No differences occurred between irrigated and non-irrigated rows.

Tab. 3.16: $CaCO_3$ content (g.100 g^{-1}) in the whole sample and in grain size fractions A, B & C according to depth and slope position, in irrigated and non-irrigated rows.

Irrigated row (IRR)												
depth	**Upslope**				**Midslope**				**Downslope**			
(cm)	**A**	**B**	**C**	**$CaCO_3$ total**	**A**	**B**	**C**	**$CaCO_3$ total**	**A**	**B**	**C**	**$CaCO_3$ total**
0-5	0.17 (2)*	0.51 (6)	7.37 (92)	8.05	0.04 (1)	0.2 (8)	2.3 (91)	2.54	0.26 (5)	0.46 (9)	4.25 (86)	4.97
5-15	0.01 (0)*	0.16 (5)	2.97 (95)	3.14	0.0 (0)	0.21 (7)	2.76 (93)	2.97	0.20 (4)	0.46 (8)	4.82 (88)	5.48
15-30 (%)	0.06 (0)	0.58 (7)	8.14 (93)	8.78	0.02 (1)	0.26 (9)	2.52 (90)	2.8	0.02 (1)	0.19 (7)	2.47 (92)	2.68
Non-irrigated row (NIR)												
depth	**Upslope**				**Midslope**				**Downslope**			
(cm)	**A**	**B**	**C**	**$CaCO_3$ total**	**A**	**B**	**C**	**$CaCO_3$ total**	**A**	**B**	**C**	**$CaCO_3$ total**
0-5	0.11 (2)*	0.55 (8)	6.24 (90)	6.90	0.15 (3)	0.41 (10)	3.82 (87)	4.38	0.31 (7)	0.45 (9)	4.05 (84)	4.81
5-15	0.13 (2)*	0.69 (10)	6.38 (88)	7.20	0.07 (2)	0.36 (9)	3.36 (89)	3.79	0.1 (2)	0.42 (8)	4.81 (90)	5.33
15-30	0.05 (1)*	0.75 (11)	5.79 (88)	6.59	0.10 (2)	0.42 (9)	4.01 (89)	4.53	0.16 (3)	0.34 (7)	4.46 (90)	4.96

* as a percentage of total $CaCO_3$ in the sample

V.1.3 *Organic carbon content*

Total organic carbon content by fraction (g.100 g^{-1}) and in proportion of the total amount by sample is shown in Tab. 3.17. Total organic carbon was lower upslope comparing to midslope and downslope (0.6 to 1.75 and 1.3 to 2.6 g.100 g^{-1} respectively). Organic carbon in fraction C represented 67 to 80 % of total Corg while 13 to 18 % for B fraction and 3 to 24 % for fraction A. OC content decreased in fractions A and B according to depth, while there was no particular trend for fraction C. According to slope, OC content in fraction A in the surface layer was lower upslope than at midslope and downslope.

No significant difference was noticed comparing IRR and NIR rows where the mean OC contents were 0.51 and 0.46 g.100 g^{-1} , respectively. In fraction A only some differences occurred with a higher OC content in IRR than in NIR row (0.23 and 0.14 g.100 g^{-1} respectively).

Tab. 3.17: Organic carbon content (g.100 g^{-1}) in the whole sample and in grain size fractions A, B & C according to depth and slope position, in irrigated and non-irrigated rows.

Irrigated row (IRR)												
depth	**Upslope**				**Midslope**				**Downslope**			
(cm)	**A**	**B**	**C**	**Corg total**	**A**	**B**	**C**	**Corg total**	**A**	**B**	**C**	**Corg total**
0-5	0.11 (12)*	0.15 (16)	0.67 (72)	0.93	0.68 (26)	0.47 (18)	1.43 (55)	2.58	0.57 (24)	0.44 (18)	1.38 (58)	2.39
5-15	0.10 (13)*	0.11 (15)	1.54 (72)	1.75	0.03 (3)	0.23 (17)	1.06 (80)	1.32	0.14 (9)	0.28 (18)	1.16 (74)	1.58
15-30	0.12 (18)*	0.09 (13)	0.46 (68)	0.67	0.10 (6)	0.30 (16)	1.37 (77)	1.77	0.26 (15)	0.27 (15)	1.27 (70)	1.80
Non-irrigated row (NIR)												
depth	**Upslope**			**Corg total**	**Midslope**			**Corg total**	**Downslope**			**Corg total**
	A	**B**	**C**		**A**	**B**	**C**		**A**	**B**	**C**	
0-5	0.16 (17)*	0.16 (16)	0.64 (67)	0.96	0.20 (12)	0.28 (16)	1.22 (72)	1.70	0.22 (12)	0.38 (20)	1.29 (68)	1.89
5-15)	0.16 (14)*	0.19 (17)	0.76 (68)	1.11	0.08 (5)	0.25 (15)	1.27 (80)	1.60	0.11 (6)	0.22 (13)	1.35 (80)	1.68
15-30	0.08 (13)*	0.09 (15)	0.44 (72)	0.61	0.08 (6)	0.20 (15)	1.07 (79)	1.35	0.15 (9)	0.24 (14)	1.27 (77)	1.66

***** as percentage of total organic carbon in the sample

V.1.4 *C/N ratio*

C/N ratios by fraction were shown in Tab. 3.18 according to soil depth and slope position. C/N ratios varied from 3.5 to 32 in the different fractions. Fraction A showed the highest ratios compared to fractions B and C (13 to 32; 5 to 15 and 3.5 to 7, respectively). C/N ratio in fraction A increased from surface to deeper layers, especially in IRR treatment at upslope and downslope positions. C/N ratio in fraction B usually decreased with depth, while it was almost constant in fraction C. According to slope, IRR and NIR rows did not follow the same pattern in C/N. In IRR row, C/N ratio in fraction A was lower upslope compared to downhill positions. Inverse pattern occurred in the deeper soil layer (5-15 cm). In NIR row, C/N ratio in fraction A did not show clear trend. In both rows, C/N ratio in fractions B and C generally increased towards downslope. C/N ratio was almost constant in B fraction according to slope and depth. C/N ratio in fraction C was the lowest upslope compared to lower positions, while no evident differences occurred according to depth. Comparing IRR and NIR rows, the main difference occurred for fraction A, with higher mean C/N ratio in IRR than NIR row (21.5 and 15 respectively).

Tab. 3.18: C/N ratio of grain size fractions A, B & C according to depth and slope position, in irrigated and non-irrigated rows.

Irrigated row (IRR)									
depth	**Upslope**			**Midslope**			**Downslope**		
(cm)	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0-5	13	11	4.4	22	13	6.8	19	13	6.9
5-15	26	10	4.1	16	12	6.3	13	11	6.5
15-30	32	10	3.6	21	12	7.0	32	12	6.9
Non-irrigated row (NIR)									
depth	**Upslope**			**Midslope**			**Downslope**		
(cm)	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0-5	15	9	4.1	15	15	6.5	12	12	6.7
5-15	17	9	5.0	14	11	7.3	17	11	7.6
15-30	18	5.0	3.5	13	10	6.7	16	11	7.3

V.1.5 *Isotopic signature of organic carbon: $\delta^{13}C$*

Isotopic fractionation measurements ($^{13}\delta C$ ‰) were performed to get information about the origin of soil organic matter. Our hypothesis was that in studied soils we had two types of organic matter: i) fresh OM mostly from apple trees and grass with low $^{13}\delta C$ (-28‰) and ii) sedimentary OM from marl with a higher $^{13}\delta C$ (-23‰) as discussed in chapter 3 III "Physico-chemical soil characteristics". Our question was how much OM in each soil sample came from the tree and how much from the marl.

$^{13}\delta C$ Isotopic composition by fraction is shown in Tab. 3.19 according to soil depth and slope position. Statistically significant differences in $^{13}\delta C$ according to fraction, depth, slope and treatment were found. According to fraction, mean value of $^{13}\delta C$ increased from fraction A to C. According to depth, mean value of $^{13}\delta C$ increased with depth. According to slope, upslope mean value of $^{13}\delta C$ was higher and differed from midslope and downslope (-25.32, -26.79 and -26.15 ‰ respectively). According to treatment, mean value of $^{13}\delta C$ was higher in IRR than in NIR rows (-25.99 and -26.18 ‰ respectively).

Tab. 3.19: $^{13}\delta C$ Isotopic composition of fractions A, B & C according to depth and slope position, in irrigated and non-irrigated rows

Irrigated row (IRR)									
depth	**Upslope**			**Midslope**			**Downslope**		
(cm)	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0-5	-26.03	-26.08	-24.48	-28.11	-28.02	-27.22	-23.68	-26.87	-25.71
5-15	-26.20	-25.04	-23.87	-27.09	-26.87	-25.35	-26.45	-26.32	-25.45
15-30	-26.18	-26.76	-23.32	-27.03	-26.84	-25.78	-26.01	-26.58	-25.55
Non-irrigated row (NIR)									
depth	**Upslope**			**Midslope**			**Downslope**		
(cm)	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0-5	-26.47	-26.04	-24.34	-27.69	-27.31	-25.65	-26.50	-26.73	-25.54
5-15	-26.62	-26.22	-25.03	-27.25	-26.98	-25.74	-27.00	-26.64	-25.62
15-30	-26.62	-24.30	-23.42	-27.26	-26.59	-25.39	-27.31	-26.97	-25.69

Distribution of fresh and sedimentary organic carbon stocks according to grain size fraction, depth, slope position and treatment

From OC content and $^{13}\delta C$ data of each fraction, the percentage of organic carbon which originated from fresh apple tree residue and from the weathering of the marl was calculated according the following mixing model for each fraction separately.

The $\delta^{13}C$ of total soil organic matter and particle-size organic matter was intermediate between that of trees and marl sedimentary bedrock. Organic carbon derived from trees (C_t) and from the bedrock (C_b) in any sample from a soil layer or soil organic matter fraction was expressed as C ($mg.g^{-1}$), or as a percentage of total C (PC_t and PC_b) of the respective layer or soil organic matter fraction, as follows :

$C_t = C_c \,.\, (\delta_c - \delta_b) / (\delta_t - \delta_b), \quad C_b = C_c - C_t$

$PC_t = 100 \,.\, C_c \,.\, (\delta_c - \delta_b) / (\delta_t - \delta_b), \quad PC_b = 100 - PC_t$

where C_C is the total C content of the sample from the cultivated soil (layer or fraction), δ_c is the $\delta^{13}C$ value of the sample analyzed, δ_b is the $\delta^{13}C$ value of sample from the corresponding bedrock from a deeper layer (60 cm depth) here taken as -23.32 ‰, and δ_t is the $\delta^{13}C$ value of the apple tree residues here taken as -28.11 ‰, used as reference.

Sedimentary and fresh organic stocks in 0 – 30 cm layer were calculated, taking into account the proportion of each fraction in a layer, the thickness of the layer and a mean bulk density for IRR and NIR rows of 1.23 and 1.28 $g.cm^{-3}$, respectively, calculated from Table III.13a&b.

Results were presented in two ways:
- first, by tons per hectare and per centimeter ($T.ha^{-1}.cm^{-1}$) for each layer (0-5, 5-15 and 15-30 cm), grain size fraction, position and treatment in Tab. 3.20 and in Fig. 3.24a &b, where the influence of depth, slope and treatment on the distribution of the two types of OC will be discussed;
- and second, by tons per hectare on the whole 0-30 cm layer for each grain size fraction, position ant treatment in Fig. 3.25 and Tab. 3.21.

According to treatment, no significant differences in stock of total, fresh and sedimentary OC in $T.ha^{-1}.cm^{-1}$ were found (Tab.3.20). Total and fresh OC stocks decreased with depth, while sedimentary OC stock was more constant through the profile. Along the slope, total, fresh and sedimentary OC stocks increased towards downslope. Both types of OC stocks were the highest in C fraction.

Tab. 3.20: Stocks of sedimentary and fresh organic carbon (OC) in T.ha^{-1}.cm^{-1} in fractions A, B & C according to depth and slope position, in irrigated (IRR) and non-irrigated (NIR) rows

Depth	OC Type	irrigated														
		Upslope					Midslope					Downslope				
		Grain size fractions			Fresh OC	Total OC	Grain size fractions			Fresh OC	Total OC	Grain size fractions			Fresh OC	Total OC
		A	B	C	Sed OC		A	B	C	Sed OC		A	B	C	Sed OC	
0 - 5 cm	Fresh	0,08	0,11	0,20	0,39	1,15	0,83	0,57	1,43	2,83	3,17	0,05	0,40	0,84	1,29	2,92
	Sedimentary	0,06	0,08	0,62	0,76		0,00	0,01	0,33	0,34		0,64	0,14	0,85	1,63	
5 -15 cm	Fresh	0,07	0,05	0,08	0,20	0,93	0,03	0,21	0,55	0,79	1,62	0,11	0,21	0,63	0,95	1,93
	Sedimentary	0,05	0,09	0,59	0,73		0,01	0,07	0,75	0,83		0,06	0,13	0,79	0,98	
15 -30 cm	Fresh	0,09	0,03	0,00	0,12	0,82	0,10	0,27	0,86	1,23	2,17	0,18	0,23	0,72	1,13	2,21
	Sedimentary	0,06	0,08	0,56	0,70		0,03	0,10	0,81	0,94		0,14	0,11	0,83	1,08	
		non irrigated														
0 - 5 cm	Fresh	0,14	0,11	0,17	0,42	1,22	0,23	0,30	0,76	1,29	2,17	0,19	0,35	0,76	1,30	2,43
	Sedimentary	0,07	0,09	0,64	0,80		0,02	0,06	0,80	0,88		0,10	0,14	0,89	1,13	
5 -15 cm	Fresh	0,14	0,15	0,35	0,64	1,43	0,08	0,24	0,82	1,14	2,03	0,11	0,19	0,83	1,13	2,15
	Sedimentary	0,06	0,10	0,63	0,79		0,02	0,07	0,80	0,89		0,03	0,09	0,90	1,02	
15 -30 cm	Fresh	0,07	0,02	0,01	0,10	0,79	0,09	0,18	0,59	0,86	1,74	0,15	0,23	0,80	1,18	2,10
	Sedimentary	0,03	0,10	0,56	0,69		0,02	0,08	0,78	0,88		0,03	0,07	0,82	0,92	

Considering that fraction C prevailed in this soil, distribution of sedimentary and fresh organic carbon stocks ($T.ha^{-1}.cm^{-1}$) in this fraction were presented according to slope, treatment and depth in Fig. 3.24a & b. Sedimentary OC stocks showed no significant variations according to depth and treatment excepted for the surface layer of irrigated row at midslope position, while it increased steadily from upslope to downslope (0.60, 0.71 and 0.85 $T.ha^{-1}.cm^{-1}$, respectively). Fresh OC stocks showed wider variations according to depth and treatment with ranges of 0.4, 0.8 and 0.2 $T.ha^{-1}.cm^{-1}$ from upslope to downslope and no clear trends according to depth and treatment. According to slope, mean fresh OC stock was very low upslope while similar to sedimentary OC stock at midslope and downslope positions (0.13, 0.63 and 0.76 $T.ha^{-1}.cm^{-1}$ respectively).

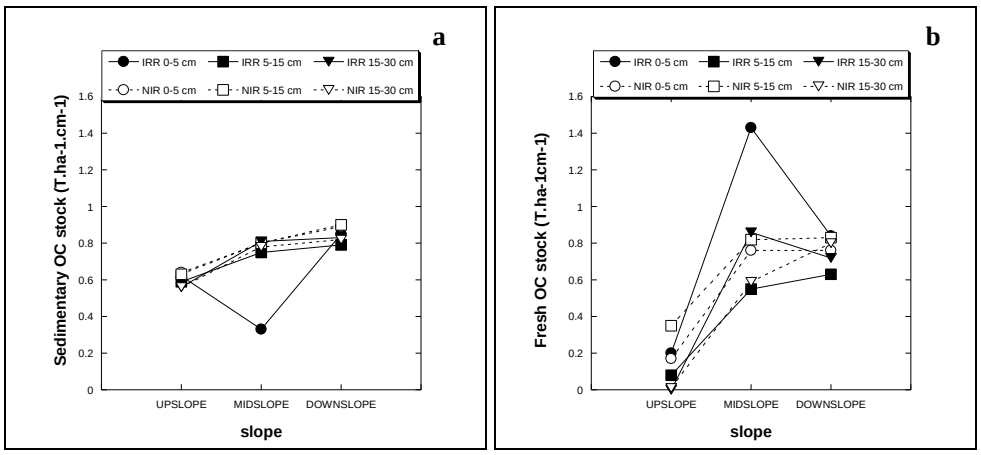


Fig. 3.24a & b: Distribution of sedimentary (a) and fresh (b) organic carbon (OC) stocks in fraction C according to slope, treatment and depth in Gačnik, October 2004.

Integration of the stocks of OC ($T.ha^{-1}$) on 0 - 30 cm showed that the ratio between sedimentary and fresh organic matter differed according to grain size fractions and slope position (Tab. 3.20 and Fig. 3.25). Focusing on the size fraction, sedimentary organic carbon prevailed in fraction C (especially at upslope). In fraction A, fresh organic carbon predominated (78 % of total OC). According to slope position, sedimentary OC strongly predominated at upslope position, where it represented 73 % of total OC. No significant differences in sedimentary and fresh OC stocks occurred between irrigated and non-irrigated row. Measurements of sedimentary and fresh OC stock (0-30 cm) according to size fraction, slope position, and treatment are presented in Annex 8.

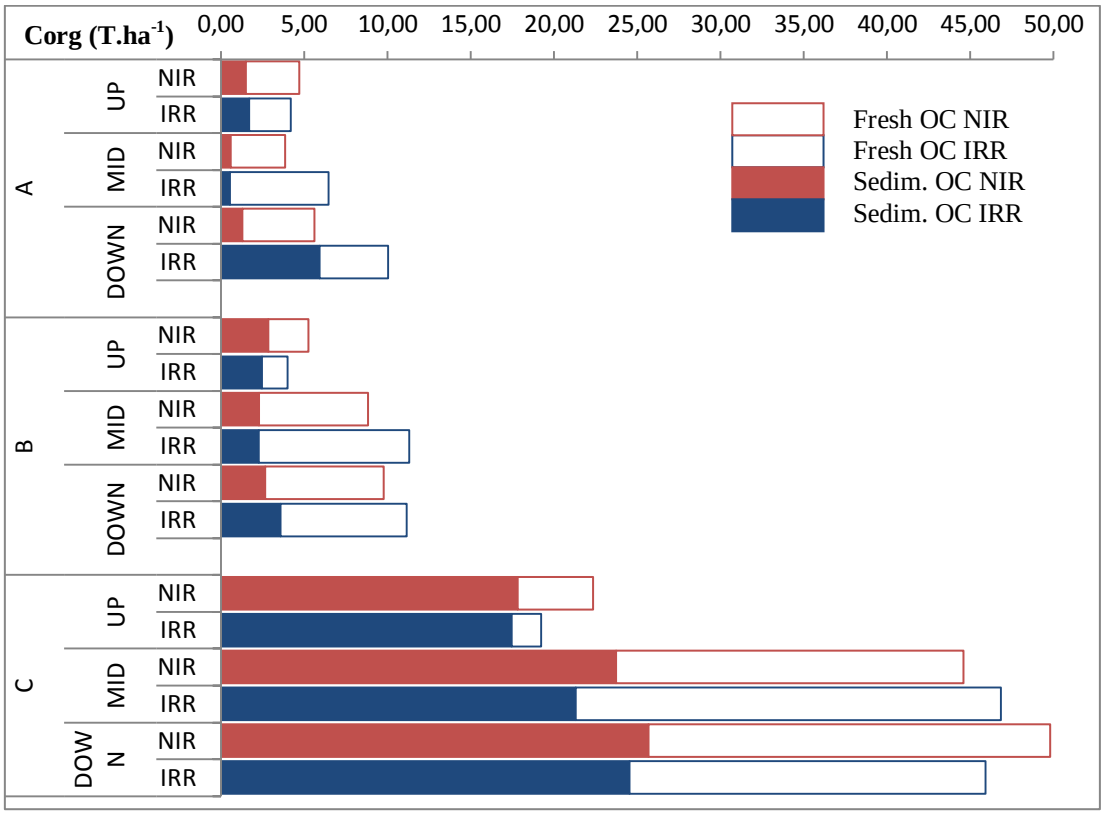


Fig. 3.25: Sedimentary and fresh organic carbon stocks (0 - 30 cm) according to grain size fractions (fraction A > 200 µm, fraction B: 200-50 µm, fraction C <50 µm), slope positions (UP = upslope, MID = midslope, DOWN = downslope) and treatments (NIR = non-irrigated, IRR = irrigated). (Table of OC stock in 0-30 cm soil layer is represented in Annex 3).

Tab. 3.21: Sedimentary versus fresh Organic carbon (OC) stock ratios in 0-30 cm layer (T.ha^{-1}) according to size fraction, depth, slope position and treatment.

		Sedimentary Organic Carbon	Fresh Organic Carbon	Total Organic Carbon	Sed/Total OC
		Stock T.ha^{-1}			Ratio %
Fraction size	A*	1,1	3,0	4,9	22
	B	2,7	5,7	8,4	32
	C	21,7	16,4	38,1	57
Slope position	U	21,9	8,0	29,8	73
	M	25,3	35,6	60,9	42
	D*	29,5	34,4	63,9	46
Treatment	NI	26,1	25,5	51,6	51
	I*	25,0	26,5	51,4	49

* minus Fraction A, irrigated downslope

(from Fig. 3.25 it can be seen that the situation according to sedimentary OM in fraction A in IRR row at downslope position differed completely from all others values from fractions A and B. It could be possibly related to the presence of a sand size particle of marl in the analysed sample)

V.2 Discussion

The fractionation of soil based on physically defined grain size fractions is used increasingly to interpret the dynamics of SOM. This method can provide useful information on SOM dynamics under natural conditions or short-term management practices (Piccolo *et al*., 2004; Sevink *et al*., 2005; Olk and Gregorich, 2006; Shrestha *et al*., 2007). For this reason, physical fractionation have been proposed to analyze the processes of organic matter stabilization in soils (Christensen, 1992; Golchin *et al*., 1997).
In our study soil texture was silty clayey, therefore fraction C (fine silt + clay) was the main fraction of the total soil mass (90 %).
Organic carbon content was the highest in fraction A, while in whole bulk soil fraction C represented the main stock of SOM. This is in accordance with other authors (Ducaroir and Lamy, 1995; Florez-Velez *et al*., 1996; Rumpel *et al*., 2004), who found that soil organic carbon is mainly located in the finest size fractions <20 μm and generally increase with decreasing particle size (Amelung *et al*., 1998; Zinn *et al*., 2007).
Fraction C was also rich in carbonates. It is generally accepted that calcium is a critical element for stabilization of SOM and aggregates (Six *et al*., 2004), inducing higher structural stability in soil samples with high carbonate content.
Fraction A was the smallest, but the most variable fraction, responding to slope (most at downslope) and depth (most in the surface layer). In fraction A, percentage of carbonates were almost negligible. The only significant difference in OC between IRR and NIR rows was found in fraction A. For this reason, the coarse fraction is an important indicator of the SOM quality, what was in agreement with Christensen (2001).

The C/N ratio is related to the degradation of fresh plant residues, and is important in Corg sequestration potential (Potter *et al*., 1998; Onweremadu *et al*., 2007). High C and N concentrations were found in the unprotected free OM fraction. Unprotected organic matter fractions are often relatively labile with high concentrations of carbohydrates and nitrogen compounds (Skjemstad *et al*., 1996; Golchin *et al*., 1994, 1995). With increasing degree of decomposition, organic matter may be transferred to more stabilized soil fractions.
In our study, C/N ratio was the highest in fraction A and it decreased in finner fractions. Our results are supported by some authors (Christensen, 1992, 2001; Buyanovsky *et al*., 1994; Plante *et al*., 2006), who found that SOM in coarse fractions is usually less decomposed and has a high C/N ratio. The nature and the turnover of the organic carbon in the finest fraction are different from the other particle size fractions probably due to less favourable conditions for mineralization (Rumpel *et al*., 2004; Balesdent, 1996). Moreover, C/N ratio in fraction C is smaller than fractions A and B, and its variations are narrower because organic carbon is relatively more stable due to sorption mechanisms (Christensen, 1992, 2001; Buyanovsky *et al*., 1994; Plante *et al*., 2006).
In our study, C/N ratio in fraction A increased with depth whatever the SOM content.
Higher C/N ratio occured in IRR than in NIR row, which indicates higher OM input from more developed irrigated trees. Very low C/N ratios were found at upslope, especially in the deepest layer, where sedimentary OM predominated.

Molecular ^{13}C signatures of organic matter (OM) are nowadays used for many purposes (Lichtfouse, 2000). Since 1985, the use of ^{13}C natural abundance technique, coupled with particle size fractionation allowed great progress in SOM turnover studies and to characterize SOM changes through time discriminated by SOM origin (Cerri *et al*., 1985; Sevink *et al*., 2005). The ^{13}C composition of SOM changes during its decay because ^{12}C is preferentially used by decomposers, resulting in enrichment in ^{13}C of the remaining SOM (Andreux *et al*.,

1990; Martin *et al*., 1990; Desjardins *et al*., 1994; Balesdent and Mariotti, 1996; Boutton, 1996; Koutika *et al*., 1997). Because of these processes, soil organic constituents can be ^{13}C-enriched by 1.5–4.3 ‰ relative to homogenous plant constituents (Lichtfouse *et al*., 1995).
In our study, we found a high δ^{13}C signature in fraction C corresponding to very low C/N ratio, which usually occurs in fresh OM like algaes and it is easily degraded by microbes. We attributed this signature to the marl bedrock which contains sedimentary OM with very low C/N ratio.

The relationships between Corg, C/N and $^{13}\delta$C are presented in fig. III.34:

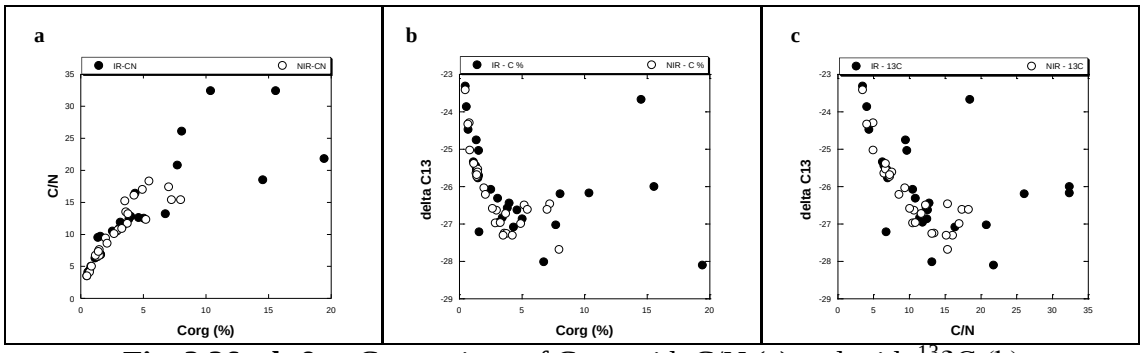


Fig. 3.26a, b &c: Comparison of Corg with C/N (a) and with $^{13}\delta$C (b) and comparison of C/N and $^{13}\delta$C (c) in Gačnik, October 2004.

As shown by Fig. 3.26a, a good correlation between Corg and C/N was found: the accumulated Corg (mainly originating from plant OM) has higher C/N ratio, while its $^{13}\delta$C decreases sharply, and is more negative than $^{13}\delta$C from marl (Fig. 3.26b). This is also illustrated by Fig. 3.26c, where OM with low C/N ratio (from marl) has higher $^{13}\delta$C than OM originating from plants, characterized by a higher C/N ratio.

Using a mixing model with δ^{13}C of marl and δ^{13}C of fresh OC, we were able to quantify the sedimentary (fossil stock) and fresh (present) OC in the soil. In accordance with a statement that older OC has higher $^{13}\delta$C, more sedimentary OC (as a concentration) was found in fraction C and more fresh OC (as a concentration) in fraction A and B, as expected. By considering soil OC stock according to proportions of each grain size fraction in this soil, higher stock of sedimentary and fresh OC occurred in fraction C. Fresh OC was mostly present in the middle of the slope and in the surface layer as a stock, where total OC was also high. The opposite situation appeared with sedimentary OC: on concentration level, it was mostly present at upslope position and in the deepest layer (similar pattern as total carbonates). When we are focusing on the stock of sedimentary OC (considering proportions of each grain fraction in this soil), no significant differences occurred in this type of OC according to depth, while small increasing at downslope was noticed.

The effect of land use on the composition and distribution of OM in size fractions is well known (Puget, 1999). The change in land use alters the rate at which the organic matter is oxidized, therefore affecting its accumulation and mineralization (Solomon *et al*., 2002). According to irrigation, no significant differences were observed in distributions of size fractions, OC content and total carbonates according to depth and slope. The impact of irrigation was noticed only inside fraction A, where higher Corg content and higher C/N ratios were noticed in IRR soil. This could be explained by a higher input of fresh organic matter from more developed irrigated trees. Previous studies have also indicated that changes in land

use (or management practices) have a marked impact on fraction A, which could serve as an early indicator to identify the impact of landuse change on soil Corg storage matter entrapped (Christensen, 2001; Solomon *et al*., 2002; Leifeld and Kögel-Knabner, 2005). In contrast, mineral-associated organic matter and organic at sites inaccessible to microbial attack or physically protected within soil aggregates belong to more stable organic matter pools with a turnover time of decades to centuries (Christensen, 1992; Piccolo, 1996; Golchin *et al*., 1997).

VI. Microbiological soil characteristics

Several microbiological soil characteristics were measured at different seasons and locations along the slope during two years to follow spatial and temporal variations of these parameters according to season, slope position and treatment. The following parameters were measured: microbial biomass (BM), labile organic matter pool (LOM) and microbial respiration and N mineralization after a 28 days incubation (Cmin, Nmin).

As a preliminary study, analyses were done in May 2004 on samples from two water treatments (IRR, NIR) and two depths (0-10 and 20-30 cm) at downslope position. In September 2004 we continued with sampling from both treatments again, while this time samples were taken at six positions from midslope to downslope and for 0-15 cm and 15-30 cm depths. Next year (2005) in May we also included the possible effect of sun exposure (sunny and shady sides of each studied row) and soil samples were taken from the upslope to downslope at eight different positions (to the same depths as previously). The last sampling for basic microbial analyses was done in September 2005: soil samples were taken from three positions on the slope (upslope, midslope and downslope) and sun exposure was still included.

VI.1 Soil physico-chemical characteristics and wetness at the sampling periods

For interpreting microbial results, soil wetness at the sampling periods and some physico-chemical soil analyses were done.

VI.1.1 *Organic carbon and nitrogen, C/N and exch. Cu*

For a preliminary study, initial soil analyses were done only at downslope position (pH, Corg, Norg and Cu) (Tab. 3.22). Soil pH was measured in all sampling periods, but the mean values were always higher than 7.5 and no differences were noticed. Therefore, pH variations were not relevant for explaining microbiological properties and activity.
Carbon, nitrogen and copper content were more or less the same to 30 cm in depth in both treatments, while slight higher values were noticed in the surface soil layer of IRR row.

Tab. 3.22: Chemical analysis of organic carbon (C_{org}), nitrogen (N_{org}), C/N ratio and $Cu_{(EDTA)}$ at downslope position in Gačnik, May 2004.

	IRR		NIR	
	0-10 cm	20-30 cm	0-10 cm	20-30 cm
C ($g.kg^{-1}$)	14.8	10.5	13.5	12.1
N_{org} ($g.kg^{-1}$)	2.01	1.53	1.84	1.71
C/N	7.37	6.85	7.35	7.07
$Cu_{(EDTA)}$ ($mg.kg^{-1}$)	8.44	3.83	6.91	4.17

VI.1.2 *Gravimetric water content*

Gravimetric water content (W) is important especially for interpreting soil microbial biomass and its activity as a consequence of weather conditions and added water by irrigation. For this reason it was measured at every sampling date, in parallel with microbial soil parameters.

In May 2004, water gradient showed a slightly higher percentage in NIR soil and in the deeper layer, but it has to be emphasized that no irrigation had been applied until this date (Fig. 3.27).

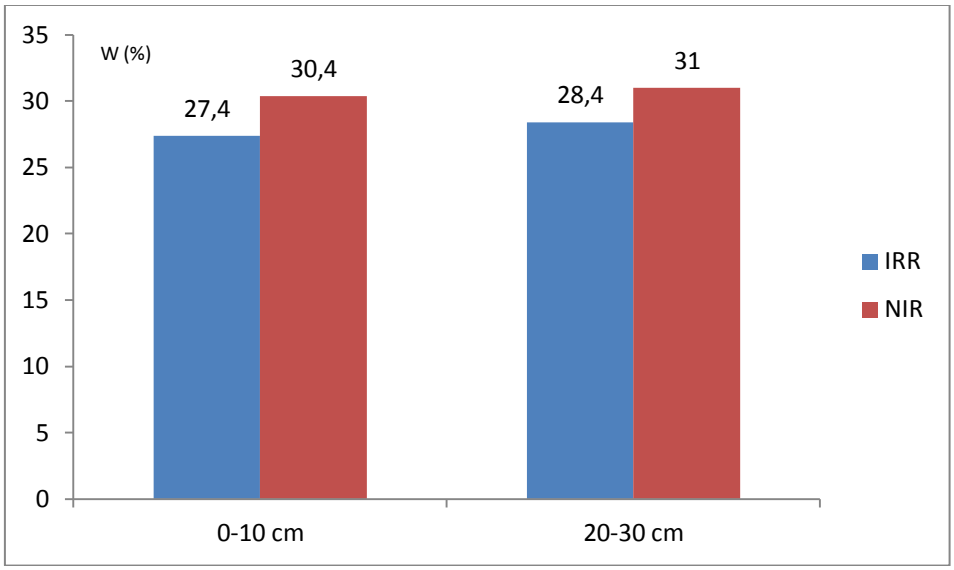


Fig. 3.27: Mean value of gravimetric water content (%) at downslope position according to depth and traitment, May 2004.

In September 2004, water gradient was measured in the lower half of slope (from midslope to downslope) with four intermediate positions. NIR row still had a significantly higher gravimetric water content than IRR row in both soil layers. In the lower half of slope, more or less the same gravimetric water content was measured in upper layer, while in the deeper layer a slight increase towards downslope was noticed. Soil moisture was higher in upper soil layer (0-15 cm) (Fig. 3.28).

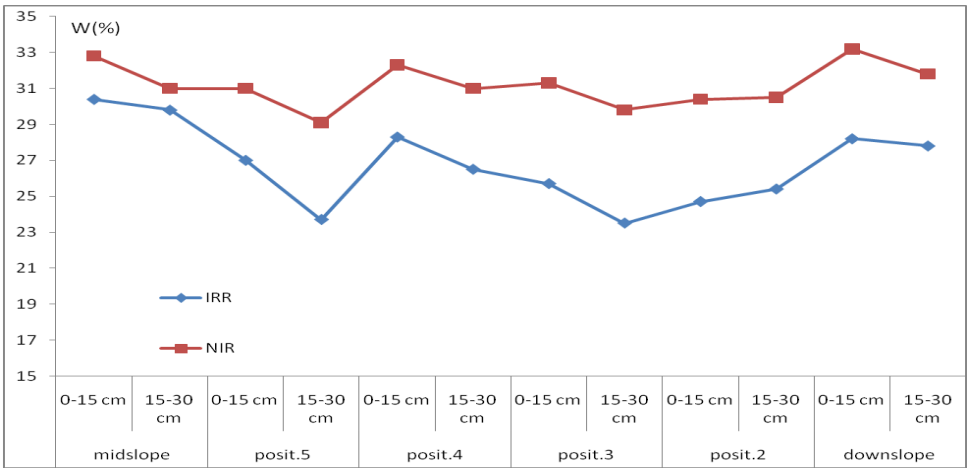


Fig. 3.28: Distribution of gravimetric water gradient (W) from midslope to downslope in Gačnik, September 2004.

In May 2005 the whole slope was sampled with 5 intermediate positions, there were no differences between soil samples according to water treatment, while gravimetric water content significantly differed along the slope, increasing from upslope to downslope. Higher gravimetric water content was observed in the deeper soil layer and at the shady side of the rows (Fig. 3.29a & b).

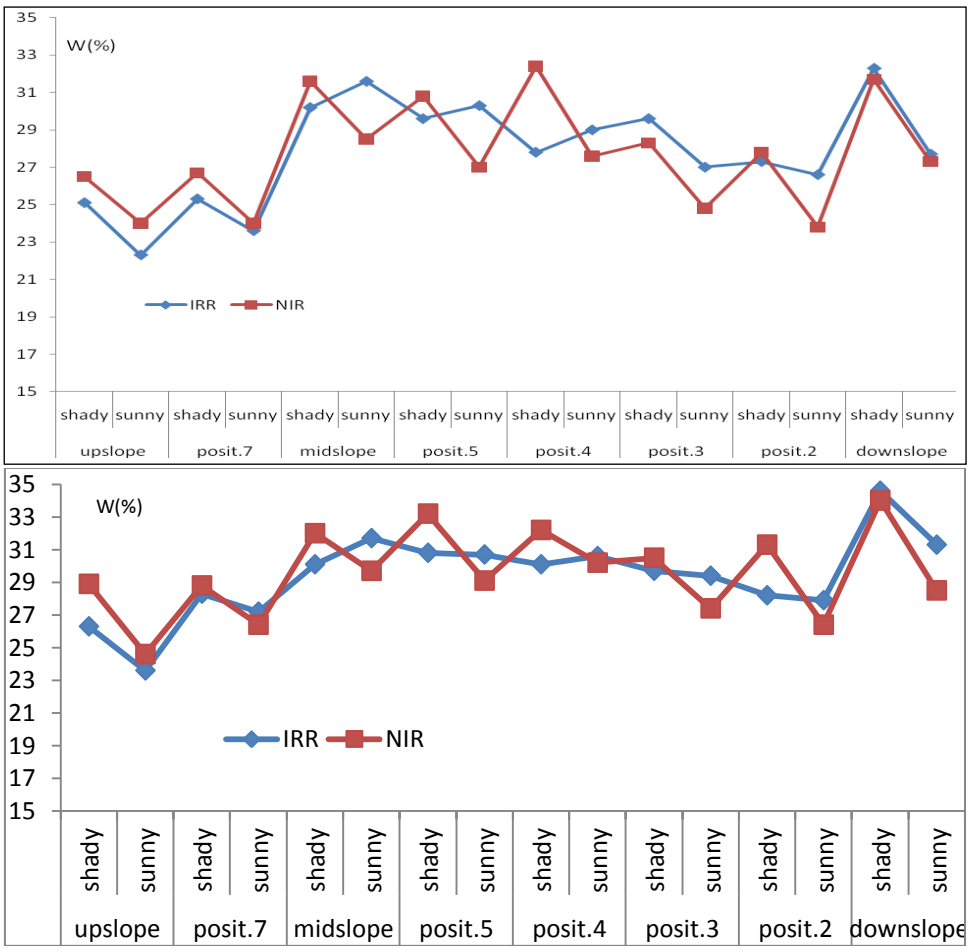


Fig. 3.29a & b: Distribution along the slope of gravimetric water content (W) in the upper soil layer (a) and in deeper soil layer (b), Gačnik, May 2005.

In September 2005, W significantly differed between water treatments: W was higher in NIR than in IRR row and showed an increasing trend towards downslope in both soil layers. There was no obvious difference according to depth but sun exposure significantly affected W: the shady side being wetter than the sunny side (Fig.3.30).

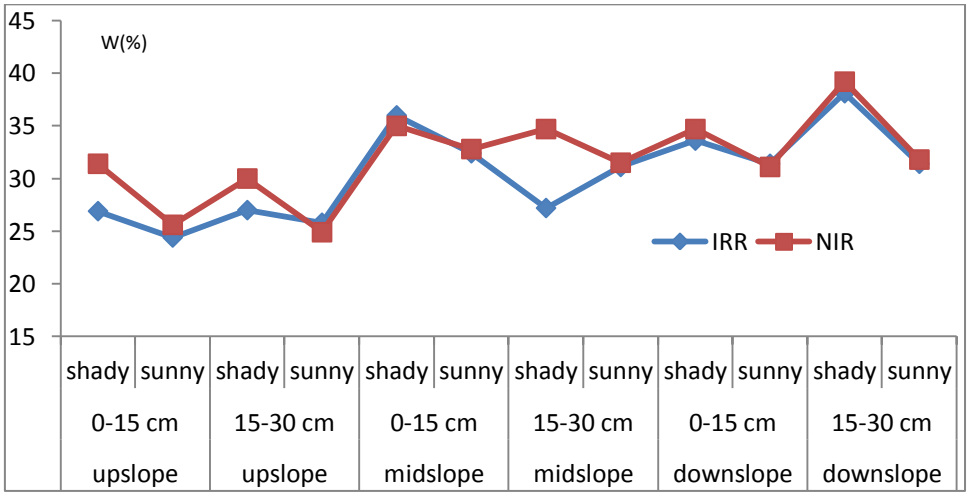


Fig. 3.30: Distribution of gravimetric water content (W) from upslope to downslope in Gačnik, September 2005.

As basic microbial properties a few parameters were measured: microbial biomass (BM), labile organic matter pool (LOM) and microbial respiration after a 28 days incubation (Cmin, Nmin).

As a preliminary study, analyses were done in May 2004 on samples from two water treatments (IRR, NIR) and two depths (0-10 and 20-30 cm) at downslope position. In September 2004 we continued with sampling from both treatments again, while this time samples were taken at six positions from midslope to downslope and for 0-15 cm and 15-30 cm depths. Next year (2005) in May we also included the possible effect of sun exposure (the sunny and the shady sides of each studied row) and soil samples were taken from the upslope to downslope at eight different positions (to the same depths as previously). The last sampling for basic microbial analyses was done in September 2005: soil samples were taken from three positions on the slope (upslope, midslope and downslope) and sun exposure was still included. Complete measurements of microbial parameters are presented in Annex 9 (in 2004) and in Annex 10 (in 2005).

VI.2 Spatio-temporal variation of the biological parameters

VI.2.1 *May 2004*

Microbial biomass (BM)

In May 2004, IRR row showed significantly higher biomass values than NIR row (Duncan test at 95 % confidence level) in the upper soil layer, while in the deeper soil layer the values were more or less the same (Fig. 3.31). Microbial biomass level was consistent with total organic matter content. In IRR treatment, BM represented 3.04% of total Corg, and only 2.75% in NIR treatment (in upper soil layer). This figure, as absolute values, confirmed a better organic status in the IRR row.

According to depth, the the upper soil layer (0-10 cm) showed a much higher microbial biomass than the deeper layer (20-30 cm), which was expected from the higher percentage of organic matter in the upper layer.

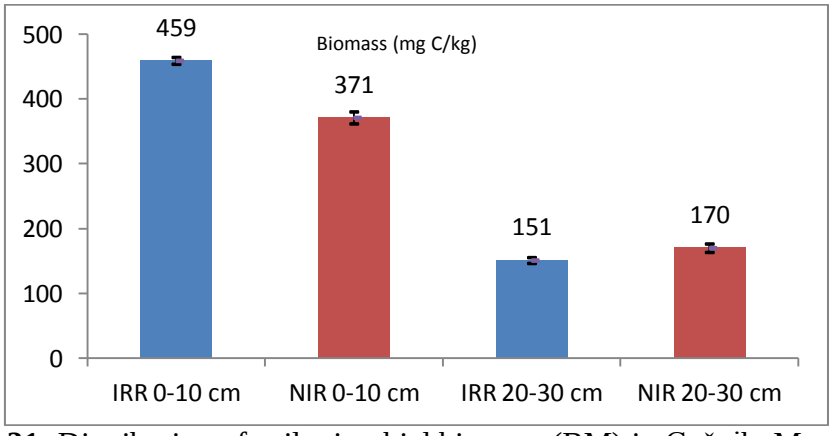


Fig. 3.31: Distribution of soil microbial biomass (BM) in Gačnik, May 2004.

Labile organic matter pool (LOM)

Labile organic matter pool was analysed only in the upper soil layer (0-10 or 0-15 cm), except in spring 2004 where the deeper layer was also included (20-30 cm) (Fig. 3.32).

In May 2004, similar pattern of LOM values occurred as with biomass. According to irrigation, statistically significant difference in labile organic matter pool (LOM) was noticed only in the upper soil layer, where more LOM was detected in IRR row than NIR one (8.33% and 7.30% of total Corg respectively). Significantly more LOM was present in the upper soil layer than in the deeper soil layer, in accordance with total soil organic matter.

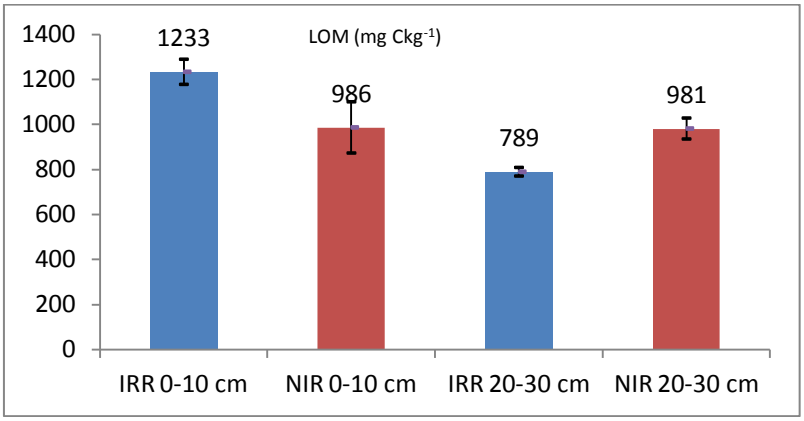


Fig. 3.32: Distribution of labile organic matter pool (LOM) in Gačnik, May 2004.

Microbial respiration (C mineralization)

In May 2004, C mineralization (respiration) was significantly more intensive in IRR treatment in both soil layers, in agreement with the results of microbial biomass. According to depth, the upper soil layer (0-10 cm) produced more mineral C by respiration than the lower layer, what is in accordance with the results of microbial biomass and labile organic carbon pool (Fig. 3.33).

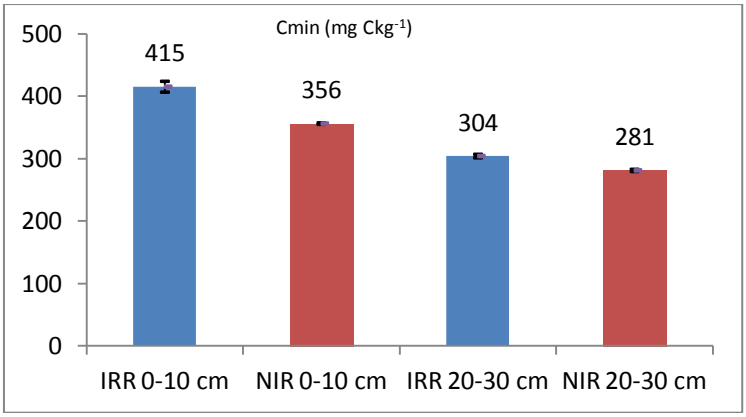


Fig. 3.33: Distribution of Cmin in Gačnik, May 2004.

Microbial respiration and microbial biomass were combined to calculate two derived parameters: specific respiration and time of turnover (Tab. 3.23). Specific respiration (qCO_2) is the amount of Cmin produced per day and per unit of microbial biomass. Time of turnover is the inverse of specific respiration: $1*(\text{spec. respiration})^{-1}$.

In IRR row, more intensive respiration of soil microbes was recorded. In the deeper soil layer, more mineral CO_2 was produced per unit of microbial biomass (Tab. 3.23). Indeed, the soil samples were incubated in optimal conditions which are not representative of *in situ* conditions, where various limiting factors (aeration, substrate availability etc.) prevailed.

Tab. 3.23: Spec. respiration (day^{-1}) and turnover time (day); Gačnik, May 2004.

	IRR	**NIR**	**IRR**	**NIR**
Soil depth (cm)	0-10		20-30	
spec. respiration (day^{-1})	0.040	0.028	0.064	0.066
turnover time (day)	25	36	16	15

Distribution of soil organic matter components in total Corg

Total soil organic carbon can be represented by three main components: microbial biomass (C-MB), labile organic matter pool (C-LOM) and stable organic matter (C-StOM). Differences may occur in proportions of these components within total Corg.

Stable OM represented the most part of total Corg, while LOM was at least twice the part of microbial biomass. The percentage of active organic carbon (microbial biomass and LOM) according to total Corg was higher in IRR row compared to NIR, especially in the upper soil layer. In the lower soil layer (20-30 cm), percentage of stable OM in total Corg increased, while microbial biomass and LOM decreased (Fig. 3.34).

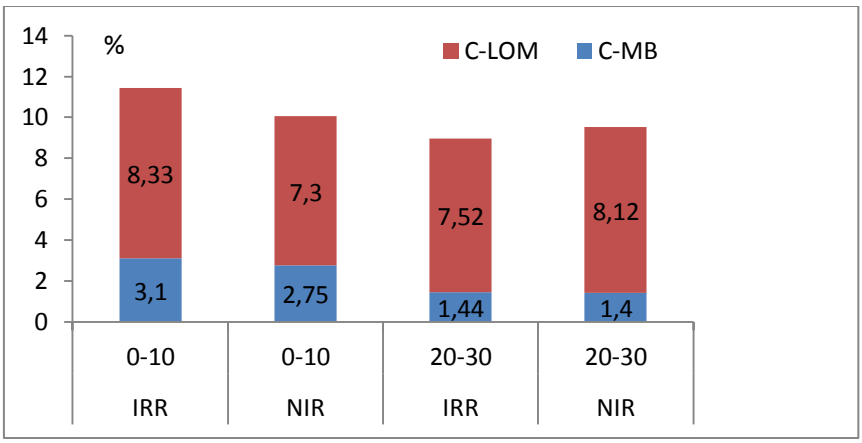


Fig. 3.34: Distribution of soil organic matter (SOM) components in total Corg, Gačnik, May 2004.

VI.2.2 *September 2004*

Microbial biomass

In autumn 2004 we continued with more detailed sampling and the analyses were performed from midslope to downslope with some intermediate positions.

In September 2004, statistical significant difference in microbial biomass according to irrigation was noticed again, with more microbial biomass in IRR soils. According to slope, a clear trend of biomass increase towards downslope in the upper layer was noticed, the highest values being detected at downslope and the lowest in midslope position. Biomass significantly decreased with soil depth (Fig. 3.35).

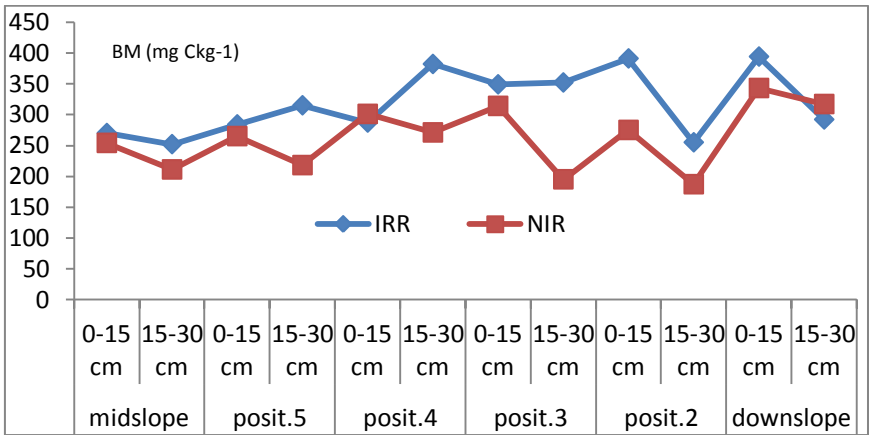


Fig. 3.35: Distribution of microbial biomass (BM) in Gačnik, September 2004.

Labile organic matter pool

LOM was analysed in the surface layer only (0-15 cm). Irrigation effect was less significant, while LOM values changed along the slope: in the first part of slope more LOM was present in IRR row, while in the upper position the situation changed (Fig. 3.36). Along the slope, some significant differences occurred: the highest LOM values were recorded downslope and the lowest at position 5 (near midslope). This is consistent with variations of some physic-chemical characteristics of the soil samples (clay content, CEC etc.) according to the position of the slope and may result from soil movement (levelling of terraces). In addition, at down slope position, fine soil particles rich in OM (and LOM) can accumulate as the result of soil erosion.

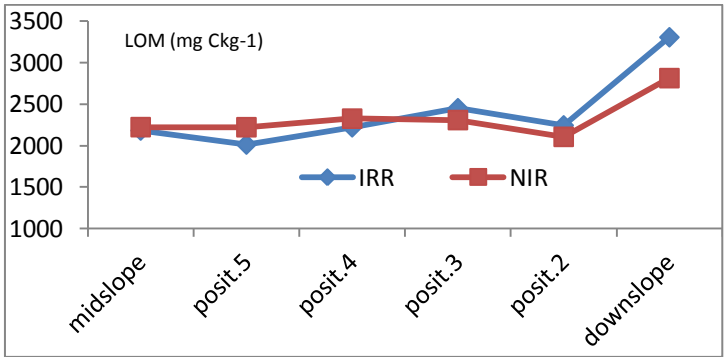


Fig. 3.36: Distribution of labile organic matter pool (LOM) from midslope to downslope, Gačnik, September 2004.

Microbial respiration (C mineralization)

Statistical significant differences in microbial respiration were noticed according to the treatment, slope and depth. IRR row released more CO_2 than NIR row, especially in the lower slope positions. The highest mineralization rate occurred downslope and it decreased towards midslope (Fig. 3.37). Cmin decreased with depth, as microbial biomass.

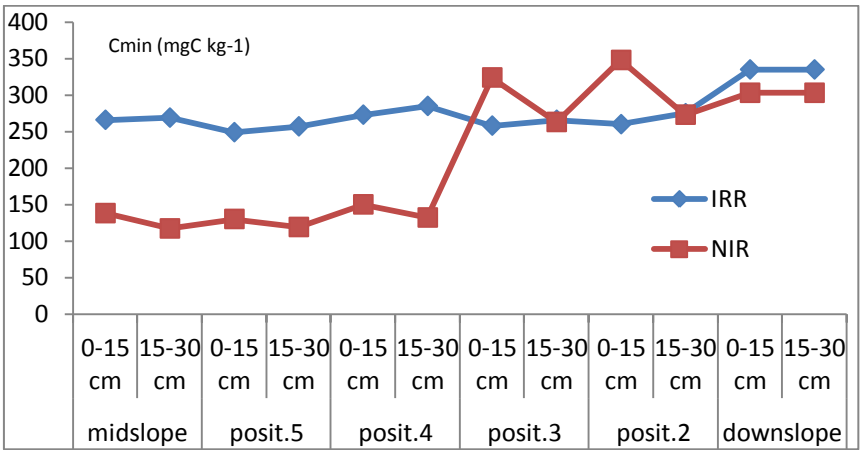


Fig. 3.37: Distribution of Cmin in Gačnik, September 2004.

More intensive respiration of soil microbes (and shorter time of Corg turnover) was noticed in IRR row, especially at midslope, which could be a consequence of added water during the summer (Tab. 3.24). Specific respiration was slightly higher in the deeper soil layer, especially downslope - the field conditions were probably not optimal like during the incubation process. Higher moisture in the deeper soil layer could be the reason for enhanced C mineralization.

Tab. 3.24: Spec. respiration (day^{-1}) and turnover time (day), Gačnik, September 2004.

	Mid-slope				**Down-slope**			
	IRR	**NIR**	**IRR**	**NIR**	**IRR**	**NIR**	**IRR**	**NIR**
Depth (cm)	**0-15**		**15-30**		**0-15**		**15-30**	
spec. respiration (day^{-1})	0.035	0.025	0.038	0.021	0.030	0.032	0.040	0.036
1*(spec. respir.)$^{-1}$ (day)	28	41	26	48	33	32	25	28

N mineralization

Values of ammonium nitrogen were negligible in this soil (NH_4^+ concentration was low in irrigation water as well), while irrigation significantly increased nitrates in soil. Along the slope, the highest values were recorded at midslope position and they significantly differed from downslope position. Intermediate positions showed zig zag pattern in nitrates values, as observed for Corg distribution along the slope (Fig. 3.38). No differences according to depth were noticed.

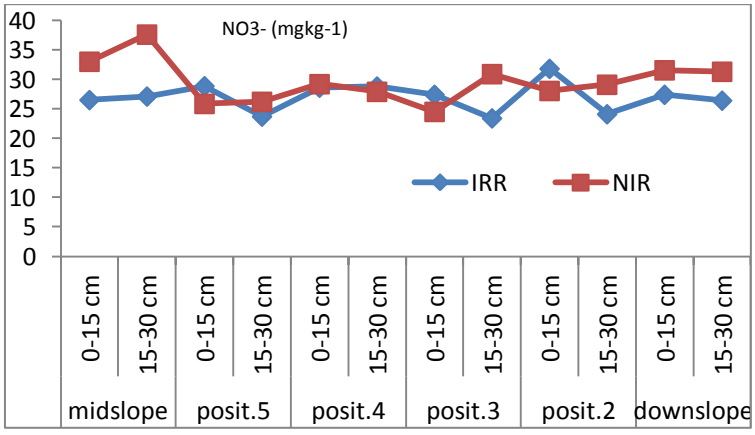


Fig. 3.38: Distribution of NO_3^- in Gačnik, September 2004.

Distribution of soil organic matter components in total Corg

A slightly higher percentage of active OM components (BM and LOM) was noticed in IRR soil, especially downslope, confirming the results from the previous sampling in May 2004 (Fig. 3.39).

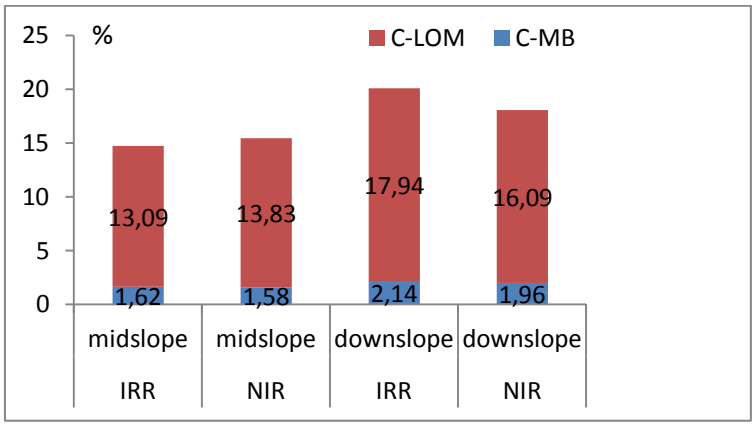


Fig. 3.39: Distribution of soil organic matter (SOM) components in total Corg in upper soil layer (0-15 cm), Gačnik, September 2004.

VI. 2.3 *May 2005*

In the year 2005, the effect of sun exposure was included in the study. For this purpose, each row (water treatment) was separated into the shady and sunny side. The top of the slope was also included in this study. As no irrigation was done this year, analyses of Cmin from microbial respiration were not performed.

Microbial biomass

In May 2005, no statistically significant differences in microbial biomass were noticed between IRR and NIR rows (Fig. 3.40a & III.48b). According to slope microbial biomass increased from upslope to downslope. Biomass also decreased with depth. Significant difference occurred between the shady and the sunny side of the row, with higher microbial biomass at the sunny side.

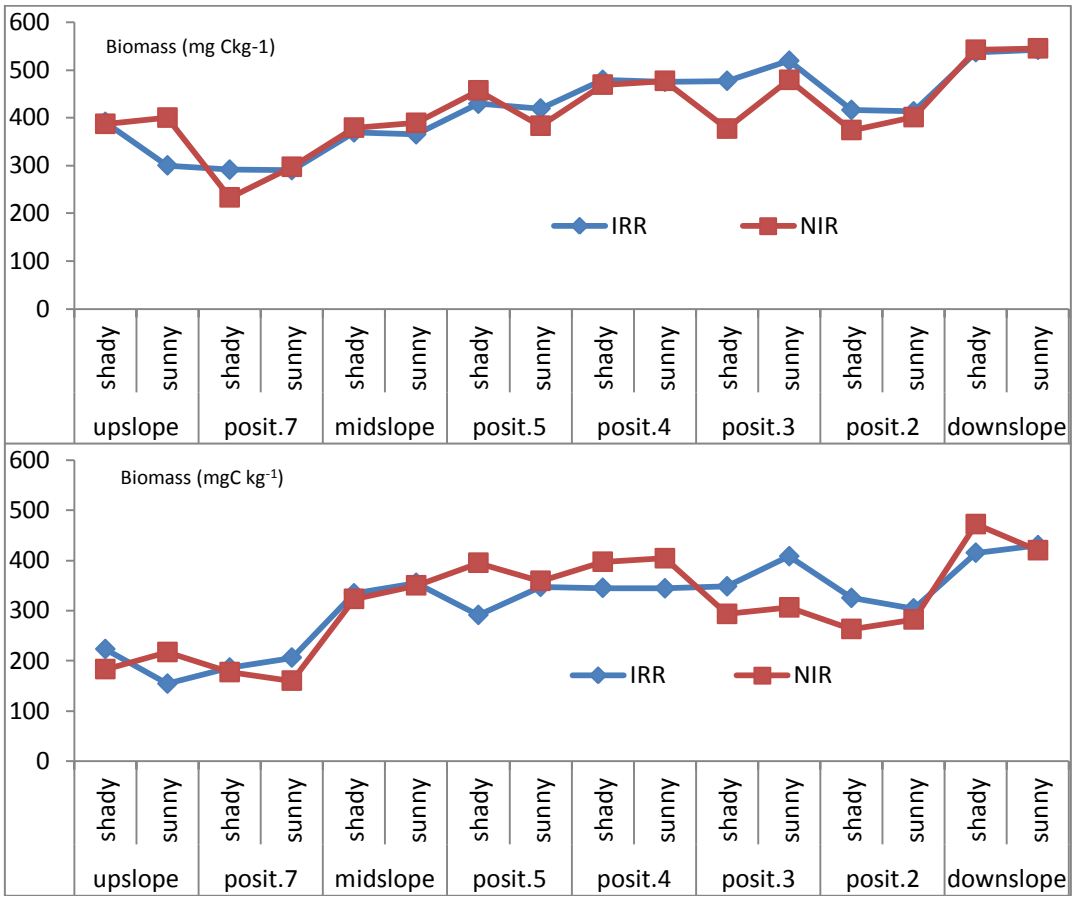


Fig. 3.40a & b: Distribution of microbial biomass (BM) in the upper soil layer (0-15 cm) (Fig. III.48a) and in the deeper soil layer (15-30 cm) (Fig. III.48b), Gačnik, May 2005.

Labile organic matter pool

No important differences in LOM were noticed between IRR and NIR rows: upslope, more LOM in NIR row was detected, while downslope the situation was the opposite (Fig. 3.41). Along the slope, LOM values increased from upslope to downslope. Sun exposure did not significantly affect LOM, only a slight tendency for higher values in the shady side was noticed.

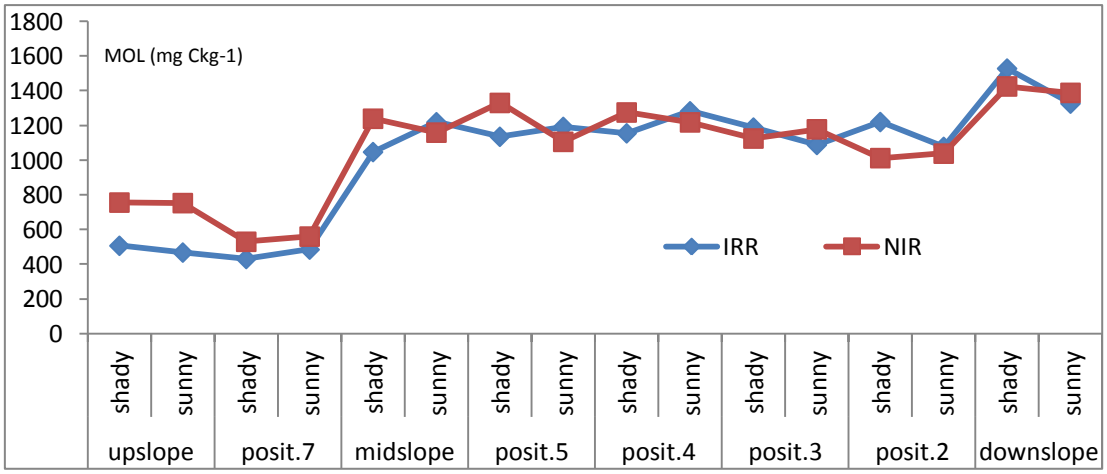


Fig. 3.41: Distribution of labile organic matter pool (LOM) in the upper soil layer, Gačnik, May 2005.

VI.2.4 *September 2005*

In September 2005, comparison of IRR versus NIR rows at three main positions of the slope was made: downslope, midslope and upslope.

Microbial biomass

Comparing soil microbial biomass under the two different water treatments, significantly higher values were detected in NIR row (Fig. 3.42a & b). Along the slope, significant differences occurred as well, biomass increasing from upslope to downslope, especially in irrigated row. Soil depth did not affect biomass, but sun exposure showed significantly higher values in the shady side. Higher values of microbial biomass mostly followed higher soil moisture.

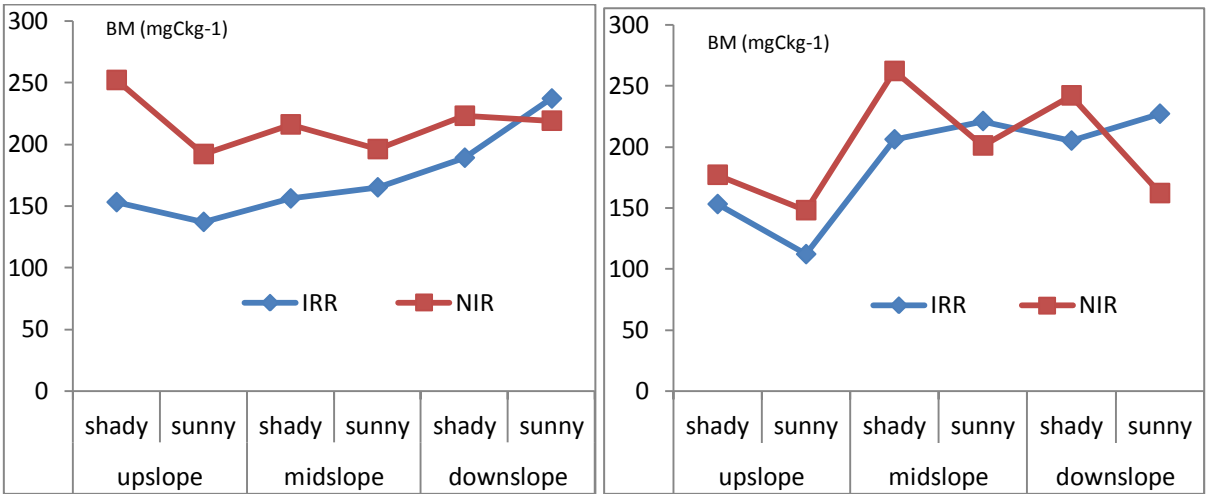


Fig. 3.42a & b: Distribution of microbial biomass (BM) in the upper soil layer (Fig. III.50a) and in the deeper soil layer (Fig. III.50b), Gačnik, September 2005.

Labile organic matter pool (LOM)

Between IRR and NIR rows statistical significant difference occurred in LOM content: NIR row contained more LOM than IRR row. LOM significantly increased towards downslope. Sun exposure affected LOM in the sense of higher values in the shady side (Fig. 3.43).

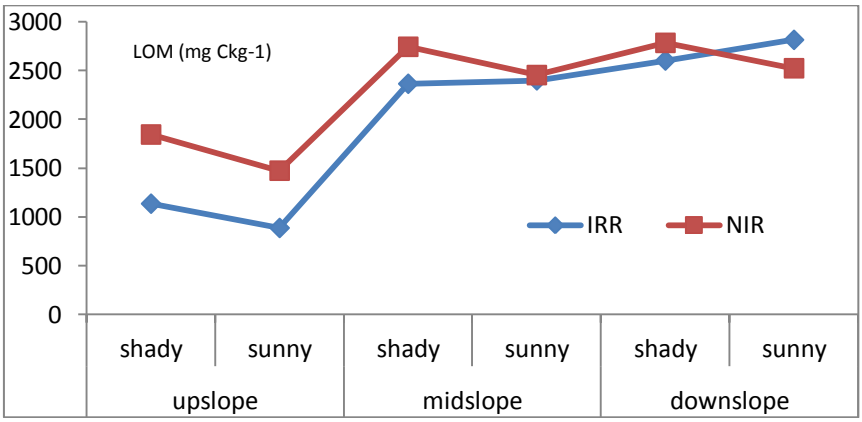


Fig. 3.43: Distribution of labile organic matter pool (LOM) in Gačnik, September 2005.

VI.3 Discussion

The activity of aerobic microbes is greatly dependent on soil water content which governs transport of oxygen and substrates (Papendick and Campbell, 1981; Skopp *et al*., 1990; Young and Ritz, 2000). Water dynamics and stress exert a major influence on microbial physiology and function (Harris, 1981; Yancey *et al*., 1982; Kempf and Bremer, 1998), however, there is still considerable debate about how water stress affects soil microbial communities, their biomass, and their overall activity. Eventually, water stress can be alleviated by irrigation in agro-ecosystems. For these reasons, gravimetric water content was measured in parallel with microbial parameters.
In spring (May 2004 and 2005), higher soil moisture was measured in deeper soil layer, the situation being the opposite in autumn (September 2004 and 2005). Distribution of soil water through the soil profile depends on the weather (rainfall, evaporation), the surface cover and structural stability. In spring, bare soils probably lost some water from the surface layer due to evaporation, while in autumn, plant cover could contribute to higher soil moisture in the surface soil layer. In spring, higher WSA was measured compared to autumn, and this could help water infiltration through the soil profile.

Along the slope, a trend of increasing soil moisture towards downslope was noticed, probably due to higher SOM content and downhill water run-off. Gravimetric water content was high at downslope and midslope positions, while soil from upslope position was the driest. Soils from the shady side of the rows always showed higher gravimetric water content, which was presumably linked with the presence of blue-green algae at this location. Indeed, both C availability and water regime are implicated as major determinants of soil microbial community structure and activity in both laboratory and field experiments (Zhang and Zak, 1998; Wilkinson *et al*., 2002; Drenovsky *et al*., 2004).

The interaction of soil microbes with their physical environment affects their abilities to respire, grow and divide. One of these environmental factors is the the soil wetness. In our study, there was a strong negative correlation between soil moisture and microbial biomass in spring 2004 (correl. coef. = -0.97 at 99% confidence level; R^2 explained 95% of the variability of MB with linear model: MB=2878-88.4*W). Too much moisture also had a negative effect on soil respiration (Cmin) (linear correl. coef.= -0.74; R^2=55%). During this period, mean water saturation ratio increased very quickly with depth (Tab. 3.11): in IRR row from 0.76 (in 20-25 cm layer) to 0.84 (in 40-45 cm layer) and in NIR from 0.82 to 0.95. It also has to be taken into account that those figures are measured in the cylinders with 100 cm3 volume, while microbes are mostly present inside the clods, where there is presumably even higher water saturation according to silt clayey texture.
Hillel (1980) found that microbial respiration was linearly related to soil-water content and log-linearly related to water potential. Obviously this linear relationship between soil respiration and water content applies only on the first part of the curve until the optimum water content (enabling also good aeration). Beyond this optimum, the relationship becomes negative, due to limited oxygen availability.

Changes in soil moisture, temperature and C input can have a large effect on the soil microbial biomass and its activity, which, in turn, affect nutrient availability due to soil organic matter turnover (Ross, 1987). Microbial biomass, labile organic matter pool and respiration rate mostly decreased with depth, as did soil organic status. Gravimetric water content showed variations with both sampling depth and season: in spring water content mostly increased with depth and decreased in autumn. Since organic matter and soil microbial

activity are typically concentrated in the top few centimetres of soil (Murphy *et al.*, 1998), there is generally a positive linear relationship between soil organic C content and microbial biomass C (Sparling, 1997; Rietz and Haynes, 2003). However this was not the case in our study, where IRR soil contained a lower SOM, but higher MB. Indeed mineralization processes, as shown by lower C/N ratio of SOM and higher respiration (Cmin) are more intensive in IRR soil, with lower remaining SOM in this treatment.
Microbial biomass, labile organic matter pool and respiration always increased from upslope toward downslope, which was positively related to SOM content. Distribution of microbial biomass, respiration rate and labile organic matter pool along the slope was similar to distribution of total Corg (zigzag pattern), and was presumably the result of soil movement when changing the previous system (with terraces) to the new one (rows on the slope).

Sun exposure had no general effect on soil microbes, even if gravimetric water content was always higher at shady side: in May 2005, higher microbial biomass was measured at the sunny side, and in September 2005 at the shady side.

To analyse the irrigation effect on soil microbial biomass, we focused on the results from 2004 (especially on September, as short-term effect), when irrigation was really performed. This period could also reflect previous irrigation seasons as well (long-term effect). In the year 2005, no irrigation was done.

At the beginning and the end of the 2004 growing season, irrigation positively affected microbial activity in sense of higher biomass, LOM and higher respiration rate in the upper soil layer. Comparing proportions of different components of SOM, differences between water treatments were observed, especially in the upper soil layer where IRR soils had more active SOM (microbial biomass and LOM). These results were not related to gravimetric water content. We can conclude that in our study organic matter status is a more important factor than water status.

In 2005, the situation according to water treatment was the opposite, but it should be emphasized again that no irrigation was done this year. In the beginning of the season 2005, no significant differences in microbial biomass and in LOM were observed between IRR and NIR rows. At the end of this season, microbial biomass and organic matter pool were higher in NIR row, but this was not a direct (short term) consequence of irrigation. Results of biomass and LOM in autumn 2005 followed the pattern of soil moisture which was itself related to SOM content. If irrigation modifies root distribution and consequently water regime, the effect of previous irrigation by modifying root system still may occurred on water regime even without irrigation. Between years, the variations of the parameters related to soil microflora were influenced by the seasonal climatic trend.

Several authors reported positive effects of irrigation on soil microbial biomass. Irrigation can increase C input to soils *via* increased crops and root production and thus increase of active microbial biomass is expected (Entry *et al.*, 2008, Melester, 2010; Ramirez-Fuentez, 2002). Martiniello (2007) showed that irrigation favoured microbial activity in Mediterranean climate. Management practices of irrigated land, including yearly fertilizer application, results in the addition of young OM to irrigated soil which contributes to the production of mineral N from labile organic matter. The labile OM acts as a substrate for microorganisms and is readily decomposed, turning over large amounts of N (Henry and Hogg, 2003). Contents of total and labile OM and microbial biomass were significantly affected by different irrigation regimes, and decreased with soil depth, while drip irrigation increased microbial biomass

(Han *et al.*, 2010; Melester, 2010; Samuelson *et al.*, 2009). Other authors (such as Swarts, 2006) found that neither microbial biomass nor respiration was significantly affected by irrigation. Intermittent irrigation did not affect soil respiration evidently, despite the significant effect of water content on soil respiration (Kucera and Kirkham, 1971, Tesarova and Gloser, 1976). Maybe the variation of soil respiration lagged behind the change of water content.

Because mineralization is performed by the microbial biomass, it is often assumed that this process is at least partly regulated by its size, specific activity or composition (Marschner and Kalbitz, 2003; Fontaine and Barot, 2005). This was the case in our study in May 2004, where positive linear correlations were found between MB and Cmin (correl. coef.=0.77; R^2=59%) and between MB and LOM (correl. coef.=0.70; R^2=49%). Results from October 2004 confirmed the previous observations, but with weaker relationships. On the contrary, Kemitt *et al*. (2008) concluded that the rate of humified organic matter mineralization is not necessarily proportional to the size, activity or composition of the microbial biomass. They hypothesized that the mineralization of organic matter is regulated by an abiological destabilizing process that transforms non-bioavailable substrates to bioavailable substrates.

Although microbial biomass is generally acknowledged to represent only a very small (0.1–5%) proportion of total carbon in the soil (Robert and Chenu, 1992; Moore *et al.*, 2000), it is characterised by its rapid turnover compared to the other components of organic matter (Sparling *et al.*, 1998). This was in agreement with our results, where microbial biomass represented from 1.4 to 3.1% of total soil carbon. The size of soil C_{MB} pool and its turnover have significant bearing on the overall productivity of soils. Labile organic C fractions (LOM) only account for a small fraction of soil organic matter (from 7.3 to 17.9% of total Corg in our study), but are used by the soil microbial community as an energy source for metabolic activity. The study of these fractions is important in agricultural soils, since they determine soil microbial activity (Janzen *et al.*, 1992) and contribute to a structural function (Metzger and Yaron, 1987). Labile organic matter pools can be considered as fine indicators of soil quality that influence soil function in specific ways and that are much more sensitive to changes in soil management practice (Haynes, 2005). In 2004, irrigation affected different Corg pools, while active organic carbon (MB and LOM) was slightly enhanced in the surface soil layer of IRR row (at downslope position).

The variations of the parameters related to the soil microflora are strongly influenced by the seasonal climatic trend (Meli *et al.*, 2002). Comparing microbial biomass and activity between May and October, higher values of MB and Cmin were measured in May, especially in the year 2004 (Annex 9). In May, soil respiration increased probably due to the enhancement of microbial activity in soil. Following soil warming, microbes were more active, and decomposable organic matter from root exudation was produced in soil, providing abundant substrate available to micro-organisms, increasing both soil microbial biomass and respiration (Yang *et al.*, 1989).

The majority of the studies dealing with the impact of farming practices on microbial communities have shown that the spatial and temporal variability in microbial processes make this impact difficult to observe accurately (Morris *et al.*, 2002). Indeed, soil biological parameters are always the result of applied farming practices, spatial soil variability and weather conditions fluctuating both within (seasonal) and between years.

VII. Structural stability of soil aggregates

Structural stability was analysed twice: in October 2004 and in May 2005, with an interest to get information about irrigation impact on structural stability and seasonal changing of this parameter. At the first sampling date, irrigated and non-irrigated rows at all three positions of the slope (upslope, midslope and downslope) and at three depths (0-5, 5-15 and 15-30 cm) were studied (Tab. 3.25). Water stable aggregates (WSA) were measured after 2 and 6 hours of wetting. In May 2005, water stable aggregates were measured after 2 hours only and at two depths (0-5 and 5-15 cm) (Tab. 3.26).
Indeed, a structural test was not performed on a large time scale till 12 to 20 hours afterwards, due to a large number of samples and due to suspecting differences in aggregating mode and not in textural differences (i.e. same type of soil).

VII.1 Variations of structural stability according to seasons

VII.1.1 *October 2004*

Gravimetric water content (W) at the time of sampling was performed systematically on each sample to take into account its influence on structural stability. W varied between 0.18 to 0.28 $g.g^{-1}$ from upslope to downslope. Slightly higher gravimetric water content was obtained in the surface soil layer and no significant differences were observed between IRR and NIR rows.

WSA varied between 5 and 72 % after 2 hours and between 3 and 44 % after 6 hours, displaying wide range of variation. Large differences in WSA were noticed at midslope position.

Tab. 3.25: Water stable aggregates (WSA) and gravimetric water content at sampling time (W) in October 2004

		UPSLOPE		MIDSLOPE		DOWNSLOPE	
Parameter	Depth	IRR	NIR	IRR	NIR	IRR	NIR
				October 2004			
WSA 2h %	0-5 cm	43±2	53±2	5±1	24±3	7±1	20±1
	5-15 cm	57±4	36±1	16±3	29±4	24±5	19±2
	15-30 cm	48±2	38±3	20±2	72±17*	27±3	20±1
WSA 6h %	0-5 cm	32±2	44±6	3±1	14±1	5±2	16±2
	5-15 cm	44±6	24±1	9±2	9±2	11±1	10±1
	15-30 cm	32±5	30±2	6±2	9±2	17±2	14±11*
W ($g.g^{-1}$)	0-5 cm	0.21	0.21	0.28	0.25	0.29	0.28
	5-15 cm	0.18	0.22	0.25	0.25	0.22	0.25
	15-30 cm	0.21	0.22	0.25	0.26	0.23	0.24

*(it could be an error at measuring-see also encircled point in Fig. III.26)

After 2 hours, higher structural stability was mostly found in the deepest soil layer with WSA values (39±19 % in average) statistically different (p=0.05) from the surface layers (25±18 %). Structural stability generally decreased from upslope to downslope, with two main patterns (Fig. 3.44a). The first pattern concerned IRR row and the surface layer of NIR row, where WSA upslope significantly differed from the two lower positions. The second pattern concerned NIR row in deeper layers. This showed high values of structural stability at upslope position, as well as at midslope position, while the lowest values were observed downslope. Generally, the highest structural stability appeared upslope (WSA=46±9 %) and it

was significantly different from the two lower positions (WSA=29±24 % midslope and 21±8 % downslope). The most important observation is that IRR row had significantly lower structural stability (WSA=26 ±18 %) than NIR row (WSA=37±8 %) (Fig. 3.45).

Structural stability significantly decreased with duration of wetting. After 6 hours, WSA percentage generally decreased, with an average WSA of 32±18 % after 2h and 18±13 % after 6h (Fig. 3.44b). The effect of slope position still remained but there was neither the effect of depth nor irrigation status present (Fig. 3.44a & b, Fig. 3.45).

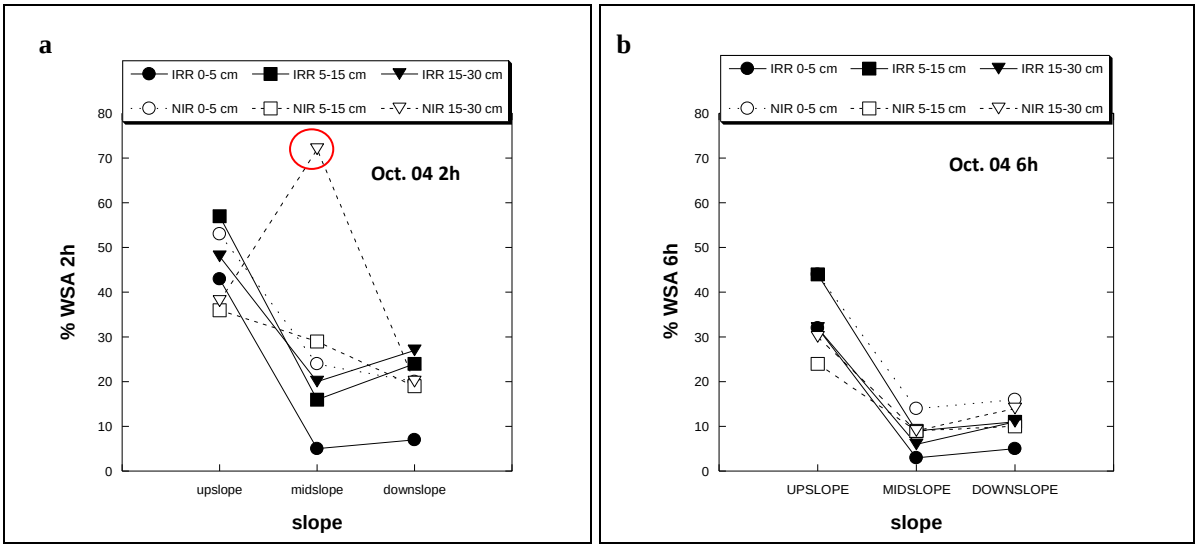


Fig. 3.44: Water stable aggregates (WSA) in October 2004 after 2 hours (a) and after 6 hours (b).

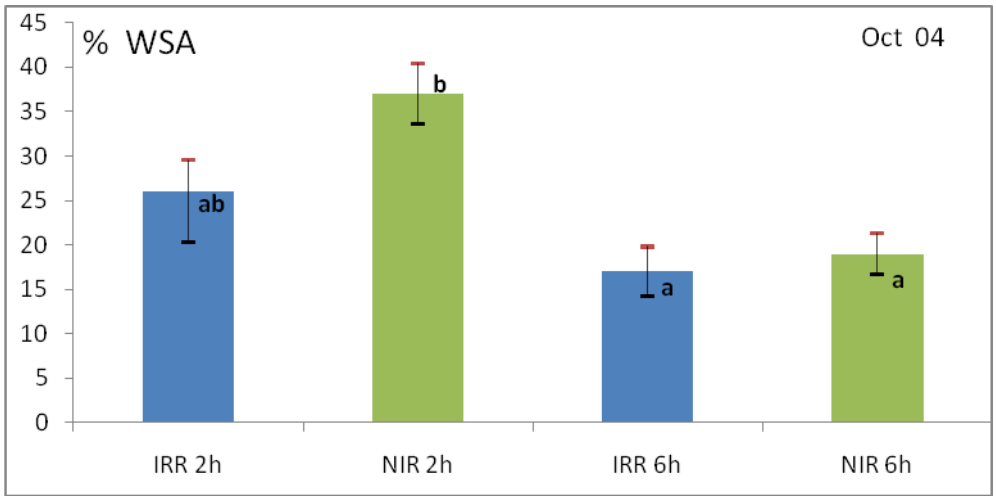


Fig. 3.45: Comparison of water stable aggregates (WSA) after 2 and 6 hours in Gačnik, October 2004.

VII.1.2 *May 2005*

From the results obtained in October 2004, structural stability in May 2005 was measured only after 2 hours of wetting, to study the effects of irrigation, slope, and depth. An additional criterion was chosen: sun exposure. In fact, depending on the position of the field to sunrise, a

side effect could be relevant to tree planting system. Persistent cover of green algae on soil surface on the shady side was observed in the field. The discussion will present the 3 criteria (irrigation, slope and depth) and will also take in account the presumed effect of sun exposure. For this sampling date, only two soil depths (0-5 and 5-15 cm) were included in our study.

Tab. 3.26: Water stable aggregates (WSA) and gravimetric water content (W) at sampling time , May 2005.

Water treatment	IRR				NIR			
	May 2005							
Soil depth (cm)	0-5 cm		5-15 cm		0-5 cm		5-15 cm	
Sun exposure	sunny	shady	sunny	shady	sunny	shady	sunny	shady
	WSA %							
UPSLOPE	11±0	27±2	54±1	54±1	30±2	20±1	57±12*	39±4
MIDSLOPE	35±2	48±2	33±9	54±3	23±1	54±3	57±7	37±4
DOWNSLOPE	27±1	30±2	44±8	31±2	39±1	32±2	29±2	50±8
	W ($g.g^{-1}$)							
UPSLOPE	0.07	0.16	0.20	0.25	0.17	0.17	0.22	0.26
MIDSLOPE	0.20	0.20	0.29	0.31	0.12	0.21	0.29	0.32
DOWNSLOPE	0.17	0.19	0.26	0.30	0.19	0.21	0.31	0.29

*(it could be an error at measuring)

In May 2005, gravimetric water content varied from 0.07 to 0.31 $g.g^{-1}$. The surface soil layer was significantly drier than sub-surface layer (0.17 and 0.28 $g.g^{-1}$ respectively). Evident differences in soil moisture occurred according to slope position: upslope soils were the driest (W = 0.19 $g.g^{-1}$) and differed significantly from midslope and downslope soils (W = 0.24 $g.g^{-1}$). According to treatment, W was similar in IRR and NIR rows (0.22 and 0.23 $g.g^{-1}$respectively). No difference between shady and sunny site of the rows occurred, except in the surface layer, where W in the shady surface layer is higher than in the sunny surface layer (0.35 and 0.28 $g.g^{-1}$ respectively). Comparing the two sampling dates down to 15 cm depth, W was more or less the same in spring than in autumn (0.22 and 0.24 $g.g^{-1}$ respectively).

WSA varied between 11 and 57 %, which represents a wide range of variation. WSA increased significantly according to depth (31 and 44 % respectively). At midslope, WSA was higher (43 %) than at upslope (37 %) or at downslope (35 %), but this difference was not statistically significant. At upslope, very high and very low values were found, yielding a larger range of variation than at midslope and downslope positions. According to irrigation, there were no significant differences in mean WSA values (37 % in IRR and 39 % in NIR rows). The effect of sun exposure was noticed when considering only the surface soil layer (0-5 cm): WSA was higher on the shady than in the sunny side (35 and 28% respectively). However, it seems that both sun exposure and irrigation regime affect WSA: on the shady side, the effect of irrigation is less pronounced than on the sunny side (Fig. 3.46a & b).

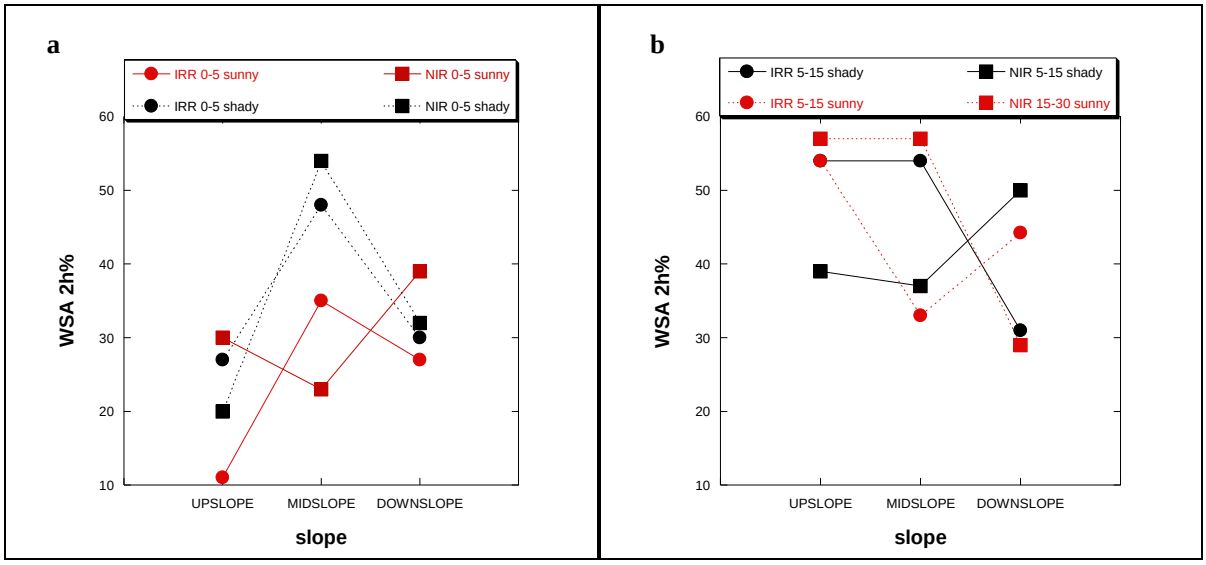


Fig. 3.46a & b: Comparison of water stable aggregates (WSA) according to sun exposure and irrigation (IRR, NIR) after 2 hours in 0-5 cm layer (a) and in 5-15 cm layer (b) in Gačnik, May 2005.

Comparing two sampling dates, structural stability of soil aggregates was a bit higher in spring (37±16 % WSA in May 2005), compared to autumn (28 ±16 % WSA in October 2004).

VII.2 Discussion

Aggregation and aggregate stability are the result of an interaction of many factors including weather environment, soil management factors, plant influences and soil properties such as mineral composition, texture, soil organic carbon, pedogenetic processes, microbial activities, exchangeable ions, nutrient reserves, carbonates and moisture availability (Bronick and Lal, 2005). Climate and landscape position influence soil structure through factors such as temperature, precipitation, elevation, slope gradient, and aspect (Bronick and Lal, 2005). In the first place, soil structural stability is related to soil texture, especially to clay content (Gollany *et al.*, 1991). Another strong positive correlation was found between structural stability and soil organic matter (Perfect *et al.*, 1990; Goulet *et al.*, 2004).

Topography was a strong soil developing factor in our study. Soils from upslope strongly differed to soil from downslope position. For this reason, certain field observations must be remembered prior to discussing structural stability: bedrock appearance, soil depth, soil colour and cracks observations according to slope. The soil from upslope was shallow (60 cm) compared to soil from downslope (more than 1 m). At upslope, the bedrock (marl) appeared already at 20 cm (as blocks), while at C horizon it appeared at 60 cm and downslope, it was the weathered saprolithe which appeared at 1 m. Differences along the slope occurred in soil colour as well: upslope soil was lighter (5Y 8/3) than downslope (10YR 7/3), suggesting a lower organic matter content at upslope position (probably due to erosion) and which was confirmed by organic carbon analyses.

On the samples from 2004, some correlations were studied between structural stability and the following parameters: Corg, clay and carbonates. In this analysis, complete results (from both water treatment and 3 depths) after two hours of wetting were included. From the figures (Fig.

3.47a & b) it can be seen that there were no significant correlations between WSA and Corg or the percentage of clay. Here it has to be taken into consideration that particle size distribution has been performed without decarbonation which means a part of the clay content is here represented by particles of $CaCO_3$ and not by mineralogical clay and therefore does not play the same physical bonding role as mineralogical clays.

In Fig. 3.47c there are obviously two groups of soils according to WSA and $CaCO_3$: one group at lower slope position and another at upslope. From different authors, it can be stated that: (a) topography by itself does not significantly affect macroaggregate stability (Cantón *et al*. 2009) but (b) higher SOM and clay content at downslope positions is reflected by higher aggregate stability (Bricchi *et al*., 2004; Eneje and Adanma, 2007, Le Bissonnais *et al*., 2002). According to topography, sloping soils are more susceptible to erosion, particularly in regions of intensive rainfall. Erosion tends to preferentially remove low density or light particles including clay and SOC that are two of the primary bonding agents in aggregation (Bronick and Lal, 2005). This was not the case in our study, where structural stability at downslope position was lower despite higher SOM content.

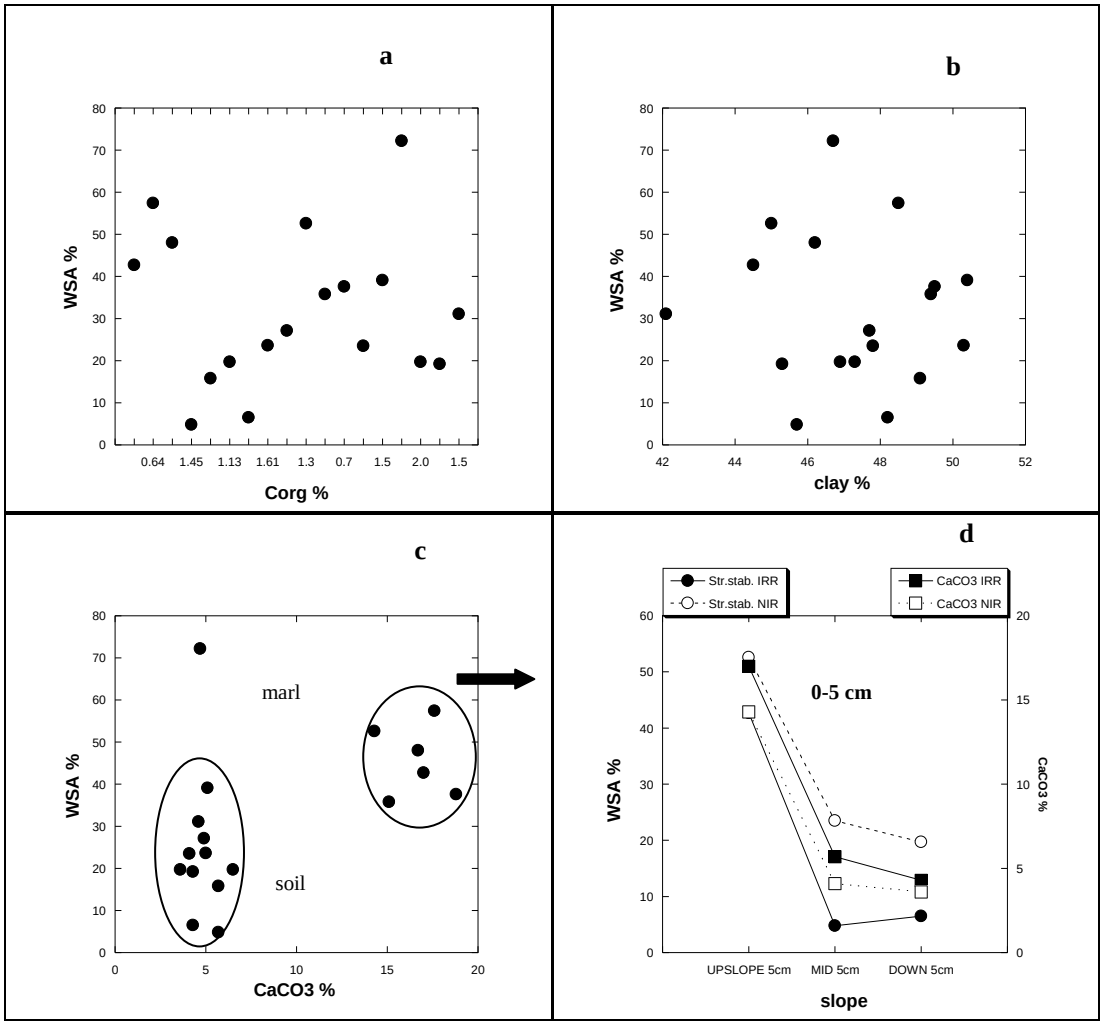


Fig. 3.47a, b, c & d: Correlations between water stable aggregates (WSA) and Corg (a), clay content (b) and total carbonates (c) from 0-30 cm; distribution of WSA and carbonates according the slope in the surface soil layer (d), 2004.

According to several authors (Bronick and Lal, 2005; Cantón *et al*., 2009), structural stability is in positive correlation with soil organic matter content. When observing relationships between structural stability and soil depth, a similar conclusion (as in the slope) about organic matter was made. Structural stability increased with soil depth (especially in IRR soils); even less SOM was present. In the literature, high structural stability in the surface soil layer was generally related to higher SOM content (Perfect *et al*., 1990). However, increases in water stable aggregates are not always related to increases in SOM of a silt loam, which led Perfect *et al.*, (1990) to suggest that some components of SOM are more actively involved in soil aggregate stabilization than others. Another possible explanation for higher structural stability in deeper layer is SOM protection by $CaCO_3$. There was a statistically significant relationship (at the 95% confidence level) between WSA and $CaCO_3$ in the surface soil layer. The R-Squared statistics indicate that the fitted model explains 70.5 % of the variability in WSA. In the surface soil layer, the equation of the linear fitted model to describe the relationship between WSA and $CaCO_3$ is the following with a correlation coefficient equals 0.84:

$$\%WSA = 2.6 + 2.7 * \%CaCO_3.$$

At low SOC concentration, macroaggregate stability is enhanced by carbonates (Boix-Fayos *et al*., 2001). High carbonate concentration enhances SOC protection, probably through decreasing SOC mineralisation and increased Ca^{2+} (Clough and Skjemstad, 2000). Upslope carbonate content was three times higher than downslope (16% versus 5%) (Fig. 3.47d), which could explain higher structural stability there. On steeper terrain, soil water content is lower, allowing a higher structural stability. This phenomenon is explained by the chemical precipitation of slightly soluble bonding agents at contacts points between soil particles (Kemper and Rosenau, 1984). These bonding agents are gypsum, silica, or carbonates of calcium and magnesium (Harris e*t al*., 1966). The precipitation of bonding agents at particle contact points as a consequence of drying is occurring in soils throughout the season, especially at upslope position.

Comparing water treatments, structural stability is in positive correlation with soil organic matter content and explains the difference in WSA between IRR and NIR. Structural stability evidently differed between IRR and NIR rows in the surface layer (WSA = 16 versus 28 %) which was probably a consequence of drip irrigation. Strong irrigation effect on structural stability was observed in October 2004 and it should be emphasized that in the season 2004, 30 days of irrigation was done (60 mm of added water), while results from spring 2005 were not related to irrigation. Lower SOM in IRR row soil could be explained by SOM mineralisation: IRR soils probably have more favourable conditions for microbial decay of SOM (lower C/N) and thus have lower SOM content at the time of sampling than NIR row. SOM input in IRR soils could be higher before sampling, but further microbial actions due to better soil conditions could change this initial situation. These results will be explained later with microbial biomass.

Structural stability is not a stable parameter, and several authors reported results of temporal changes in aggregate structural stability (Brown *et al*, 1995; Lehrsch and Jolley, 1992; Perfect *et al*., 1990; Yang end Wander, 1998). It is now well known that aggregate stability varies for some soils over a growing season (Ellsworth et al., 1991; Perfect *et al*., 1990) or in longer periods (Bullock *et al*., 1988). The majority of these studies have focused on the growing season (Brown *et al*, 1995; Lehrsch and Jolley, 1992; Perfect *et al*., 1990; Yang end Wander, 1998), although some information is also available on the influence of winter due to freezing and thawing processes (Bullock *et al.*, 1988; Coote *et al*., 1988).

For this reason, a comparison in WSA between the beginning and the end of the growing season was done in our experiment. Comparing two studied sampling dates, the trend of structural stability was not the same. Higher WSA values were measured in May than in October. The pattern of structural stability according to depths were the same at autumn sampling, while in spring two soil layers showed differed trends: the surface layer had higher WSA at midslope , while deeper layer showed strong variability in the slope. This statement is in agreement with Perfect *et al*. (1990) which reported a decrease of structural stability during the growing season although some authors reported different patterns of temporal variation in structural stability: Brown *et al*. (1995) measured low structural stability in early spring, with a maximum in early summer. Lehrsch and Brown (1995) observed the maximum structural stability at the end of fall.

Many previous investigators pointed out the strong influence of soil water content at the time of sampling: in general, aggregate stability decreases with increasing water content. In our experiment, this pattern was found along the slope: downslope more humid conditions resulted in lower structural stability. Furthermore, moisture at sampling dates was in different correlation with structural stability: in autumn 2004 (Fig. 3.48a), low WSA percentage in humid soil conditions corresponds to significant negative correlation between W and WSA ($r = -0.62$ after 2 hours and $r = -0.60$ after 6 hours at $p<0.05$); while in spring 2005 (Fig. 3.48b), a positive correlation between W and WSA ($r = +0.55$ at $p<0.05$) occurred. The first relationship is in agreement with Perfect *et al*. (1990) and Coote *et al*. (1988), who found a significant negative correlation between soil moisture and WSA. Chan *et al*. (1994) reached a similar conclusion and asserted that seasonal variation in soil aggregate properties were significantly influenced by soil water content at the time of sampling. The second relationship explained that soil moisture content alone has no consistent effect on soil aggregate stability while soil moisture interacted with other factors to influence aggregation (Yang and Wander, 1998). The parameter of soil water content is not necessarily directly linked with water conditions in the sampling period, but can be masked with some other processes in soil (positive correlation between WSA and W might be only artificial, because it can be also affected by algae, not only by water content by itself). For this reason, gravimetric water content at the time of sampling was not a pertinent parameter for explaining structural stability. Anyway, the measure was done on air dried soils.

Changes of structural stability (WSA) may vary between years due to different climatic conditions: rainfall, temperature or frost occurrence. Variability of seasonal changing is mostly driven from rainfall or with soil water content (Yang and Wander, 1998). When relatively dry aggregates wet quickly by the advancing furrow stream, they are unstable (Kemper *et al*., 1984) and, upon disintegration, likely contribute to the high sediment loss rates. In our experimental location, heavy rains are typical and could contribute to lower structural stability in the surface layer with possible surface crusting (Le Bissonnais and Arrouays, 1997).
In our study, winter 2004/05 was dry (280 mm rainfall from Nov. 2004 till May 2005) compared to the 30-year average (465 mm). This data could be also important for explaining higher structural stability in spring. Unger (1991) pointed out the importance of soil physical properties over a relatively cold but dry winter, while over a wetter winter he detected a decrease of structural stability. The freezing and thawing process may be responsible for the changes in aggregate stability occurring during winter (Chan *et al*., 1994).
Weather conditions probably influenced marl behaviour and as a consequence higher structural stability at midslope position (in spring 2005). Differences in structural stability according to slope between October 2004 and May 2005 were presumably related to special

behaviour of bedrock during the year (temperature, soil moisture). In dry conditions, more vertical cracks appeared and structural stability was stronger compared to wet conditions.

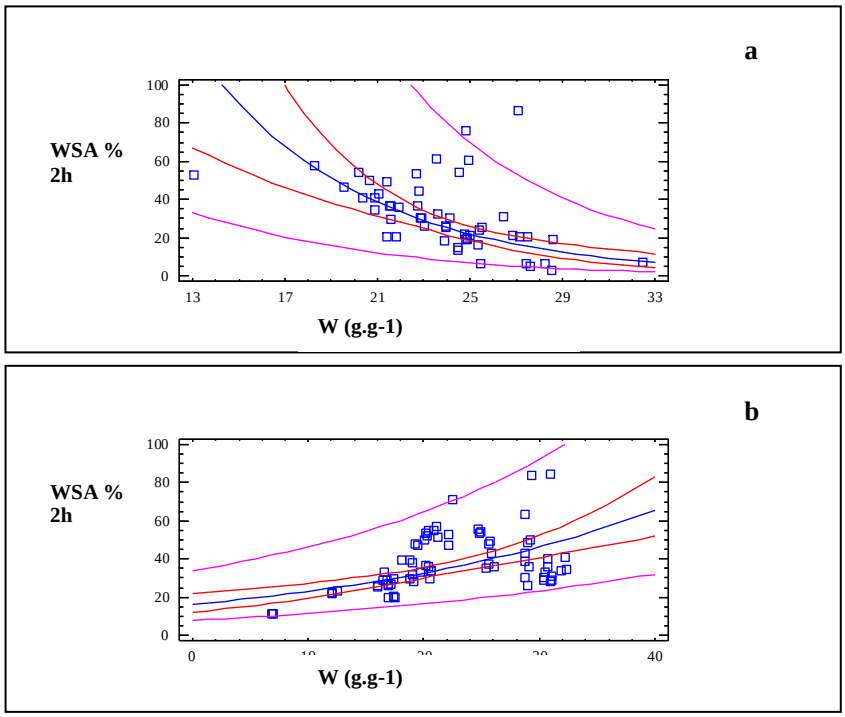


Fig. 3.48a & b: Correlations between gravimetric water content (W) and structural stability (WSA) in October 2004 (a) and in May 2005 (b).

Some conclusions from both sampling date were confirmed for our study site:

1. Large range of variation of the structural stability was noticed in space (at the slope scale) and in time (according to water conditions during the season).
2. Structural stability was positively correlated with soil calcium carbonates content.
3. Structural stability was not always positively correlated with soil organic matter content.
4. Water content at the sampling date was not a pertinent parameter (and justifies the normalised methods working on air dried soils).
5. According to sun exposure, structural stability in the surface layer was higher at shady side of the rows (which could explain positive relation with soil moisture in May 2005).

VIII. Discussion and conclusions - Gačnik site

The study field in Gačnik was very heterogeneous due to rough human intervention to soil reorganisation and topography. For this reason, the results were sometimes masked or not as representative as expected in an ideal situation. Anyway, our study was a good opportunity to learn how to study soil behaviour in the field according to irrigation and how to approach the real situation in practise.
Regarding the hilly terrain and land levelling, we were dealing with two groups of soil: one at upslope and another one at mid and downslope. Considering soil characteristics, slope effect was more expressed than irrigation effect.
Lower gravimetric water content in irrigated row could be explained by the modification in root distribution and development due to drip irrigation. According to Isbérie (1995), drip irrigation may favour a specific distribution of the root system, more superficial around the dripper. This distribution may favour drier superficial soil after irrigation due to root system water extraction. Moreover, this drier water regime may favour higher porosity in these loamy clay soils. The influence of drip irrigation on root system distribution may be rapid, especially in young plantation.

In our study, irrigation differently affected soil organic matter considering time periods. Supplementary drip irrigation increased microbial biomass and its activity (short time effect) but gently decreased total SOM content and structural stability (long time effect). Indeed mineralization processes, as shown by lower C/N ratio of SOM and higher respiration (Cmin), are more intensive in IRR soil, with lower remaining SOM in this treatment. Previous statements were supported by several authors. Kochsiek *et al.* (2009) and Mandal *et al*. (2008) reported that irrigated management regimes not only led to greater litter-C inputs but also greater decomposition rates. According to grain size fraction, only fraction A showed significant difference in Corg between IRR and NIR rows in favour of irrigation. Fraction A is an important indicator of the SOM quality, which was in agreement with Christensen (2001). This could explain lower organic matter content in irrigated row. Because mineralization is performed by the microbial biomass, it is often assumed that this process is at least partly regulated by its size, specific activity or composition (Marschner and Kalbitz, 2003; Fontaine and Barot, 2005). Contents of total and labile organic matter and microbial biomass are significantly affected by different irrigation regimes, while drip irrigation increases microbial biomass (Han *et al.*, 2010; Melester, 2010; Samuelson *et al.*, 2009; Entry *et al*, 2008 and Martiniello, 2007). Management practices of irrigated land, including yearly organic fertilizer application or (indirectly) through grass in inter-rows, result in the addition of young, labile OM to irrigated soil which contributes to the mineralization of organic N. The labile OM acts as a substrate for microorganisms and is readily decomposed, turning over large amounts of N. However, the total organic matter content of the irrigated treatment did not increase. Constant OM levels, increased mineralization, relatively high NO_3-N levels, and increased uptake of available N in the irrigated treatment stress the importance of OM quality. The rapid cycling of OM in irrigated soils results in increased plant uptake of readily available nutrients and greater soil fertility (Henry and Hogg, 2003).

Organic matter reduction is, in turn, associated with the soil structure degradation (Albiach *et al.*, 2001; Mandal *et al.*, 2008), which was also found in our study. Improvement of soil structure through organic matter input has been found to be of primary importance in the type of soil on which the study was conducted. Soil structure had high susceptibility to degradation by the action of water or tillage despite the fact that these soils have more than 50% total porosity (Gomez *et al.*, 2001). Irrigation results in aggregate destabilization either through

rapid organic matter (OM) breakdown and mineralization, or continuous years of water-drop impact. In our study, the results of soil structural stability according to irrigation confirmed the previous statements.

Among chemical properties of irrigation water, only the amount of nitrates was high, which probably contributed to higher mineral nitrogen values in IRR soils. The significant difference of NO_3-N levels in IRR row may be attributed to greater mineralization of N in irrigated land than dryland (Henry and Hogg, 2003).
Before explaining structural stability results according to slope and depth, emphasis needs to be placed on decreasing of soil organic matter and increasing of carbonates towards upslope and with depth. As mentioned before, high structural stability is related to the organic matter content (Perfect *et al*., 1990). However, in our study, SOM was negatively related (r = - 0.89) to structural stability according to slope and depth (Fig. 3.49).

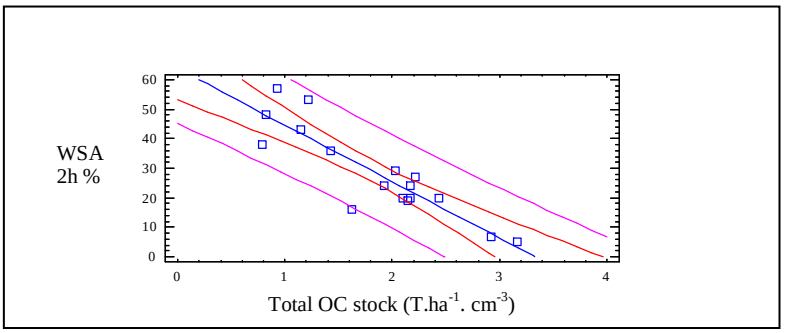


Fig. 3.49: Correlation between structural stability (WSA) and total OC stock ($T.ha^{-1}.cm^{-1}$).
*Data of WSA from 15-30 cm in NIR row at midslope position (72 %) was excluded from the correlation due to strong standard deviation.

This was explained by Perfect *et al.*, (1990), who suggested that some components of organic carbon pool are more actively involved in stabilizing aggregates than others and total organic carbon is not necessary positively linked with WSA. A specific fraction of the organic pool may be the main stabilizing agent (Roldan *et al.*, 2003), and therefore the measurement of total organic carbon content may not be sufficiently discriminating (Janzen *et al*., 1992). In our study we were dealing with different pools of organic matter: (a) young organic matter from trees and microbial biomass and (b) sedimentary organic matter from the weathering of the marl bedrock. To accertain the respective role of these two fractions, correlations between structural stability and fresh or sedimentary OC stock was studied. Clear negative correlation between fresh OC and WSA (Fig. 3.50a) and no relation between WSA and sedimentary OC (Fig. 3.50b) were found. Therefore, we can assume that sedimentary OC didn't have an effect on structural stability.

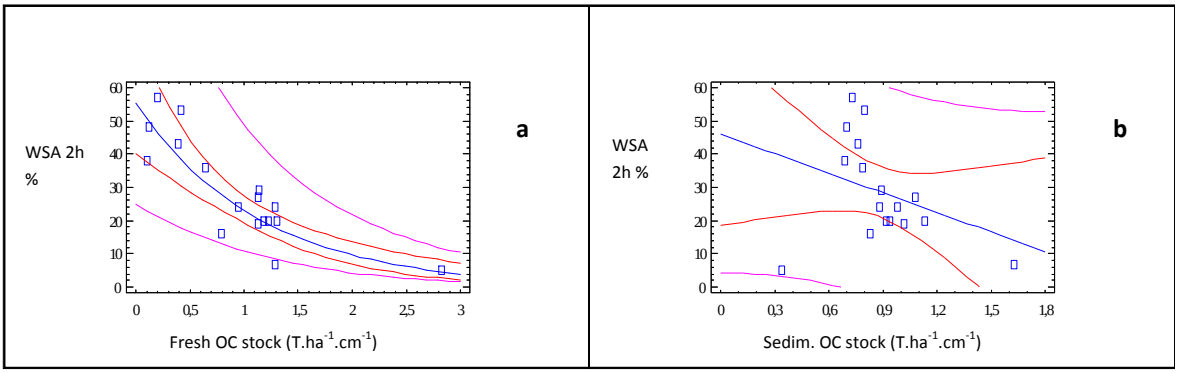


Fig. 3.50a & b: Correlation between structural stability (WSA) and fresh OC (a) or sedimentary OC stock ($T.ha^{-1}.cm^{-1}$).
*Data of WSA from 15-30 cm in NIR row at midslope position (72 %) was excluded from the correlation due to very high standard deviation.

But anyway, fresh OC still displayed an even more negative correlation to structural stability which remains unexplained. The answer may lie in the positive correlation between WSA and carbonates, even if it was not very strong (Fig. 3.51).
High percentage of total carbonates in soil could also contribute to higher structural stability through protecting SOM from mineralization. It is generally accepted that calcium is a critical element for stabilization of SOM and aggregates (Six *et al*., 2004), inducing higher structural stability in soil samples with high carbonate content. Soils from our study had a great potential to contain high content of protected SOM due to a large proportion of fine particles: 45-50% clay and 25-35% fine silt. A positive correlation between WSA and $CaCO_3$ (r = +0.52) was determined (Fig. 3.51).

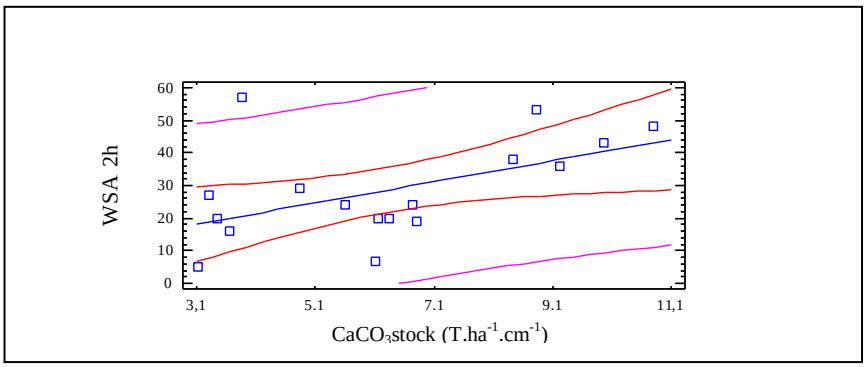


Fig. 3.51: Correlation between structural stability and total carbonates ($CaCO_3$) stock ($T.ha^{-1}.cm^{-1}$).

After discussing different types of OC, there is still one hypothesis which can help interpret lower organic matter content in irrigated row. In fraction C, which is the dominant fraction in whole bulk soil, sedimentary OC represented about a half of total organic carbon (Fig. 3.52). When comparing IRR versus NIR sites by pairs, they showed similar sedimentary OC stocks, except downslope for fraction A where the very high sedimentary stock was attributed to a possible presence of sand size particles of marl, and fraction C at midslope and downslope. It could be explained by the higher microbial biomass and activity in irrigated row. Microbes may attack sedimentary organic matter and this could explain lower TOC value there. Mineralisation of sedimentary OC is very important in relation to general cycle of organic

carbon by producing additional CO_2, going to atmosphere. Organic matter, which is entrapped in geological substratum (marl) may be mineralised by irrigation process, which has to be taken into account according to the environmental point of view.
At upslope, the amount of sedimentary OC in both treatments was more or less the same, which gives some doubts about arriving irrigation water to this point.

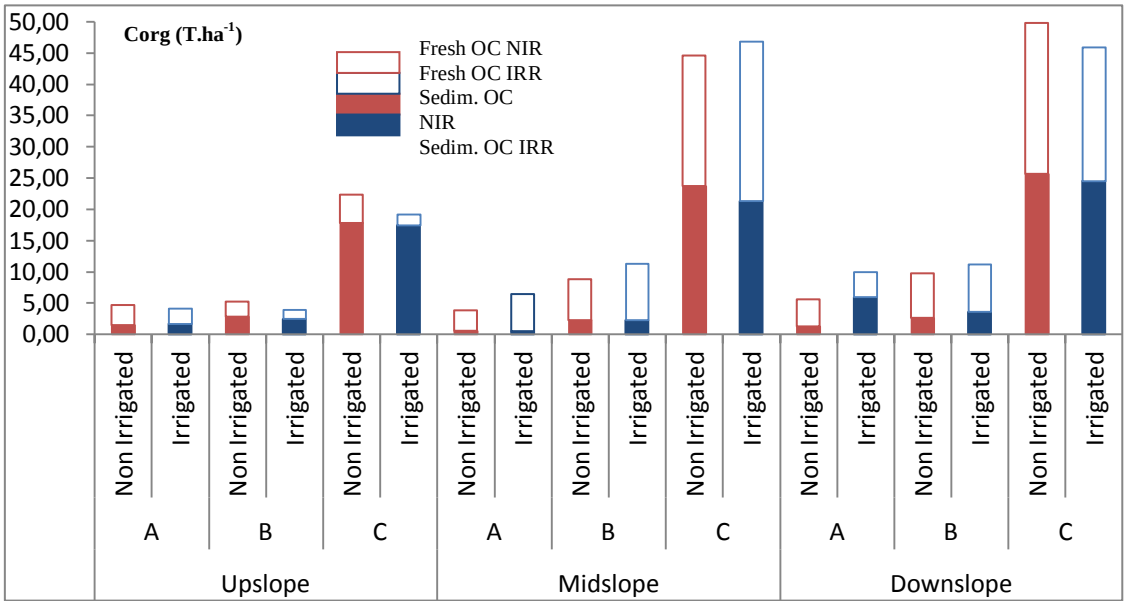


Fig. 3.52: Distribution of fresh (white) and sedimentary (coloured) OC stock ($T.ha^{-1}.cm^{-1}$) according to fraction, slope and treatment.

Soil structural stability is not a constant parameter; it is changing within season and between years. Structural stability variation with moisture content may be due to sampling, physico-chemical, or biological factors (Perfect *et al.*, 1990). For this reason, reliable assessment of structural stability should not rely only on one time measurement. Additional research will be needed to pinpoint the mechanisms of structural stability and its temporal changes according to irrigation. Seasonal variations of structural stability are difficult to explain with soil water content only, however rainfall and microbial biomass information could be helpful. In our study, higher structural stability was measured in spring 2005 than in autumn 2004. An explanation for this result might be the dry winter 2004/2005, as previously observed by Unger (1991). Another explanation of higher structural stability in spring 2005 is the higher microbial biomass in this period. In the surface soil layer, a strong positive correlation between WSA and soil moisture was found (r=0.93 with double reciprocal model: $WSA = (-0{,}0035 + 0{,}64/humidity)^{-1}$), which could probably be explained by the presence of green algae on the shady side, where WSA and soil moisture were higher. Contribution of micro-organisms to soil aggregate stability is a well-known phenomenon. Microbial biomass has been reported as responsible for soil aggregation (Gupta and Germida, 1988), but relationships between soil microbial biomass and aggregate stability are not very consistent and tend to be mainly site-specific (Carter *et al.*, 1999). Two major mechanisms are suggested by Lynch and Bragg (1985): the ability of some micro-organisms, mostly filamentous, to mechanically bind soil particles, and the production of binding agents by some others (bacteria). In general, fungi are reported to be the most effective in soil aggregation (Lynch and Bragg, 1985).

In the end, some doubts still remain about irrigation practice: a) if enough irrigation in one season was done to measure its actual (short-term) impact; b) if enough seasons of irrigation was performed to study long-term effect of irrigation; c) if irrigation was really needed in this type of soil; d) if irrigation water really reached upslope in the same amount as downslope; e) if sampling was reliable to compare irrigated versus non-irrigated soils?
In 2004, only 30 days of irrigation were performed, which was more than twice less than one season before (70 days). The irrigation effect we measured in our study was the consequence of only a few previous irrigation seasons. Before 2004, 6 years of irrigation were performed, which could participate to changes in soil properties. This time period is long enough to modify the root system of young plantation and therefore modify water regime at soil surface and keep a long term effect even without irrigation.

According to the soil texture in our study, irrigation is not the most recommended practice. Such soils are susceptible to structural damage, if they are intensively cultivated (Kostopoulou and Zotos, 2005). The fine silt fraction could contribute soil sealing. A good interpretation of irrigation effects should include additional information about the changes in porosity redistribution and the break of water circulation channels in the soil profile (Ramoz *et al*., 2007). Regarding significantly lower soil humidity at upslope, it might have happened that in irrigation pipes there was not enough pressure to push water to the top of the hill. For a reliable study, it could be useful to compare the treatments (IRR versus NIR) on several rows and not on only one row for each treatment. In our case, the situation has been already settled in manner way for six years and used by us as a preliminary study.

Conclusions

1. Soils along the slope were very heterogeneous due to land and soil reorganisation.
2. Slope effect had much stronger effect on soil quality than irrigation. According to slope, we were dealing with two groups of soil: one at upslope and another one at mid and downslope.
3. Supplementary drip irrigation gently decreased total SOM content and structural stability (long time effect), but increased microbial biomass and its activity (short time effect).
4. A high percentage of total carbonates in soil contributes to higher structural stability.
5. Higher structural stability is not always explained by higher total organic matter content, which was found according to slope and depth.
6. Information from isotopic composition of carbon (^{13}C) can be a successful tool for distinguishing different pools of organic matter, namely fresh and sedimentary organic matter.
7. Structural stability is not a constant parameter; it is changing within season and between years. For this reason, a serious assessment in structural stability should not rely only on one time measurement.
8. For improving structural stability in irrigated soils, adding organic fertilizers probably would be helpful.
9. Basic chemical parameters in irrigation water should be regularly monitored to avoid structural breakdown and soil pollution with nitrates.

Chapter 4

Results in Pohorski dvor

Chapter 4: Results in Pohorski Dvor

The study in Pohorski dvor is dealing with the possibility of enhancing microbial biomass and nitrogen nutrition with different combinations of organic fertiliser and liming. Among different applied organic fertilisers, Compo guano was chosen as a reference fertiliser due to its high nitrogen content (11% N).

For a better understanding the results of our study, it is very important to describe morphological properties of soils first and represent soils in general. Two pits were dug and soil morphology in the inter-row space in blocks I (laying at downslope) and III was described for this purpose (Tab. 4.1).

I. Soil morphology

For a simple characterisation of soil properties in the study area, two soil pits were dug in inter-row space in blocks I and III (where tensiometers were settled). A simple description of some morphologic soil properties are presented in Table 4.1. There was rather important soil heterogeneity in this orchard in relation to the shape of the slope. An obvious difference occurred between block I and block III. Compare to block III, soil from block I was more humid, loam texture was observed till deeper layer, fine granular structure was observed in the shallower part of the soil, consistence became compact from 10 cm of depth onwards and the colour had the appearance of hydromorphic features from 50 cm. In block III, soil colour indicated more SOM and a lot of weathering material were noticed in the soil profile (mica from schist).

Tab. 4.1: Comparison of soil morphological properties between block I and block III, Pohorski dvor 2005.

	0-30 cm		**30-60 cm**		**60-90 cm**	
	bl I	**bl III**	**bl I**	**bl III**	**bl I**	**bl III**
Humidity	dry to fresh	dry	fresh	Dry	wet	fresh
Texture	loam	loam	30-40 cm: loam, below silt loam	silt loam	silty loam	silt loam
Structure	0-8 cm: fine crumble structure, then subangular polyedric and polyedric	0-30 cm: fine crumble structure, then subangular polyedric and polyedric	mixture of subangular polyedric and polyedric	Subangular polyedric till 40 cm, then polyedric	polyedric	polyedric
Consistence	0-10 cm: fragile, crumbly, after more compact	fragile, crumbly	compact	Fragile	compact	compact
Color	10 YR3/3 and 10 YR4/3	10 YR4/4	30-40 cm: 10 YR3/3, after 10 YR5/8 and 10 YR5/2	10 YR4/6	10 YR5/2	10 YR5/8
Hydromorphic features	no	no	from 50 cm	No	from 60-80 cm: very explicitly	no

II. Physico-chemical soil characteristics

II.1 General soil characteristics

At the beginning of the experiment, some preliminary soil analyses were done. Samples were taken after the first treatment of fertilizing and liming.

II.1.1 *Comparison among blocs*

Physico-chemical soil analyses in Pohorski dvor according to blocks is presented in Tab. 4.2. Texture grade was unique in all blocks (clay loam) till 30 cm of depth, but small differences occurred for clay, silt and loam within this texture grade. Soil from block I contained the lowest percentage of sand (17.5%) and the highest percentage of silt (52.3%). The opposite situation was recorded in block III: the highest percentage of sand (32.8%) and the lowest percentage of silt (40.3%) and clay (26.9%). Block II contained the highest clay content (32.8%). There were no differences in soil texture with depth. Corg, Norg and available phosphorus significantly decreased with depth. Exch. Mg, K and Cu were also higher in upper soil layer.
No differences were detected in initial soil pH (in water and KCl) according to blocks. Soil pH was very low: the grand mean was 5.85 (in water) and 4.83 (in KCl).

Tab. 4.2: Physico-chemical soil analyses at Pohorski dvor in the studied blocks, May 2004.

Block	I		II		III	
Depth (cm)	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**
% **clay**	30.0	29.7	32.9	32.6	27.1	26.7
% **silt**	53.1	51.4	43.8	42.7	40.8	39.7
% **sand**	16.9	18.1	23.3	24.7	32.1	33.6
Corg (gkg^{-1})	21.7	19.3	24.2	21.9	19.8	17.6
Norg (gkg^{-1})	2.02	1.82	2.20	2.00	1.76	1.60
C/N	10.8	10.6	11.0	11.0	11.3	11.0
NO_3^- (mg $N.kg^{-1}$)	1.19	1.10	1.48	1.68	1.14	1.06
P_2O_5 (gkg^{-1})	0.027	0.018	0.033	0.027	0.039	0.032
CEC ($cmolkg^{-1}$)	12.2	12.2	13.5	13.5	9.7	9.4
Ca^{2+} ($cmolkg^{-1}$)	9.5	9.6	10.6	11.1	7.6	7.5
Na^+ ($cmolkg^{-1}$)	0.07	0.06	0.04	0.06	0.03	0.04
Mg^{2+} ($cmolkg^{-1}$)	1.68	1.45	1.71	1.53	1.34	1.11
K^+ ($cmolkg^{-1}$)	0.21	0.12	0.25	0.15	0.31	0.14
Al^{3+} ($cmolkg^{-1}$)	0.07	0.07	0.12	0.14	0.24	0.37
H^+ ($cmolkg^{-1}$)	0.07	0.07	0.08	0.08	0.08	0.12
Cu_{EDTA} ($mgkg^{-1}$)	6.04	4.18	6.94	5.62	6.58	5.25
Mn^{2+} ($mgkg^{-1}$)	0.12	0.17	0.13	0.14	0.18	0.14

Corg and Norg were the highest in block II (23.03 and 2.10 $g.kg^{-1}$, respectively). The lowest Corg and Norg were measured in block III (18.7 and 1.7 $g.kg^{-1}$ respectively), which was not in accordance with previous observations of dark soil colour in this block. In general, soils from this experimental field contain good levels of soil organic matter (grand mean = 20.7 $g.kg^{-1}$ Corg) and a C/N ratio (grand mean = 10.9) indicating a fair microbial activity. Nitrates reached the highest values in block II.
For available phosphorus, significant differences were noticed among the blocks: block I had the lowest value (0.02 $g.kg^{-1}$ P_2O_5) and block III the highest value (0.035 $g.kg^{-1}$ P_2O_5). CEC showed similar differences among blocks as clay content and Corg: the lowest value was measured in block III (9.5 $cmol.kg^{-1}$). Exch. Ca, Na and Mg followed the CEC pattern; K, H and Al reached the highest values in block III. Extractable copper (EDTA) had the lowest value in block I.

II.1.2 *Comparison between treatments*

Physico-chemical soil analyses in Pohorski dvor according to treatments is presented in Tab. 4.3. According to the texture, no strong differences among treatments were noticed till 30 cm of depth. Texture grade was always clay loam. No significant differences in Corg, Norg, C/N, pH (in water and KCl), CEC, Ca^{2+}, Mg^{2+} and Al^{3+} were noticed among treatments. Compo guano contributed to higher available phosphorus (P_2O_5), especially in upper soil layer. Compo guano increased exch. Na and K (in upper soil layer), while lime increased Mg. Values of exchangeable Mg and K were significantly higher in the upper soil layers. Manganese and copper did not show any differences

Tab. 4.3: Physico-chemical soil analyses at Pohorski dvor according to treatments, May 2004.

Treatment	**Pure control (no Compo guano, no lime)**		**No Compo guano, lime**		**Compo guano, no lime**		**Compo guano, lime**	
	5		**10**		**1**		**6**	
Depth (cm)	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**	**0-15**	**15-30**
% **clay**	31.2	30.6	31.2	31.3	29.2	28.4	28.5	28.3
% **silt**	46.6	44.0	44.7	43.7	46.6	46.4	45.8	44.2
% **sand**	22.2	24.2	24.2	24.9	24.2	25.2	25.7	27.5
Corg (gkg^{-1})	22.3	20.6	21.7	19.3	21.7	18.4	21.9	19.4
Norg (gkg^{-1})	2.04	1.91	1.94	1.81	1.97	1.7	2.02	1.80
C/N	10.9	10.8	11.2	11.0	11.0	10.8	10.9	10.8
NO_3^- (mg $N.kg^{-1}$)	1.16	1.31	1.07	1.08	1.23	1.24	1.62	1.50
P_2O_5 (gkg^{-1})	0.028	0.026	0.025	0.023	0.039	0.025	0.040	0.027
CEC ($cmolkg^{-1}$)	12.13	11.95	11.73	11.90	11.7	11.44	11.50	11.42
Ca^{2+} ($cmolkg^{-1}$)	9.42	9.46	9.41	9.57	9.19	9.32	8.88	9.16
Na^+ ($cmolkg^{-1}$)	0.051	0.046	0.030	0.043	0.048	0.069	0.057	0.608
Mg^{2+} ($cmolkg^{-1}$)	1.64	1.45	1.52	1.35	1.62	1.39	1.52	1.25
K^+ ($cmolkg^{-1}$)	0.249	0.134	0.197	0.139	0.314	0.134	0.261	0.130
Al^{3+} ($cmolkg^{-1}$)	0.170	0.259	0.148	0.180	0.104	0.169	0.143	0.166
H^+ ($cmolkg^{-1}$)	0.070	0.133	0.070	0.070	0.070	0.093	0.080	0.060
Cu_{EDTA} ($mgkg^{-1}$)	7.01	5.67	6.12	4.84	6.64	4.62	6.29	4.94
Mn^{2+} ($mgkg^{-1}$)	0.146	0.113	0.174	0.124	0.122	0.140	0.131	0.219

II.2 Soil pH evolution according to treatment and year

For interpreting microbial parameters, soil pH (in water and in KCl) was measured in parallel with microbial analysis (during two seasons).

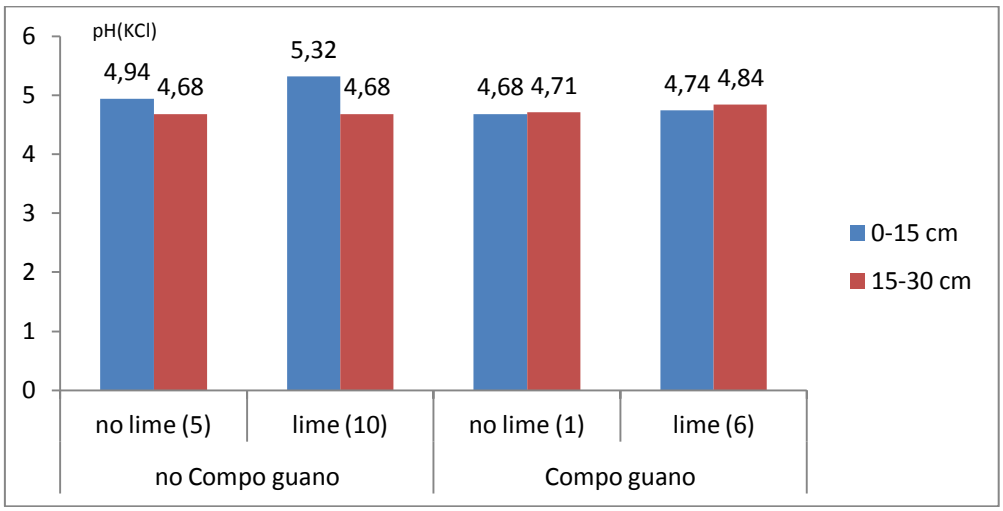


Fig. 4.1: Soil pH_{KCl} at Pohorski dvor, May 2004.

In May 2004, no significant differences in pH_{KCl} occurred according to the treatments (combinations of Compo guano and lime) and according to soil depth (Fig. 4.1)

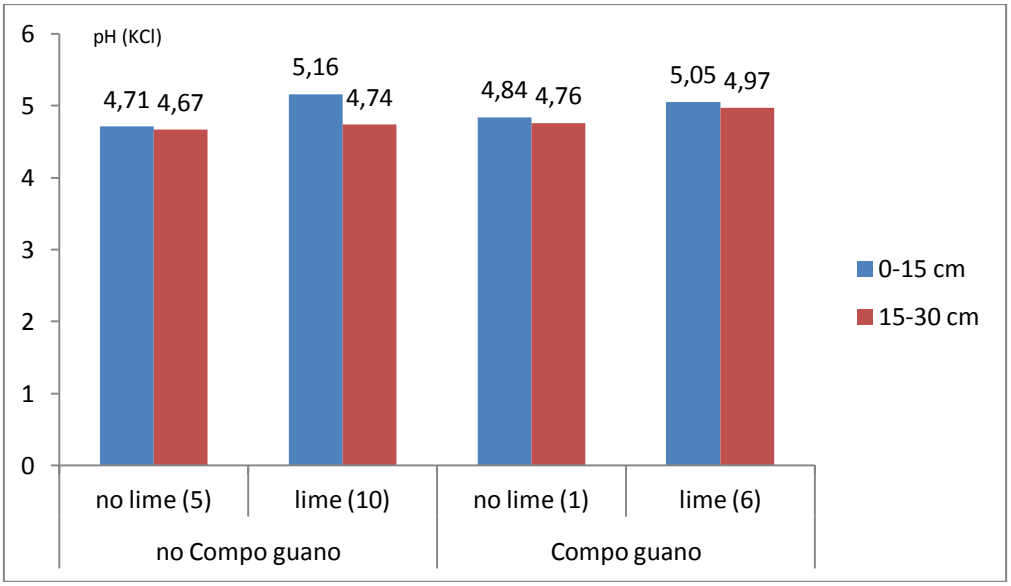


Fig. 4.2: Soil pH_{KCl} at Pohorski dvor, November 2004.

In November 2004, differences occurred between treatment 5 (pure control-no Compo guano, no lime) and 6 (Compo guano + lime): liming increased significantly soil pH (Fig. 4.2). For these two treatments, pH values were quite similar in both soil layers.

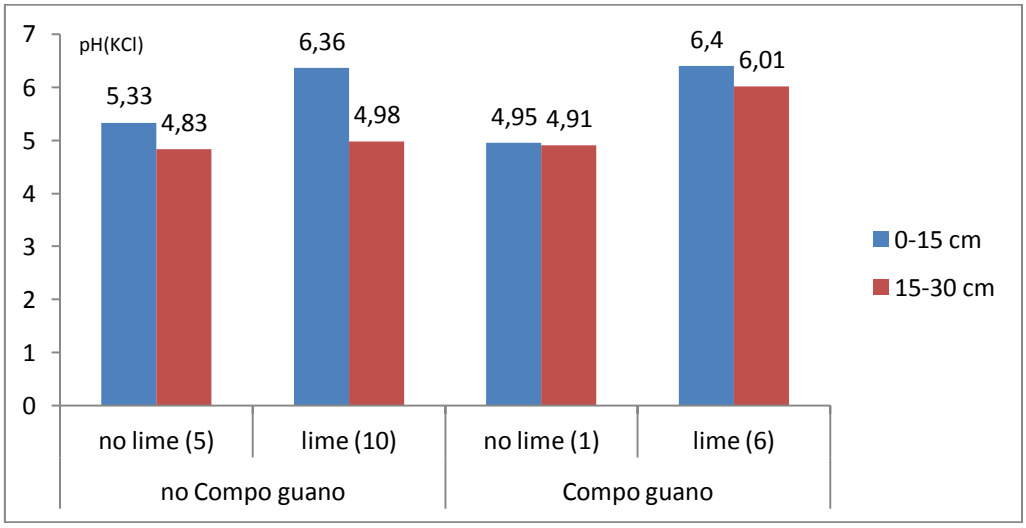


Fig. 4.3: Soil pH_{KCl} at Pohorski dvor, May 2005.

In May 2005, more significant differences were observed: treatment 5 (pure control) and treatment 1 (Compo guano without lime) had similar pH values, but they significantly differed from treatment 10 (lime only) which exhibited a higher pH value in the upper layer (Fig. 4.3). Treatment 6 (Compo guano + lime) significantly differed from all the other treatments with the highest pH value. In general, Compo guano decreased soil pH, while liming increased soil pH.

The upper soil layer showed significantly higher pH values compared to the deeper layer. Changes mostly occurred in the upper soil layer, which was expected because fertilisers and lime were incorporated till this depth.

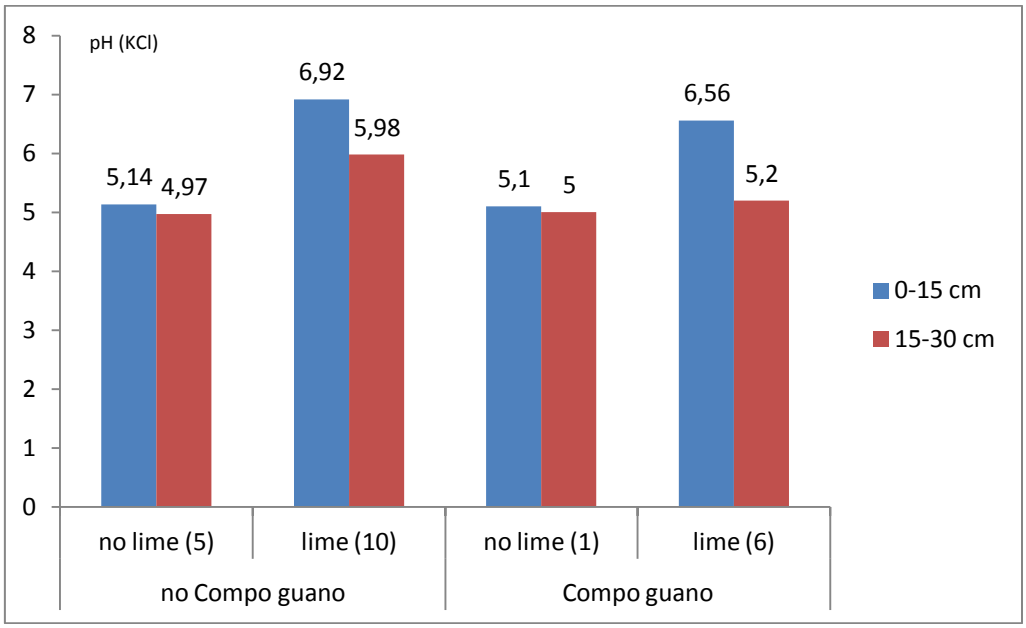


Fig. 4.4: Soil pH_{KCl} at Pohorski dvor, November 2005.

In November 2005, the same pattern occurred as in May 2005: soil pH values increased with liming and decreased with Compo guano application (Fig. 4.4).

II.3 Discussion

The soil of our experimental field was very heterogeneous according to physico-chemical properties. Significant differences occurred mostly between block I and block III or even among all three blocks. Block I had the lowest sand and the highest silt percentage, the lowest P_2O_5 and the highest CEC and pH. Block III had the highest sand and the lowest silt and clay percentage, the lowest Corg, Norg and CEC and the highest P_2O_5. Block II had the highest clay content. A slight decrease of clay and Corg content in direction from block III to block I could probably be explained by erosion process along the slight (5%) slope, block I being settled at downslope position. pH_{KCl} in block I (5.2) was higher than in the two other blocks (4.6 in block II and 4.7 in block III). Even when properly mixed with soil, lime has little effect on pH if the soil is dry.
However, only one application was done before sampling, so it's difficult to explain some differences as a real consequence of organic fertilising and liming in such short period.

pH was measured along both seasons of the experiment, so temporal distribution of this parameter is represented in Fig. 4.5. Treatments with liming slightly increased soil pH (Fig. 4.5).

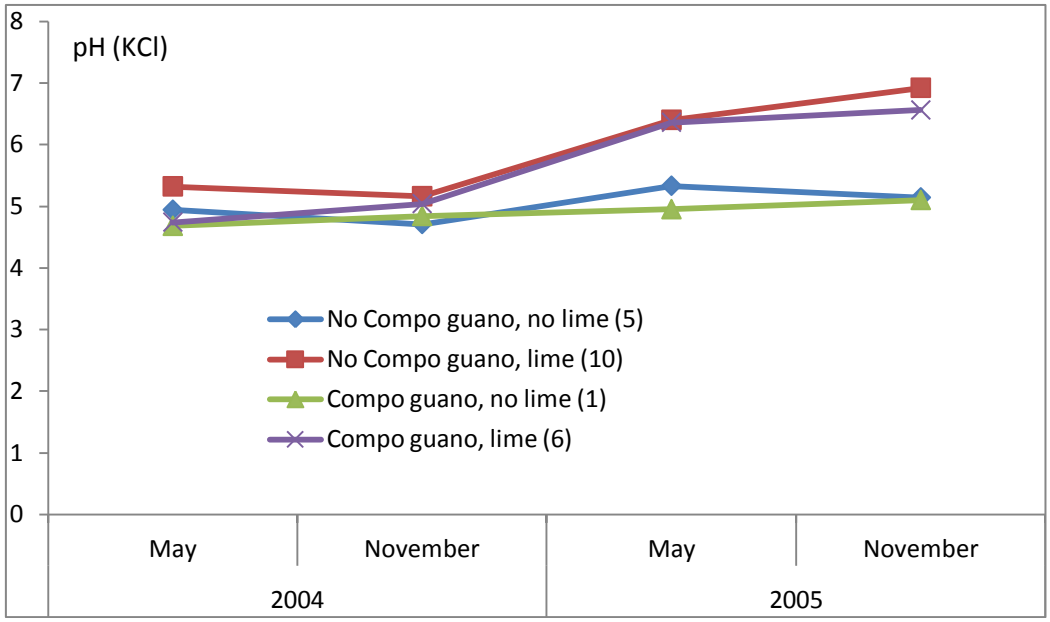


Fig. 4.5: Time distribution of soil pH_{KCl} in upper soil layer (0-15 cm) at Pohorski dvor.

The strongest effect on soil pH was observed for the treatment 10 (lime without Compo guano), for which all pH values increased during the experiment. The immediate reaction between soil and lime lead to an immediate increase in pH of the soil water due to partial dissolution of carbonates (Ola, 1978). However, the completion of the reaction is slow and their effect may show up in days, months, or years (Ola, 1978).The same pattern was observed in the treatment 6 (Compo guano with lime), but the values were somewhat lower. Benefits of compost amendments to soil also include pH stabilization and faster water infiltration rate due to enhanced soil aggregation (Stamatiadis *et al.*, 1999). In the treatments without liming, Compo guano alone induced lower soil pH values comparing to treatments 6 (Compo guano with lime) and 10 (lime only).

III Physical soil characteristics

Soil temperature

Soil temperature was measured at 5 cm of depth at the sampling date. Soil temperatures were more or less the same for all the studied area: 12.5°C (standard error: 0.1°C).

III.1 Soil water characteristics

III.1.1 *Soil water retention curve (SWRC)*

Water retention curve was done in soils from pure control (treatment 5) in two blocks: block I and block III. Both curves were done at 6 pressure points: 33, 100, 200, 300, 500, 1000 and 1500 kPa for three depths separately (20-25, 40-45 and 80-85 cm).

Tab. 4.4: Volumetric water content (θ) at different pressure points in blocks I and III (treatment 5=pure control), at Pohorski dvor 2004.

θ	**Pressure** (kPa)											
	33		**100**		**300**		**500**		**1000**		**1500**	
Depth (cm)/block	I	III	I	III	I	III	I	III	I	III	I	III
20-25	46.8	41.86	34.72	34.33	25.45	24.45	23.31	22.62	19.34	19.47	19.75	17.38
40-45	46.15	46.69	33.66	38.02	23.83	28.43	21.86	26.23	17.19	23.12	16.74	21.79
80-85	58.10	44.92	44.43	39.42	31.80	26.51	28.97	25.18	24.52	21.47	26.75	19.81

θ ($cm3.cm^{-3}$) = volumetric water content

Generally, in block I, volumetric water content was higher than in block III (Tab. 4.4).
In block I, the values of volumetric water content were higher in the deepest layer (80-85 cm), while in the upper layers (20-25 cm and 40-45 cm) values were lower and very similar. In block III, the mid-layer (40-45 cm) showed the highest values of volumetric water content, while the sub-surface layer (20-25 cm) showed the lowest values.
Water retention curves in block I and III are presented in Fig. 4. 6a & b.

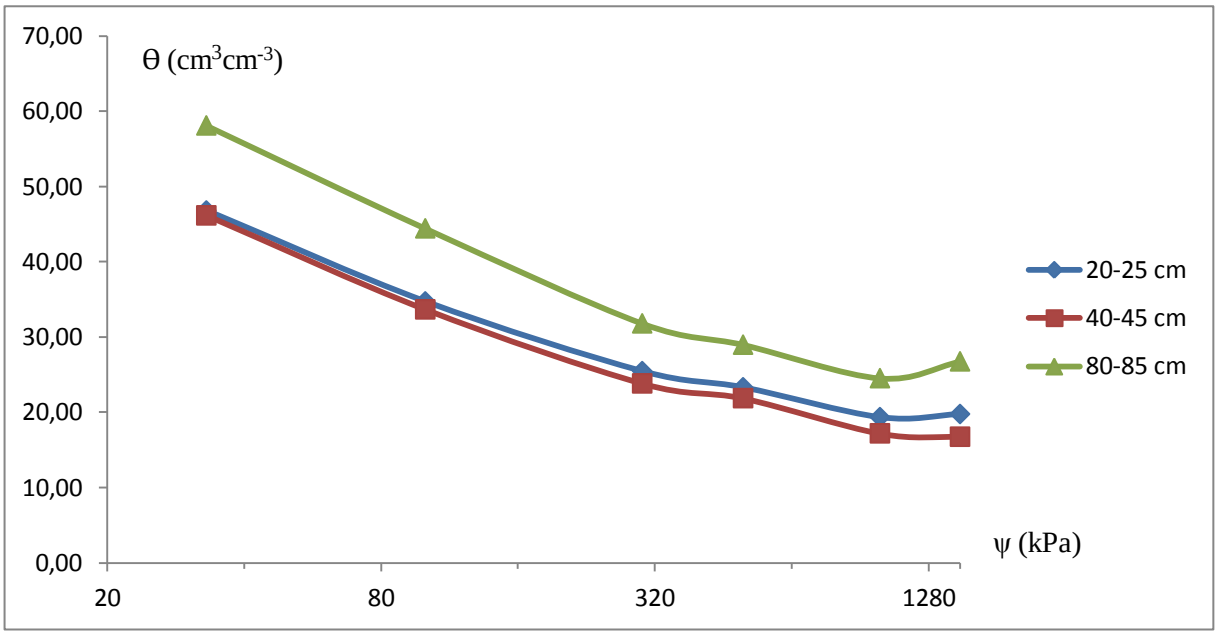


Fig. 4.6a: Water retention curve in block I, at Pohorski dvor 2004.

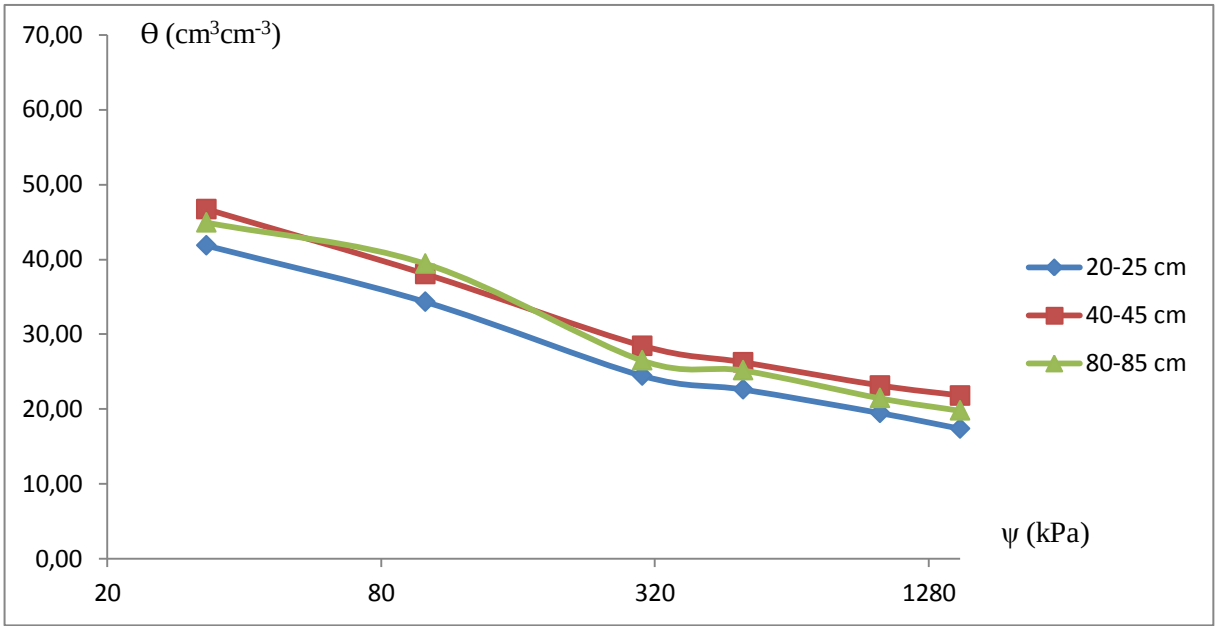


Fig. 4.6b: Water retention curve in block III, at Pohorski dvor 2004.

The previous results were used to calculate the available water content (AW). The deepest layer contains the highest value of available water (31 $cm^3.cm^{-3}$) in block I, compared to upper layers (29 $cm^3.cm^{-3}$ in 40-45 cm and 27.05 $cm^3.cm^{-3}$ in 20-25 cm layer). Similar situation occurred in block III, but here the differences in available water content were very small: 25 $cm^3.cm^{-3}$ in 80-85 cm, 25 $cm^3.cm^{-3}$ in 40-45 cm and 24 $cm^3.cm^{-3}$ in 20-25 cm layer.

III.1.2 *Soil water potential measurements*

Measurements of water potential (ψ) in soils from treatment 5 (pure control), treatment 10 (lime only) and inter-row in blocks I and III were compared, at three depths: 20-25 cm, 40-45 cm and 80-85 cm during the season 2004. In 2005, tensiometers were damaged due to mechanical cultivation in the rows. Distributions of soil water potential according to treatment and season with rainfall distribution by decades are presented in Fig. 4.7a, b & c and Fig. 4.8a, b & c. Measurements of water potential are presented in Annex 6.

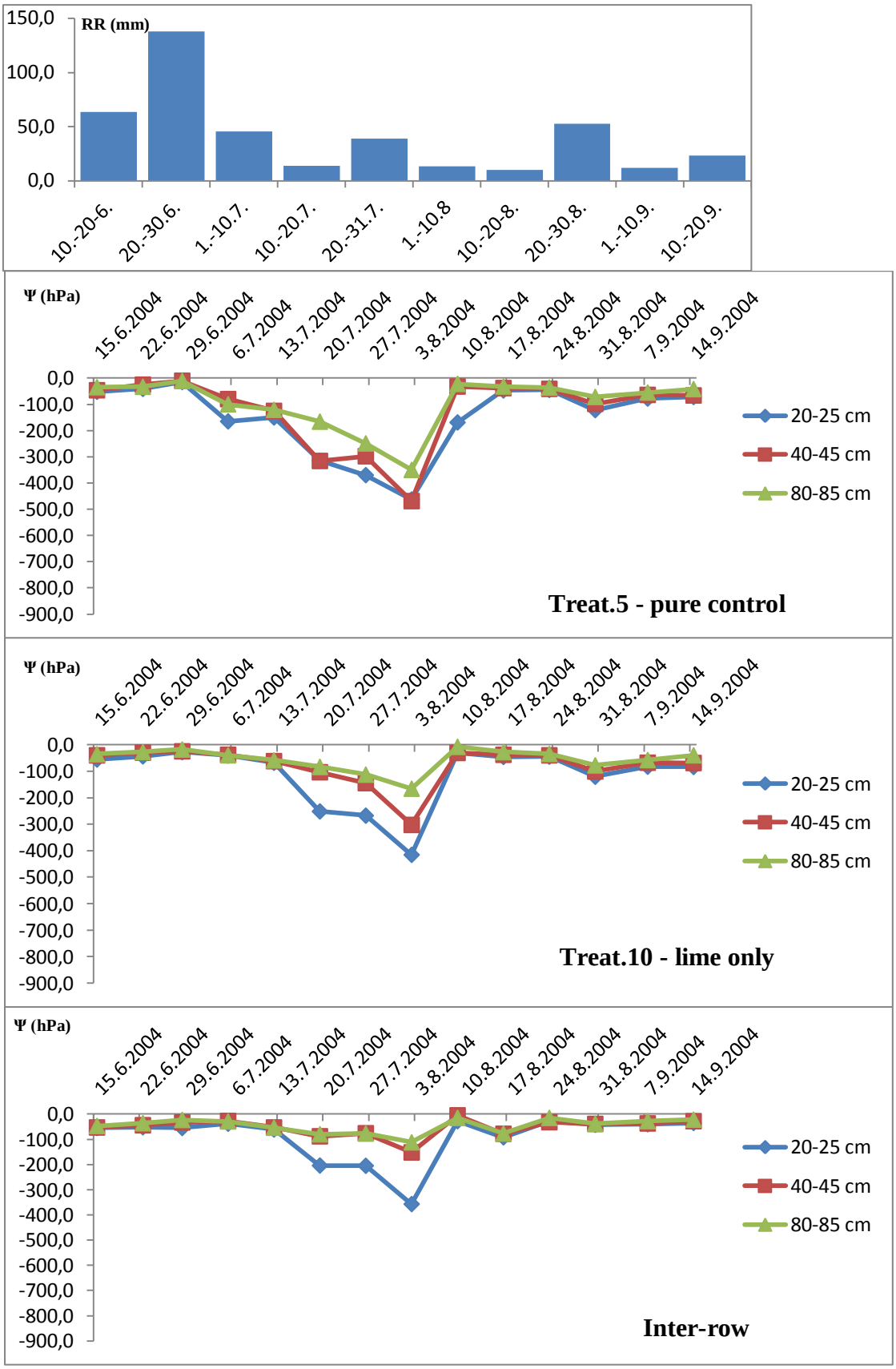


Fig. 4.7 a,b,c: Measurements of water potential in soils from treatment 5 (pure control) (a**)**, treatment 10 (liming only) (b**)** and from inter-row (c**)**, in block I with distribution of rainfall (RR) at Pohorski dvor 2004.

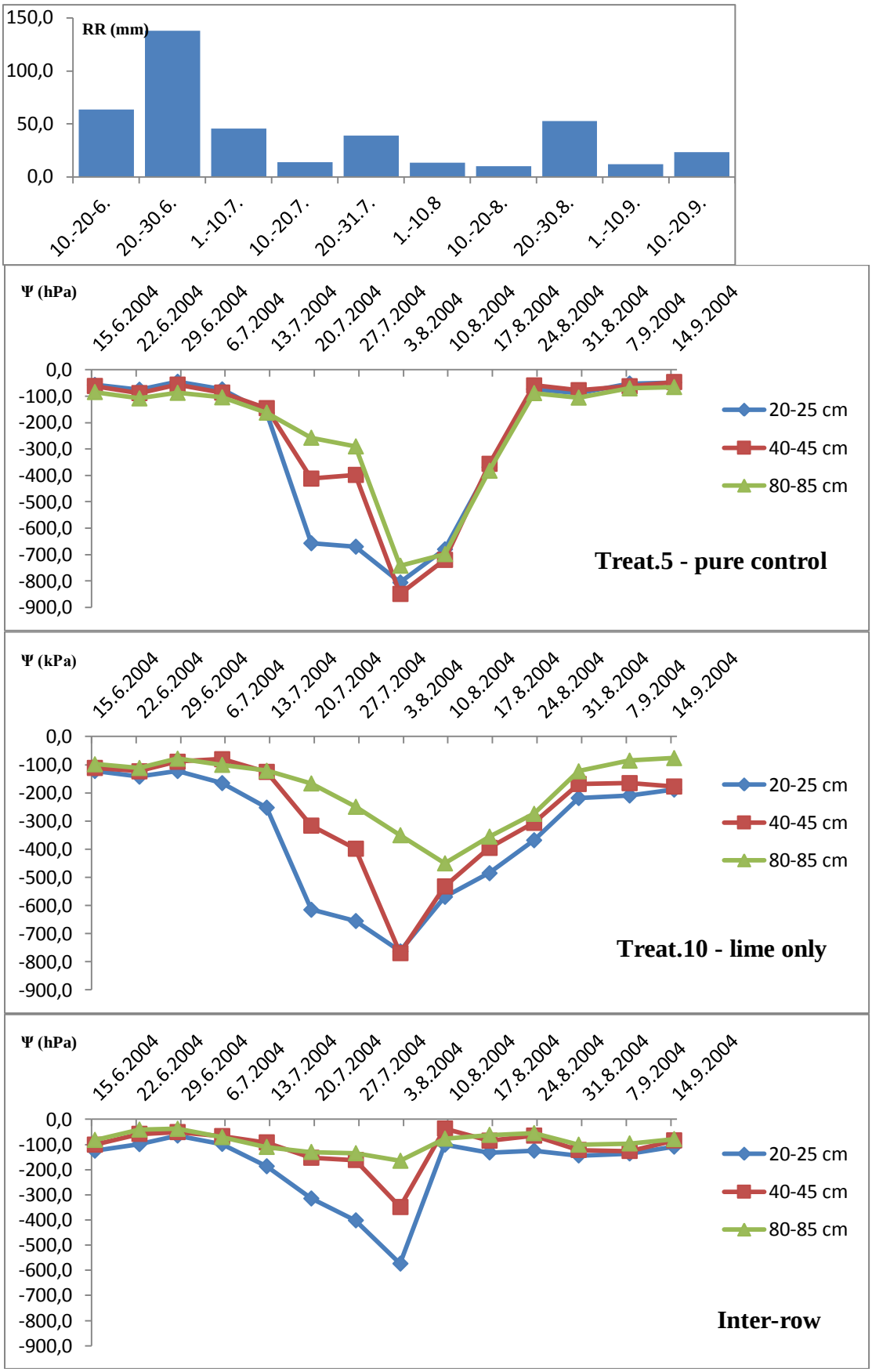


Fig. 4.8 a,b,c: Measurements of water potential in soils from treatment 5 (pure control) (a**)**, treatment 10 (liming only) (b**)** and from inter-row (c**)**, in block III with distribution of rainfall (RR) at Pohorski dvor 2004.

In June 2004, 232 mm of rainfall was measured, 98 mm in July, 77 mm in August and 99 mm in September. One main minimum of rainfall was noticed: in the beginning of August, where the lowest water potential was measured, mainly in the upper soil layer (20-25 cm).
In block I, water potential measurements in soils from all treatments soils (treat. 5-pure control, 10-lime only and inter-row) were very similar till the last rainfall in June. In July, water potential in treatment 5 (pure control) decreased more intensively, especially in the two upper layers (20-25 and 40-45 cm), and in the beginning of August the lowest value was reached. In this minimum water potential peak differences were observed according to treatments. In treatment 5 (pure control), water potential values in upper soil layers were very similar, while in the deepest layer the highest water potential was measured. In treatment 10 (lime only), a large difference between the deepest and the shallowest soil layers was noticed: the highest water potential was measured in the deepest layer and the lowest in the shallower layer. In the soil from inter-row, the sub-surface soil layer showed a value of water potential lower than for deeper layers, where similar measurements were observed. Later, values from all three soil layers followed rainfall simultaneously. Soil from inter-row showed higher water potential compared to soils from the rows. In soil from treatment 5 (pure control), the lowest water potential was measured compared to treatment 10 (lime only).

In block III, water potential measurements were similar till the last rainfall in all treatments and layers. Later, water potential decreased more rapidly in treatment 5 (pure control) like in block I, especially in the sub-surface soil layer. The pattern of decreasing water potential was very similar in treatments 5 (pure control) and 10 (lime only): the deepest soil layer had always the highest water potential and the sub-surface the lowest water potential. A difference occurred in the minimum peak in the beginning of August, when one week delay in the deepest soil layer in treatment 10 (lime only) was noticed. The two upper layers showed more or less the same values. Rainfall in the second half of July induced an increase of water potential in all three layers. Further differences among soil layers were more obvious in treatment 10 (lime only). In the inter-row, the highest water potentials were measured compared to the two other treatments (5-pure control and 10-lime only).

III.2 Evolution of gravimetric water content according to treatment and year

Significant differences in water content were noticed among blocks in both soil layers, where block I showed the highest soil moisture and block III the lowest soil moisture (Fig. 4.9).

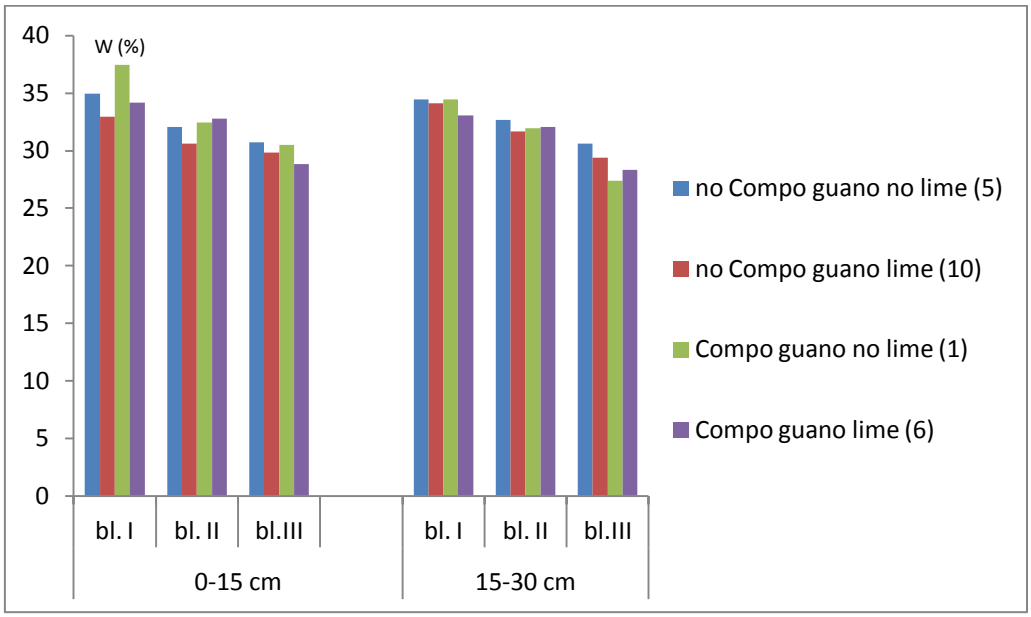


Fig. 4.9: Distribution of gravimetric water content (W) by treatments in blocks at Pohorski dvor, May 2004.

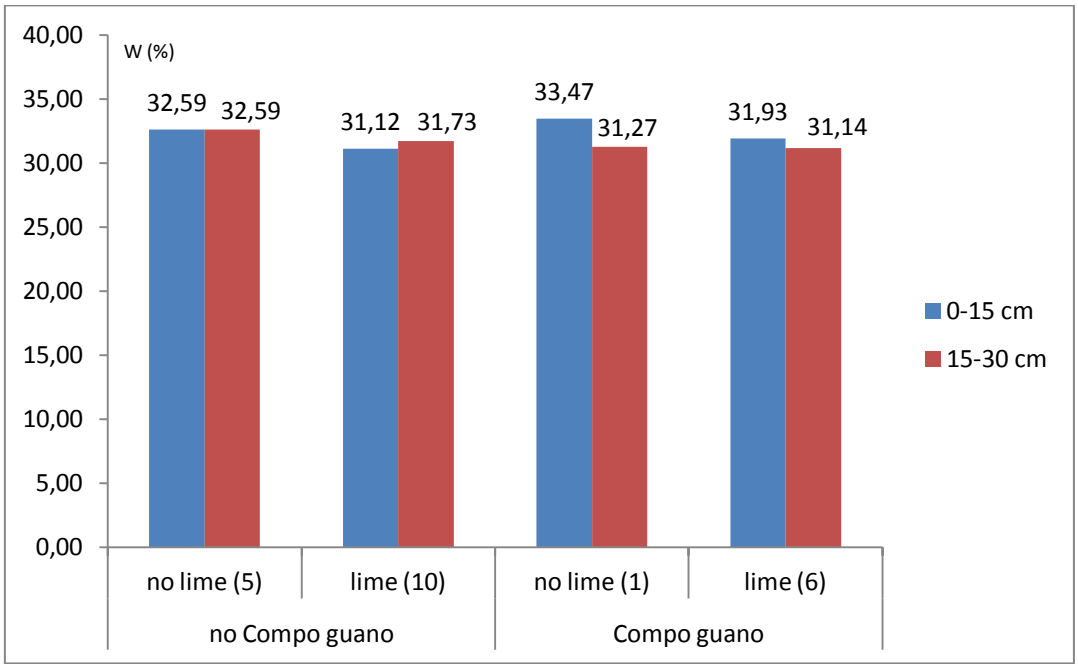


Fig. 4.10: Gravimetric water content (W) at Pohorski dvor, May 2004.

In May 2004, two interesting differences were found: (1) lower values of gravimetric water content in case of liming, (2) higher soil moisture in upper soil layer in case of Compo guano (Fig. 4.10).

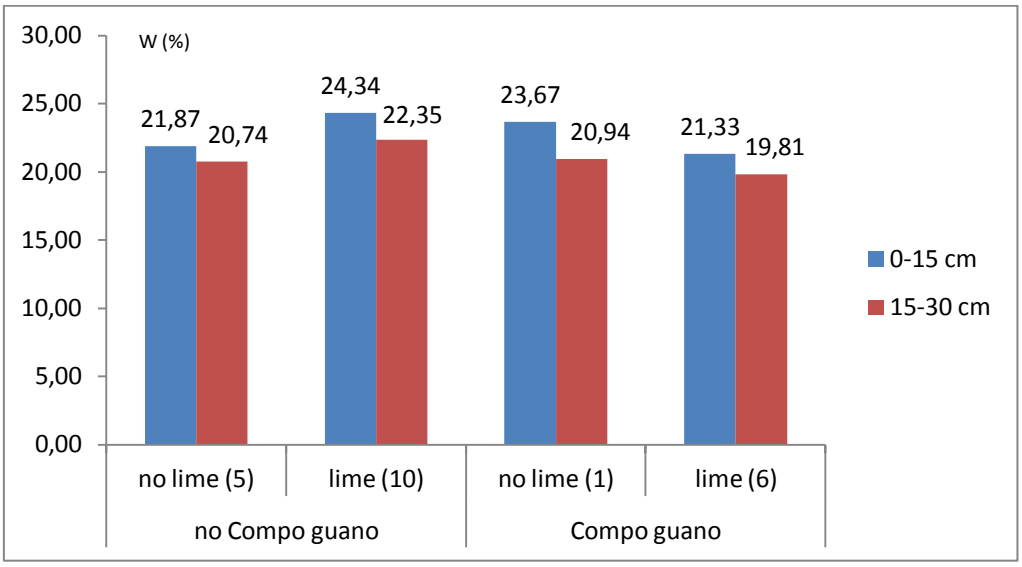


Fig. 4.11: Gravimetric water content (W) at Pohorski dvor, October 2004.

In October 2004, no effect of treatments was observed, while upper soil layer contained significantly higher soil moisture (Fig. 4.11).

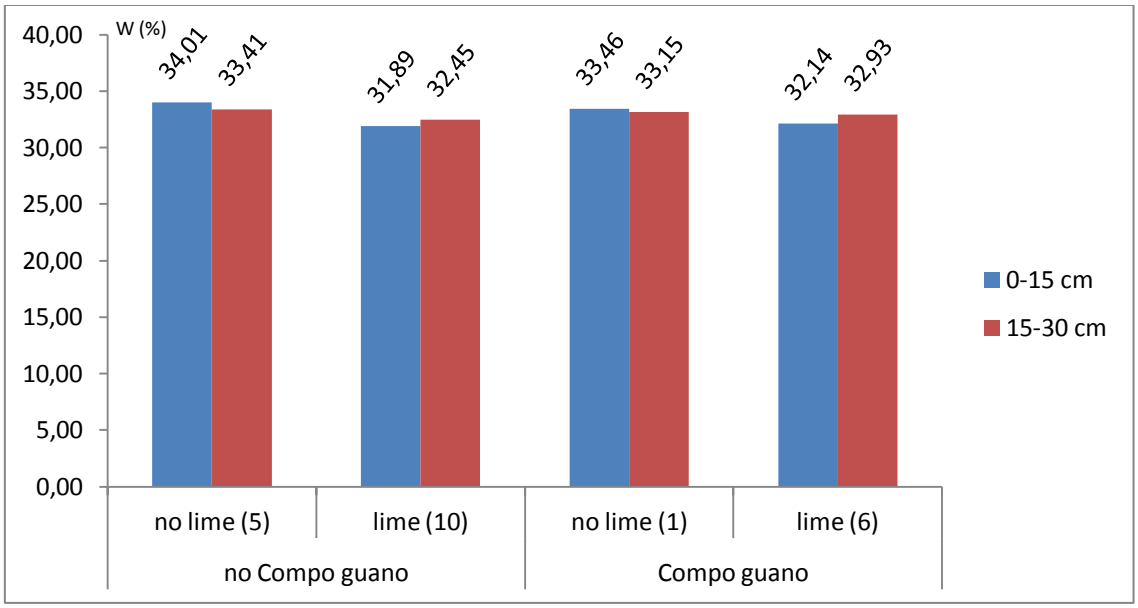


Fig. 4.12: Gravimetric water content (W) at Pohorski dvor, May 2005.

In May 2005, a significant difference was found between treatment 5 (pure control) and 10 (lime only), liming slightly decreasing gravimetric water content (Fig. 4.12).
No differences were observed according to depth.

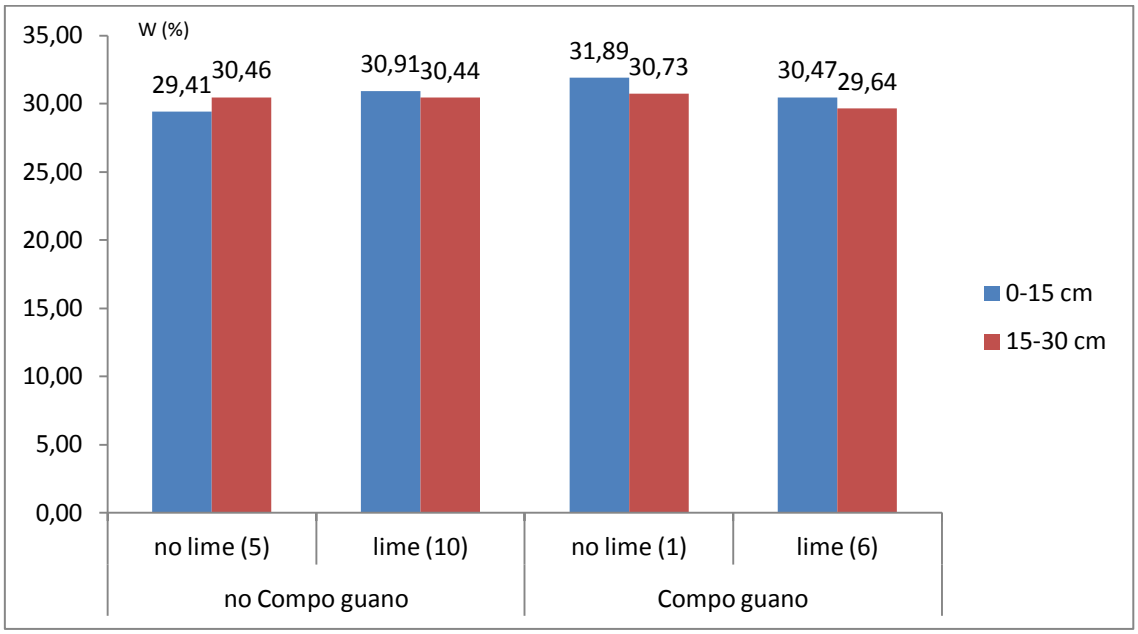


Fig. IV.13: Gravimetric water content (W) at Pohorski dvor, October 2005.

In October 2005, no differences in gravimetric water content were noticed according to treatments and depth (Fig. 4.13).

III.3 Discussion

Significant differences in gravimetric water gradient occurred among blocks: block I had the highest soil moisture and block III the lowest. Beside some physico-chemical properties (like slightly higher % clay and SOM), this was probably also due to the slope gradient (5 %), block I being located at the lowest position. Slightly lower values of soil moisture were observed in case of liming. Liming decreased gravimetric water content probably through the improvement of soil structure and this could affect pore space in sense of better water drainage. It is already known that liming improves soil structure (McLean, 1971).

Comparing the slope of water retention curves from different soil layers, the deepest layer (80-85 cm) exhibited a more pronounced slope in block I, i. e. it contained the largest amount of available water. In block III, the deepest layer contained intermediate values of volumetric water content, but the highest amount of available water. Soil from block I had a finer texture than in block III, contributing to more humid conditions. Morphological observations detected hydromorphic features in deeper soil layer in block I. Soil in block I was also more compact due to polyedric structure.

Some differences occurred in pattern of water potential measurements in different treatments and blocks according to dry periods. Water potential very clearly followed the rainfall distribution. However, comparing water potential curves in blocks I and III, water potential measurements were higher in block I (more humid soils). Comparing water potential curves in different soil layers in both blocks, the sub-surface soil layer (20-25 cm) showed the biggest variation. Soil from the deepest soil layer (80-85 cm) had the highest water potential compared to soils from upper layers, especially in dry periods (appearance of hydromorphic features in block I). Soil from inter-row showed higher water potential compared to soil from

the rows. The reason lies probably in less evaporation due to natural vegetation growing in inter-row.

The lowest water potential was measured in soil from treatment 5 (pure control), compared to treatment 10 (lime only). A possible explanation could be the better structure in treatment 10 (lime only), due to liming, which allowed easier water movement through the soil profile and less water stagnation. Moisture is essential for the lime-soil reaction to occur. Significant increase of pH was noticed in the block I, where pH_{KCl} was higher (5.2) compared to blocks II and III (4.6 and 4.7 respectively). Even when properly mixed with soil, lime will have little effect on pH if the soil is dry (http://www.herbgardeningguru.com/soil-ph.html), so this could explain the higher pH observed in block I.

IV Microbiological soil parameters evolution according to treatments and seasons

As basic microbiological properties, microbial biomass (BM), labile organic matter pool (LOM) and mineralised carbon from microbial respiration after 28 days (Cmin) were determined. Complete measurements of microbial parameters are presented in Annex 11 (in 2004) and in Annex 12 (in 2005).

IV.1 May 2004

Microbial biomass (BM)

In May 2004, no differences in microbial biomass were detected according to the treatments. Microbial biomass was significantly lower in deeper layer (Fig. 4.14).

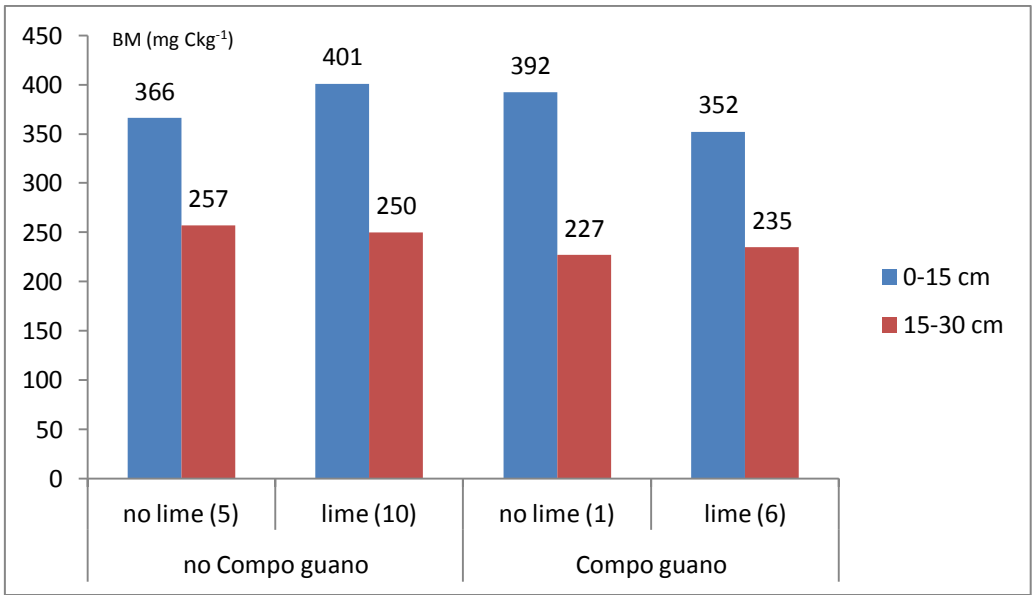


Fig. 4.14: Microbial biomass (BM) at Pohorski dvor, May 2004.

Labile organic matter pool (LOM)

LOM was determined in the upper soil layer only. No differences according to treatments were noticed (Fig. 4.15).

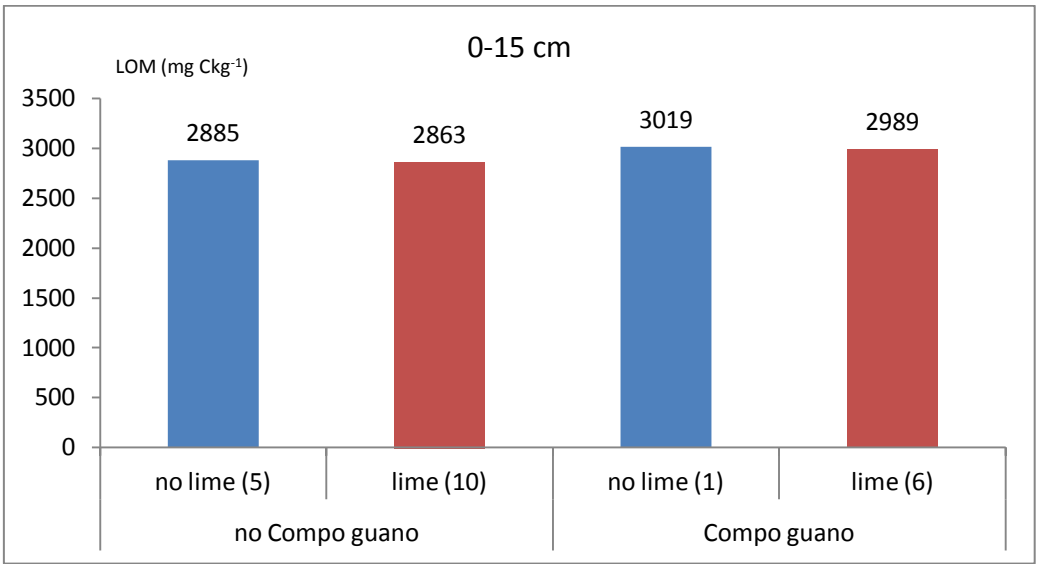


Fig. 4.15: Labile organic matter pool (LOM) in upper soil layer at Pohorski dvor, May 2004.

Cmin

Cmin, released by microbial respiration, showed no significant differences according to treatments, while a slight increase of Cmin was noticed in the treatment 10 (lime only). The effect of liming with Compo guano was not clear due to their interaction.
Cmin significantly decreased with depth.

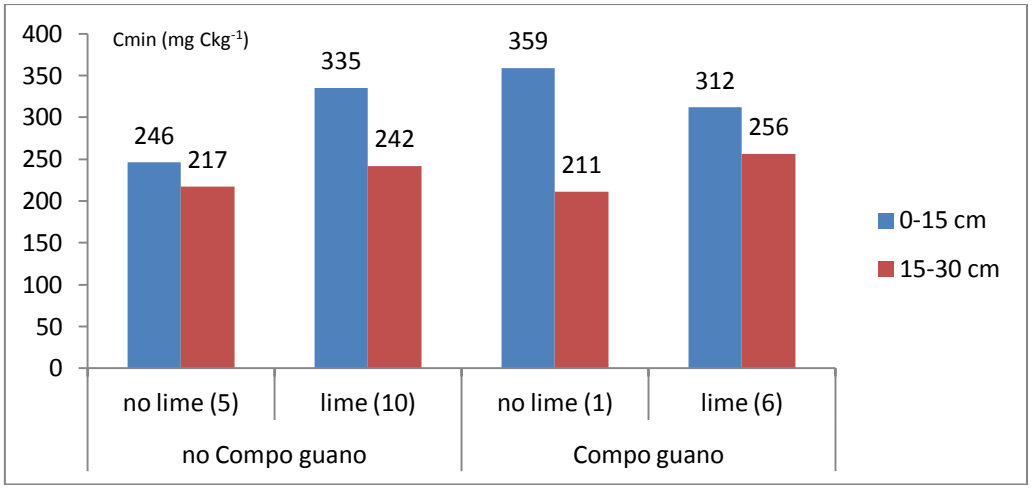


Fig. 4.16: Cmin at Pohorski dvor, May 2004.

In treatment 6 (Compo guano with lime), the most intensive respiration of soil microbes was detected (Fig. 4.16 and Tab. 4.5). In the upper soil layer, the effects of the treatments were more pronounced, inducing larger differences in soil respiration than in the lower layer.

Tab. 4.5: Spec. respiration (day^{-1}) and time of turnover (day); Pohorski dvor, May 2004.

	No Compo guano, no lime (5)		No Compo guano, lime (10)		Compo guano, no lime (1)		Compo guano, lime (6)	
Soil depth (cm)	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
spec. resp. (day^{-1})	0.043	0.039	0.04	0.04	0.046	0.042	0.05	0.044
1*(spec. respir.)$^{-1}$ (day)	23	25	25	25	22	24	20	23

Distribution of soil organic matter components in total Corg

In Fig. 4.17, stable OM represented the main part of total Corg (about 83% in upper layer and nearly 99% in deeper layer), for this reason only active organic compartments are presented (BM and LOM). Lime (without Compo guano) increased active Corg, while in combination with Compo guano, liming decreased active Corg. Compo guano slightly increased part of labile Corg, especially LOM (more evident without liming). The expected effect of lime was not clear with Compo guano due to their interaction.

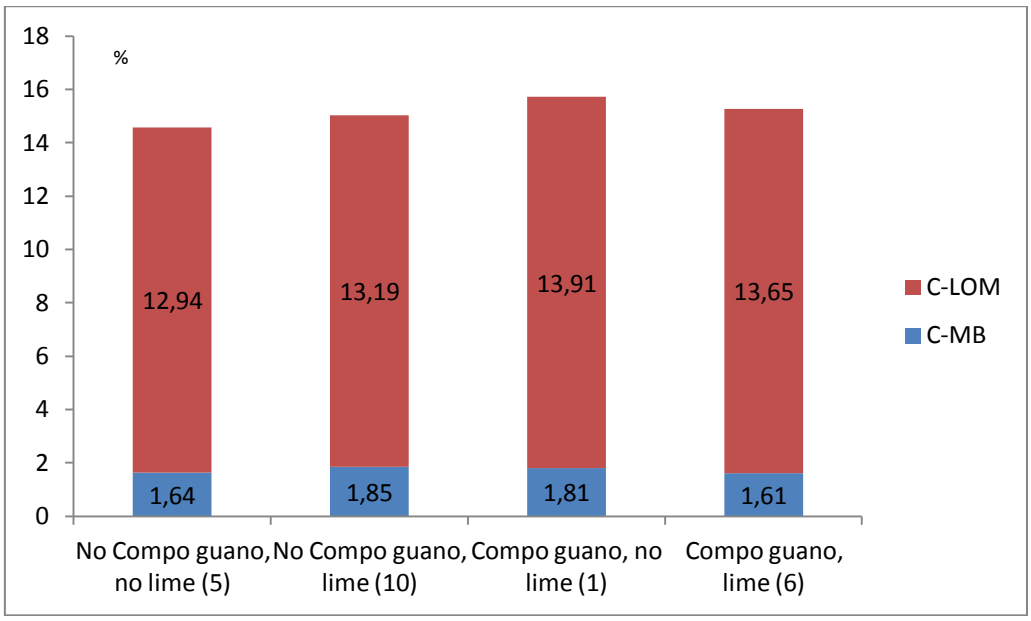


Fig. 4. 17: Distribution of soil organic matter (SOM) components in total Corg (0-15 cm), Pohorski dvor, May 2004.

IV.2 September 2004

Microbial biomass (BM)

In September 2004, microbial biomass did not significantly differ according to the treatments; only a slight increase in the treatment 10 (liming without Compo guano) was noticed (Fig. 4.18). The upper soil layer contained significantly more microbial biomass.

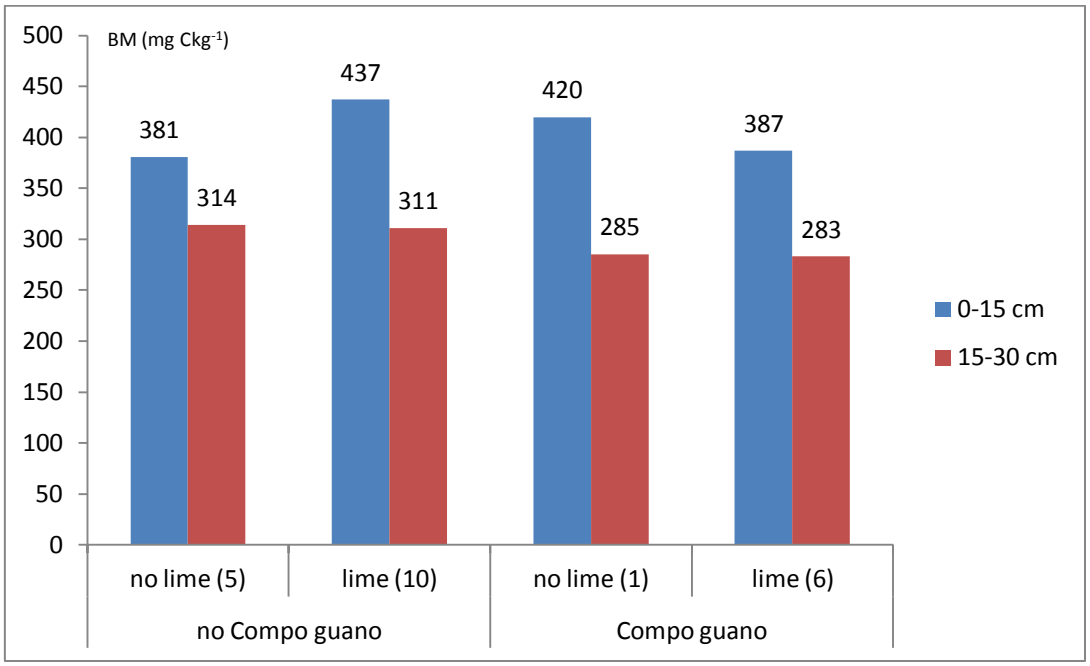


Fig. 4.18: Microbial biomass (BM) at Pohorski dvor, September 2004.

Labile organic matter pool (LOM)

In September 2004, LOM didn't differ significantly according to treatments (Fig. 4.19).

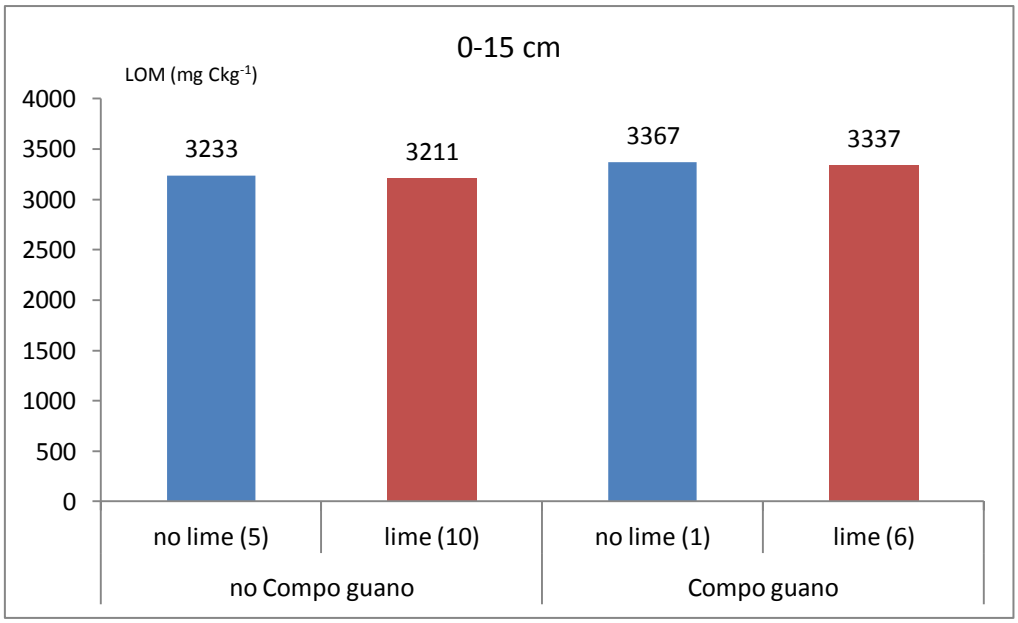


Fig. 4.19: Labile organic matter pool (LOM) in upper soil layer at Pohorski dvor, September 2004.

Cmin

In September 2004, statistical significant differences occurred regarding the released CO_2 from microbial respiration: more Cmin was detected in treatment 1 (Compo guano without lime) compared to treatment 5 (pure control). Compo guano contributed to higher microbial respiration. Without Compo guano, liming still increased Cmin, while in combination with Compo guano the opposite effect was observed (Fig. 4.20).

Significantly higher values of Cmin were noticed in the upper soil layer.

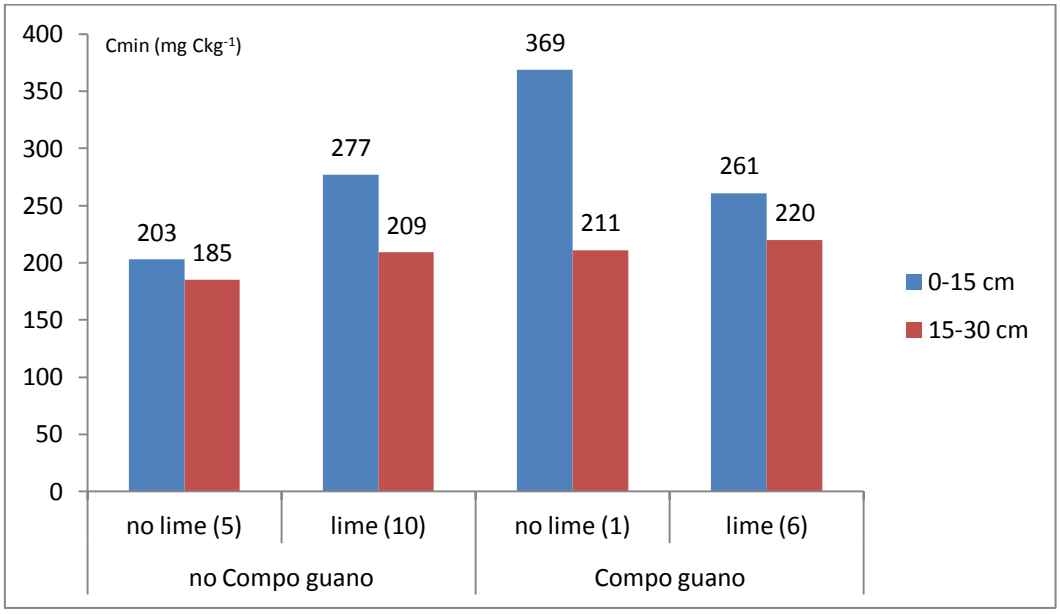


Fig. 4.20: Cmin at Pohorski dvor, September 2004.

Tab. 4.6: Spec. respiration (day^{-1}) and time of turnover (day); Pohorski dvor, September 2004.

	No Compo guano, no lime (5)		No Compo guano, lime (10)		Compo guano, no lime (1)		Compo guano, lime (6)	
Soil depth (cm)	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
spec. resp. (day^{-1})	0,019	0,021	0,022	0,023	0,031	0,027	0,024	0,027
1*(spec. respir.)$^{-1}$ (day)	62	56	47	49	32	38	43	44

A significant difference was observed for specific respiration, which was lower in treatment 5 (pure control) than in treatment 1 (Compo guano without lime). No differences according to depth were noticed (Tab. 4.6).

IV.3 May 2005

Microbial biomass (BM)

In May 2005, significant differences occurred for microbial biomass: (a) between treatment 1 (Compo guano without liming) and treatment 6 (Compo guano with liming); (b) between treatment 1 (Compo guano only) and treatment 10 (liming only). Liming obviously contributed to higher values of microbial biomass (Fig. 4.21).
In the upper soil layer significantly higher microbial biomass was observed.

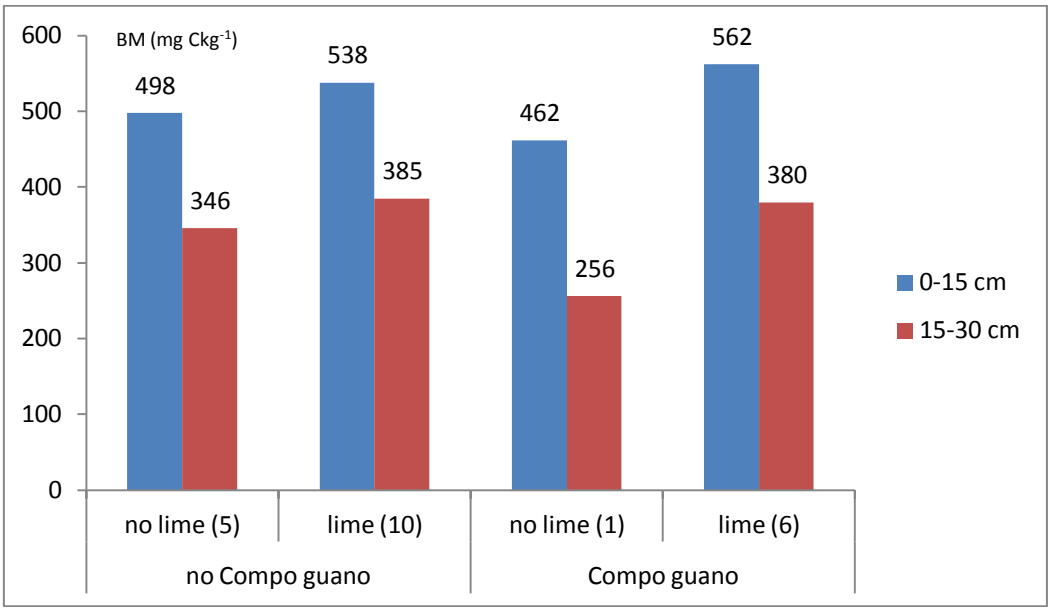


Fig. 4.21: Microbial biomass (BM) at Pohorski dvor, May 2005.

Labile organic matter pool (LOM)

In May 2005, treatment 10 (lime only) differed from all the other treatments and showed the lowest values of LOM (Fig. 4.22).

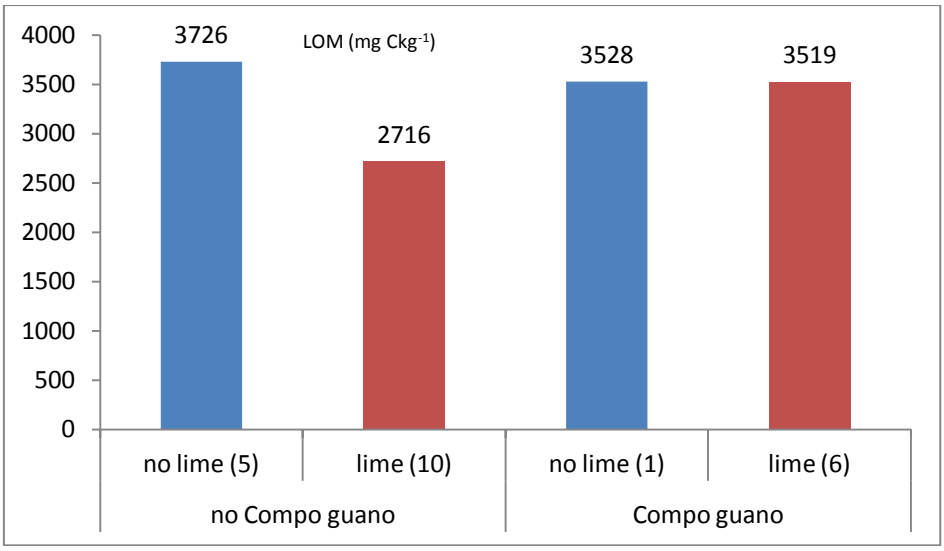


Fig. 4.22: Labile organic matter pool (LOM) in upper soil layer at Pohorski dvor, May 2005.

Cmin

In May 2005, no significant differences according to treatments were noticed. From the Fig. 4.23 can be noticed that liming (without Compo guano) decreased mineralisation, probably due to a decrease of LOM. With the addition of Compo guano, the situation was the opposite, liming increased C mineralisation.

The upper soil layer contained significantly more Cmin.

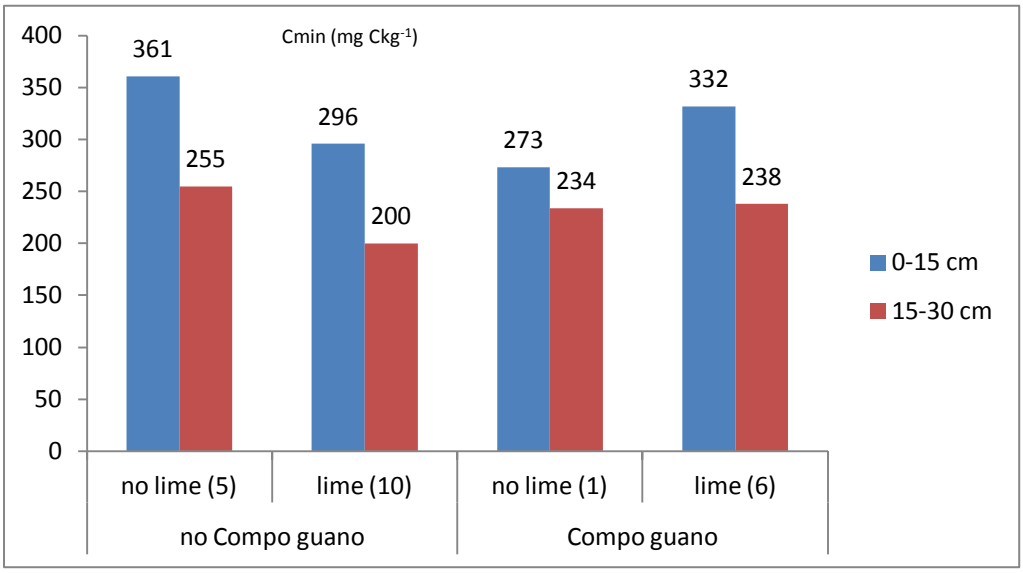


Fig. 4.23: Cmin at Pohorski dvor, May 2005.

Tab. 4.7: Spec. respiration (day^{-1}) and time of turnover (day) at Pohorski dvor, May 2005.

	No Compo guano, no lime (5)		No Compo guano, lime (10)		Compo guano, no lime (1)		Compo guano, lime (6)	
Soil depth (cm)	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
spec. resp. (day^{-1})	0.029	0.030	0.041	0.028	0.024	0.026	0.028	0.027
1*(spec. respir.)$^{-1}$ (day)	35	34	25	36	42	40	36	37

In May 2005, a significant difference occurred between treatments 10 (lime only) and 1 (Compo guano without liming), where liming increased specific respiration (Fig. 4.23 and Tab. 4.7). No differences according soil depth were observed.

IV.4 September 2005

Microbial biomass (BM)

In September 2005, a statistically significant difference in microbial biomass occurred between treatment 5 (pure control) and treatment10 (lime only), with lower values for liming. Microbial biomass also significantly decreased with soil depth (Fig. 4.24).

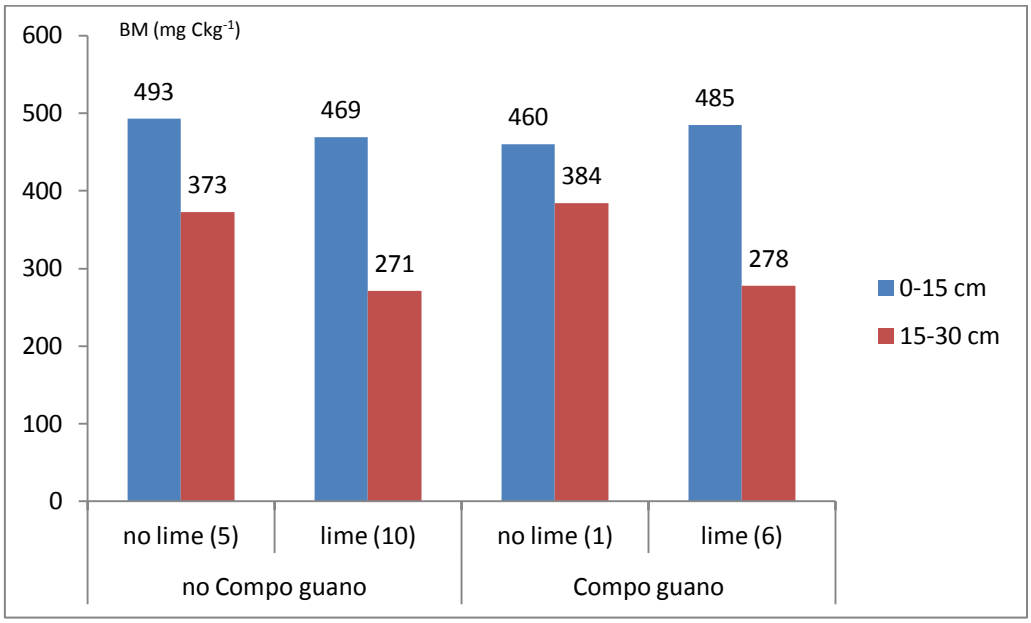


Fig. 4.24: Microbial biomass (BM) at Pohorski dvor, September 2005.

Labile organic matter pool (LOM)

In September 2005, LOM didn't differ significantly according to treatments, but we still could notice a low LOM value due to liming without Compo guano (Fig. 4.25).

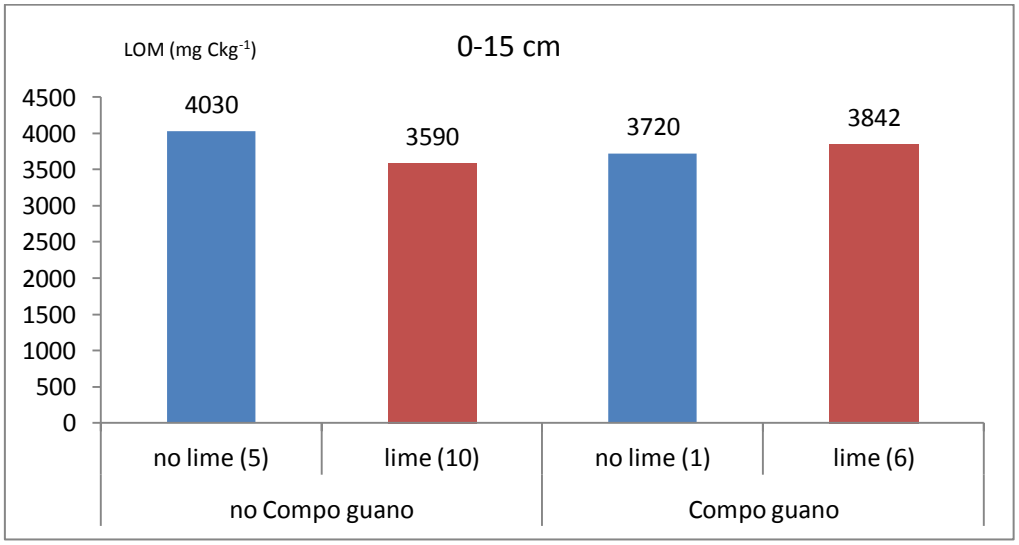


Fig. 4.25: Labile organic matter pool (LOM) in upper soil layer at Pohorski dvor, September 2005.

Cmin

No significant differences in Cmin were observed among the treatments, but it could be noticed that liming generally stimulated C mineralisation (Fig. 4.26 and Tab. 4.8). Significantly higher Cmin was detected in the upper soil layer, where specific respiration was also the highest.

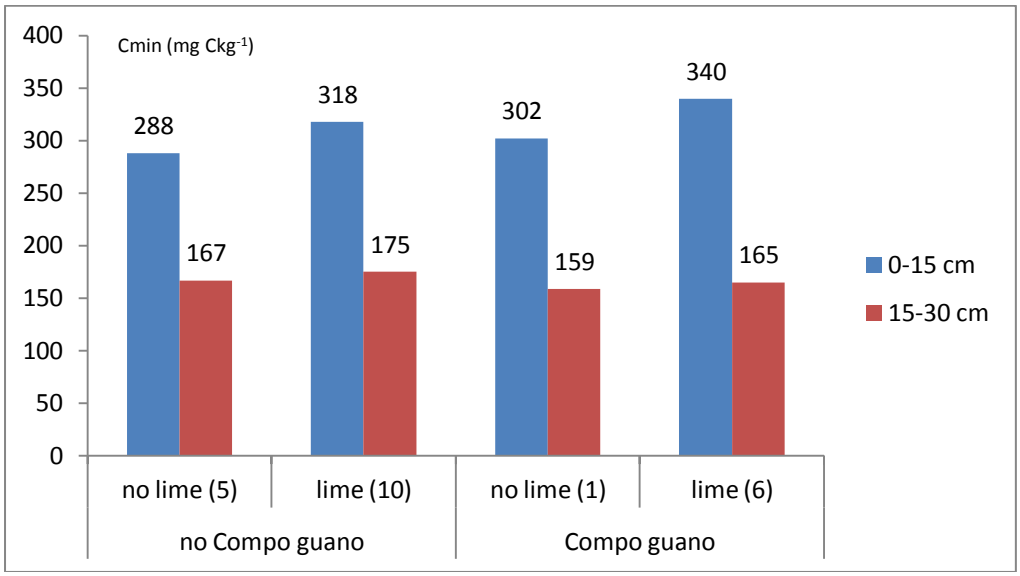


Fig. 4.26: Cmin at Pohorski dvor, September 2005.

Significant differences occurred between treatments, liming increasing specific respiration, while Compo guano had no effect.

Tab. 4.8: Spec. respiration (day^{-1}) and time of turnover (day) at Pohorski dvor, September 2005.

	No Compo guano, no lime (5)		No Compo guano, lime (10)		Compo guano, no lime (1)		Compo guano, lime (6)	
Soil depth (cm)	0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
spec. resp. (day^{-1})	0.032	0.025	0.038	0.036	0.037	0.023	0.041	0.034
1*(spec. respir.)$^{-1}$ (day)	31	40	26	28	27	44	25	30

Nmin

Significant difference occurred between treatment 6 (Compo guano with liming) and treatment 10 (liming without Compo guano). In case of liming, Compo guano contributed to higher nitrate concentration in soil. No differences in nitrates were observed according to soil depth (Fig. 4.27).

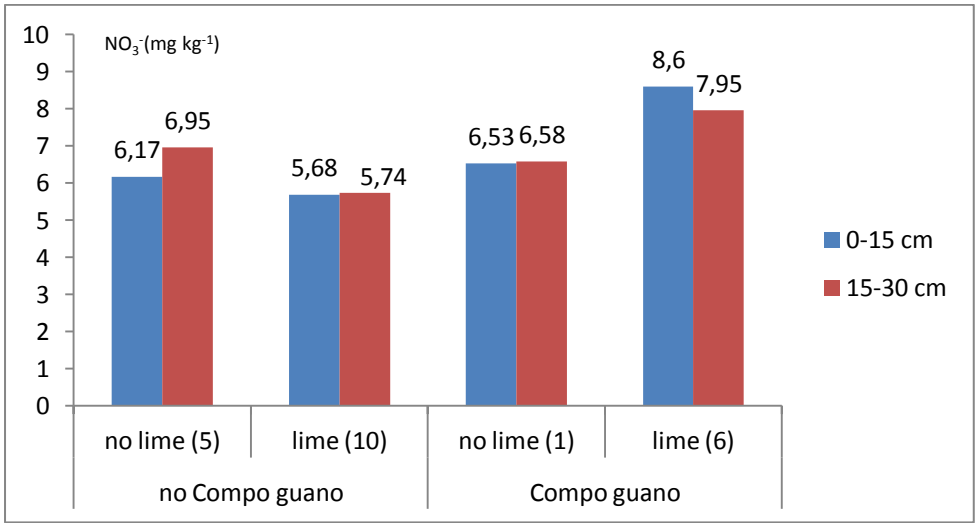


Fig. 4.27: Nmin (NO_3^-) at Pohorski dvor, September 2005.

IV.5 Discussion

At the beginning of the experiment (spring 2004), values of BM, LOM and Cmin were uniform among blocks, while after applied treatments (autumn 2004), the lowest values of these parameters were found in the block III. Microbial parameters in this block were in accordance with the lowest Corg and the lowest available water content. Humidity and organic matter could be limiting factors of microbial biomass and activity here.

In May 2004, microbial biomass, LOM and released Cmin from microbial respiration were not significantly affected by treatments yet (after just one application of fertiliser and lime). Lime alone slightly increased C mineralisation and part of active Corg, while in combination with Compo guano the effect was unclear, presumably due to interactions between the organic amendment and liming. Compo guano slightly contributed to faster mineralisation process, increased LOM and more active Corg in the upper layer.

In September 2004, treatments did not affect microbial biomass significantly, but a slight increase of BM for liming without Compo guano was still noticed. Significant differences in Cmin occurred, where Compo guano alone increased values of released CO_2. Compo guano stimulated soil respiration as well. Liming or Compo guano alone stimulated C mineralisation, but not in combination (unclear effect due to their interaction). In September 2004, LOM did not differed significantly according to treatments. This was expected while LOM usually do not change over short periods.

In May 2005, liming had a positive effect on microbial biomass and a negative effect on LOM, due to previous stimulation of C mineralisation. However, there was no extra input of Corg in the treatment 10 (lime only), so LOM was decreasing due to accelerated SOM turnover. LOM was presumably consumed more intensively by microbial biomass.

In September 2005, a slight decrease of BM in case of liming was probably a consequence of the observed decreased of LOM in May 2005. Low values of LOM occurred probably due to more intensive C mineralisation, leading to less microbial biomass.

Liming alone had a positive short-term effect on BM, but on the cost of lower LOM after intensive respiration. During the longer period, liming alone decreased both BM and LOM. Anderson (1998) also found that the microbial activity measured as basal respiration was higher after liming as compared to the control, and Kreutzer (1995) noticed that the C pool of the organic surface layer and upper mineral soil decreased (root biomass not included) after long lime application. In the combination with Compo guano, unclear effect on BM, LOM and Cmin was found due to their interaction.

Generally, Compo guano contributed to more intensive microbial respiration and to small increase of active Corg. In general, the addition of compost and amendment applications stimulated microbial respiration (Gonzales, 2010; Marinari *et al*., 2000). Most of the carbon supplied by this amendment is partially decomposed material, easily degradable and used as energy and nutrient source by soil microorganisms, resulting in an increased soil microbial respiration (Stevenson, 1986).
It is well known that biomass increases when organic materials are applied to soil (Jenkinson and Ladd, 1981). While organic amendments generally increase BM (Mazzarino *et al*., 1993; Goshal and Singh, 1995), there are diverse reports regarding the effect of mineral N fertilizers on the size and activity of soil microbial biomass. In our experiment, Compo guano alone did

not significantly increase BM, probably due to low soil pH. Application of organic fertilizer as well as various composts generally caused an increase in microbial populations compared to mineral fertilizer, but the patterns varied depending on the type of organic fertilizer (Krishnakumar *et al*., 2005), application rate (Lee *et al*., 2004; Mondini *et al*., 2008), soil type to which it was applied (Perez-Piqueres *et al*., 2006) and the time scale of investigations (Kokalis-Burelle and Rodriguez-Kabana, 1994). In some cases, compost or farmyard manure did not significantly enhance any microbial density more than mineral fertilizer (Lalfakzuala *et al*., 2008). This result also showed that the effect of the organic fertilizer on microbial populations was not always the same or consistent over the whole season (Lee, 2010).

Obviuosly, at Pohorski dvor, where soil pH is low, the effects of addition of organic matter on soil metabolism are mitigated by soil pH. It must be pointed out that Compo guano alone increased soil acidity. The effects on labile pools of OM (BM and LOM) are therefore different for limed or unlimed situations. In combination with liming, Compo guano contributed to higher nitrate values.

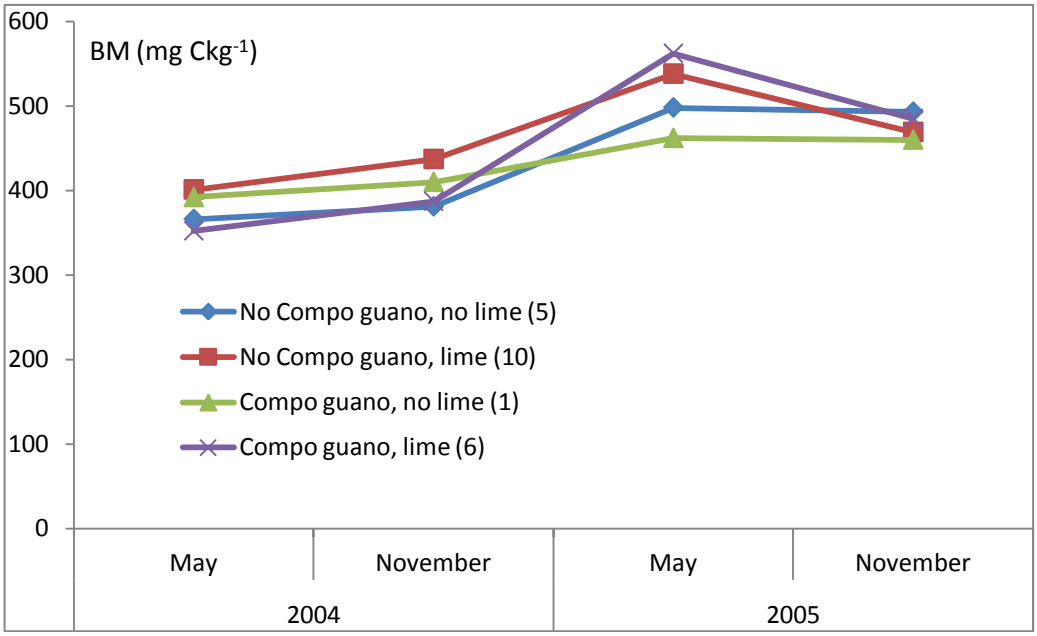


Fig. 4.28: Time distribution of microbial biomass (0-15 cm) at Pohorski dvor.

From Fig. 4.28 it can be seen that BM increased during the experiment, following the addition of fertiliser or lime (as observed for soil pH). The best combination was Compo guano with lime (only in the second season); being an easily available source of Corg, Compo guano increased BM while lime avoided excessive soil acidification which is detrimental to soil organisms. Liming was more efficient in the upper soil layer (positive effect on soil pH).

Generally, nitrate values were very low. Possible explanations could be: (1) the soil was very wet and nitrates could be leached or (2) mineralisation was disturbed due to excessive soil moisture and low pH or (3) due to denitrification. Compo guano contributed to the highest nitrate values when in combination with liming. Gunapala and Scow (1998) also reported a positive effect of improved conditions for microbial life on soil nitrogen in fields under organic production.

Cropping history, type of plant cover, plant age, and climatic changes are also well known to influence soil microbial biomass and its activity, thus leading to considerable seasonal

fluctuations in BM (Franzluebbers *et al*., 1994; Xu & Juma, 1993; Mendes *et al*., 1999; Moore *et al*., 2000; Piao *et al*., 2000). However, only a few long-term field studies exist to ascertain the practical significance of these fluctuations on crop growth and nutrient cycling (Kaiser & Heinmeyer, 1993; Joergensen *et al*., 1994; Goshal & Singh, 1995). The temporal trend of C availability, soil microbial biomass and its activity could be much more pronounced as compared to treatment effects. Treatment effects on BM could be also associated with temporal variations in the microbial community structure.

V. Discussion and conclusions - Pohorski dvor site

In this experimental field, a large heterogeneity according to soil moisture and chemical-physical properties was observed. This could mask the real effect of applied fertilisers and liming on soil properties.

Our goal in the experiment was: 1) enrich soil with organic matter as a source of plant nutrients (especially with nitrogen), 2) increase pH for improving conditions for microbial activity (especially nitrification) and 3) speed up the mineralisation processes for releasing plant nutrients (especially nitrogen). The best solution to improve soil microbial activity and to avoid exhaustion of Corg (BM and LOM) in long term is to use liming with Compo guano, even if at the beginning the results were not encouraging.

The first time (2004), combinations of liming and Compo guano did not show any differences in BM and LOM, while liming without Compo guano slightly increased C mineralisation. The second time (2005), liming first increased microbial biomass and decreased LOM (in May). Later (in September), the consequence was also a decrease of BM and LOM due to liming.
However, in addition to providing substrate for the decomposers, organic amendments also can improve soil water retention (Carter, 2007; Rawls *et al*., 2003). This was also partly found in our measurements of water potential, where additional organic matter from the grass in inter-row contributed to higher available water content in upper soil layers. In our study we also found that liming also favoured drainage. Thus, amendments contribute to improved soil health through a lot of direct and indirect effects (Magdoff, 2001; Snapp *et al*., 2005).

The aim of this research was to develop an efficient and fast methodology combining physical, chemical, and microbiological soil parameters to evaluate soil quality as a function of organic matter addition and liming in agricultural soils. Results showed that microbiological parameters are sensitive indicators of changes and improvements in soil fertility in a very short period of time. These results confirm that this investigation should be accompanied by the simultaneous determination of basic physical and chemical soil characteristics such as pH, CEC, EC, and FC. These abiotic characteristics are complementary for a better understanding of the biological and biochemical properties and support the final evaluation of soil quality (Mäder *et al*., 1997; Filip, 1998; Kumar & Goh, 2000).

General conclusions

1. A large heterogeneity according to the soil moisture and chemico-physical properties was observed on the experimental field.
2. Liming increased soil pH (especially in upper soil layer), while Compo guano slightly decreased soil pH.
3. Soil pH was higher in the upper soil layer.

4. Liming alone decreased soil moisture and increased water potential probably due to effects on pore space, inducing better water drainage and better structure.
5. Liming (without Compo guano) increased microbial biomass and decreased LOM as a short time effect. The long time effect was decreasing LOM and MB.
6. Compo guano (without liming) increased microbial mineralisation.
7. The interaction of Compo guano and lime together was not clear, but in long term this is probably the best solution because it had positive consequences on both soil pH and available nitrogen, while preserving fair levels of BM and LOM.

Chapter 5

Pathogenic microbes in soil

Chapter 5: Pathogenic microbes in soil

According to the fact that one of the goals of our study is also the assessment of soil health, some analyses of specific microbes were done in both locations. In Gačnik, irrigation water was the potential source of pathogens, while in Pohorski dvor it was organic fertilising.

I Gačnik site: effect of irrigation

The effect of irrigation water and slope on specific microbes was studied. Specific microbial analyses in Gačnik were done in July 2006 in the surface soil layer (0-5 cm) in two treated rows (IRR=irrigated and NIR=non-irrigated row) at three slope positions (upslope, midslope, downslope). This summer no irrigation was done due to enough water input from precipitation. In this situation, the short term effect of irrigation couldn't be studied, but analyses were done with allowing a possibility that pathogens could remain in soils as a consequence of irrigation in previous seasons.

I.1 Summary of previous results

Before presenting the specific microbes at this location, some basic previous results should be remembered:

- Soil gravimetric water content increased from upslope to downslope and it was higher in non-irrigated row.
- Soil pH didn't vary according to slope and treatment.
- Total carbonates were three times higher at upslope, comparing lower slope positions. Irrigated row had higher total carbonates than non-irrigated row.
- Total organic carbon and C/N ratio increased from upslope towards downslope and the values of both were higher in non-irrigated row.
- Higher Corg and C/N in fraction A (>200 μm) were noticed in irrigated row.
- Soil mineral nitrogen (ammonium and nitrates) were higher at upslope and in irrigated row positions.
- Soil bulk density at upslope was lower comparing lower slope positions and it was higher in non-irrigated row.
- Structural stability was higher upslope and in non-irrigated row.
- Microbial soil biomass and microbial respiration increased towards downslope. Higher microbial biomass was found in irrigated row.
- Irrigation water contained high amount of nitrates (on the borderline by Official Gazette of Republic Slovenia) and high concentration of chemical (COD) and biochemical oxygen (BOD) demands.

Parallel with the analyses of specific microbes, soil pH, temperature and gravimetric water content were measured in the surface soil layer at three slope positions. No significant difference was noticed between sampling locations regarding soil temperatures (5 cm depth) and soil pH. The average soil temperature (at the sampling date) was 25.5°C and soil pH (in water) was 8.15.

Statistically significant differences were noticed in gravimetric water content, where values increased from upslope towards downslope (Fig. 5.1), what confirms previous results.

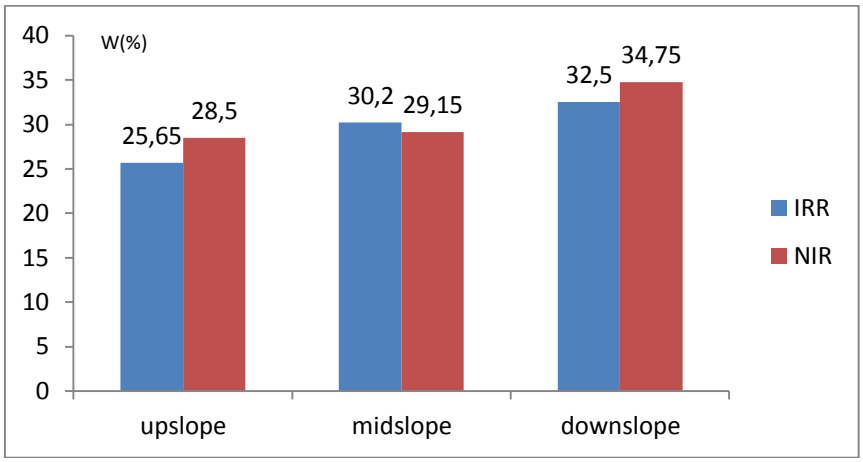


Fig. 5.1: Gravimetric water content in Gačnik, July 2006 (IRR=irrigated, NIR=non-irrigated row).

I.2 Specific microbes' characterization

I.2.1 *Enumeration of Fungi*

The number of soil fungi followed the pattern of gravimetric water content in the slope, increasing towards downslope. No significant differences occurred between irrigated and non-irrigated rows, but there was a slight trend to higher fungi numbers in NIR row (Fig. 5.2). Fungal population density decreased from irrigation pond towards the end of the irrigation system.

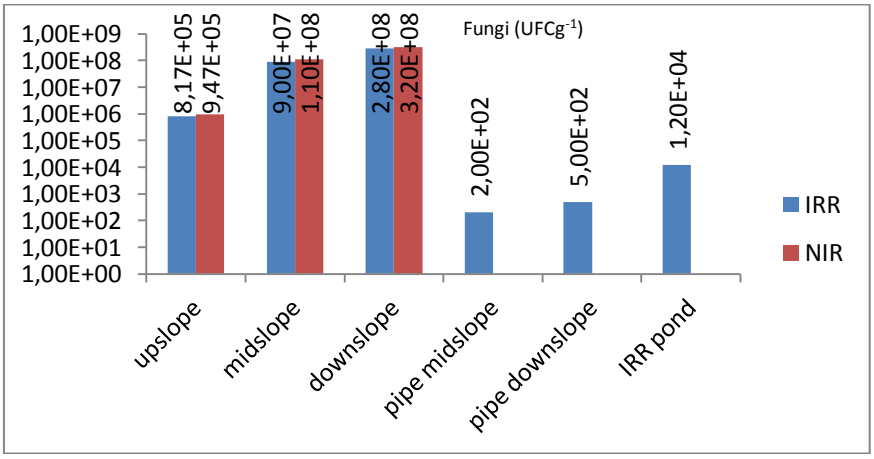


Fig. 5.2: Fungal population density in soil (0-5 cm) in Gačnik, July 2006.

I.2.2 *Enumeration of Bacteria*

Aerobic bacteria

Aerobic bacteria abundance was the highest at mid and downslope positions, while soil from upslope contained significantly less aerobic bacteria (Fig. 5.3). No significant differences occurred between irrigated and non-irrigated rows, but there was a slight trend to higher aerobic bacteria numbers in NIR row. Water from irrigation pond contained significantly less aerobic bacteria than water from irrigation pipe.

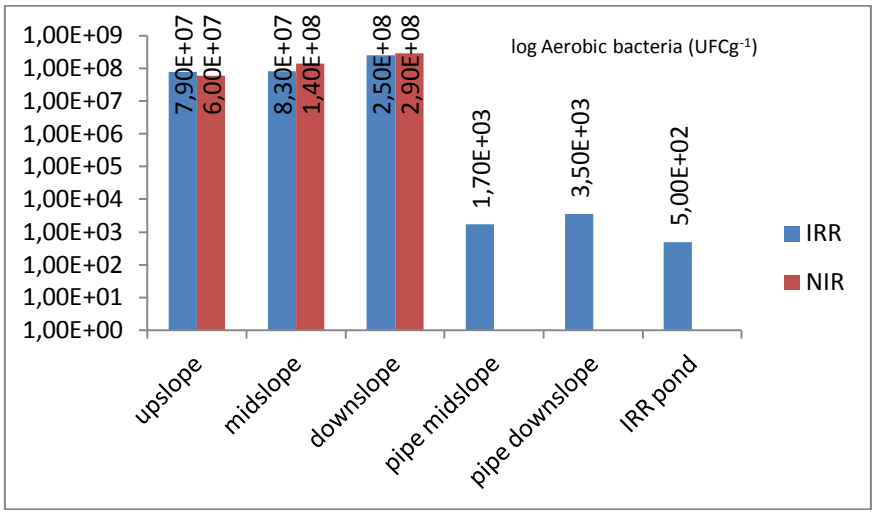


Fig. 5.3: Aerobic bacteria enumeration in soil (0-5 cm) in Gačnik, July 2006.

Anaerobic bacteria

Numbers of anaerobic bacteria showed significant differences along the slope, the lowest value being found upslope and the highest value downslope (Fig. 5.4). No significant differences occurred between irrigated and non-irrigated rows, but there was a slight trend to higher anaerobic bacteria numbers in NIR row. Water from irrigation pond contained the highest number of anaerobic bacteria and this number decreased in water moving to irrigation pipes.

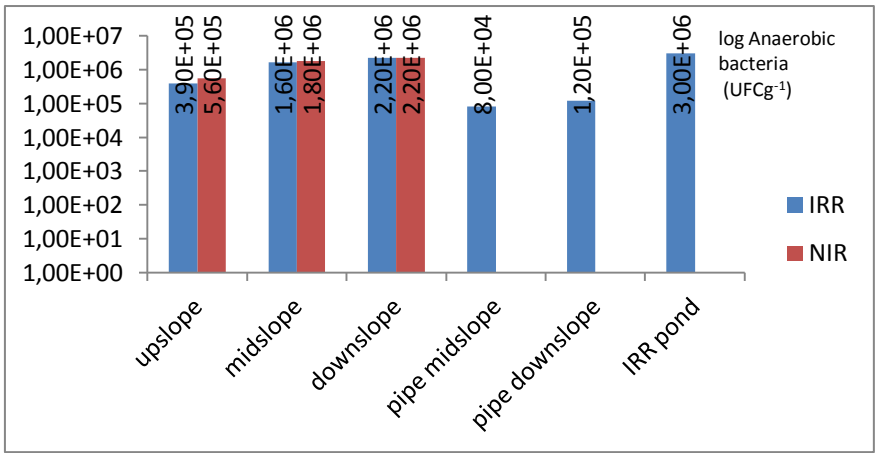


Fig. 5.4: Anaerobic bacteria enumeration in soil (0-5 cm) in Gačnik, July 2006.

Total coliforms

Total coliforms significantly increased towards downslope, where IRR row showed higher number compared to NIR row (Fig. 5.5). The number of total coliforms in irrigation water increased while moving from water source to middle of irrigation system.

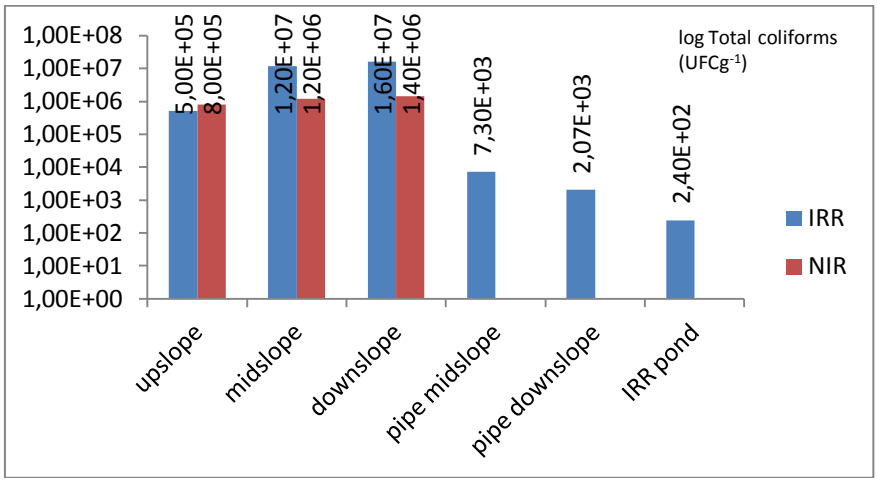


Fig. 5.5: Total coliforms in soil (0-5 cm) in Gačnik, July 2006.

Faecal coliforms and E.coli

Faecal coliforms and *E. coli* were obviously present in higher numbers at downslope position, similarly to data of total coliforms (Fig. 5.6 & 5.7). Enumeration of faecal coliforms and *E. coli* gave higher numbers in IRR row again (at lower slope positions). Following the presence of these bacteria in irrigation water, irrigation pond was obviously the source of faecal coliforms.

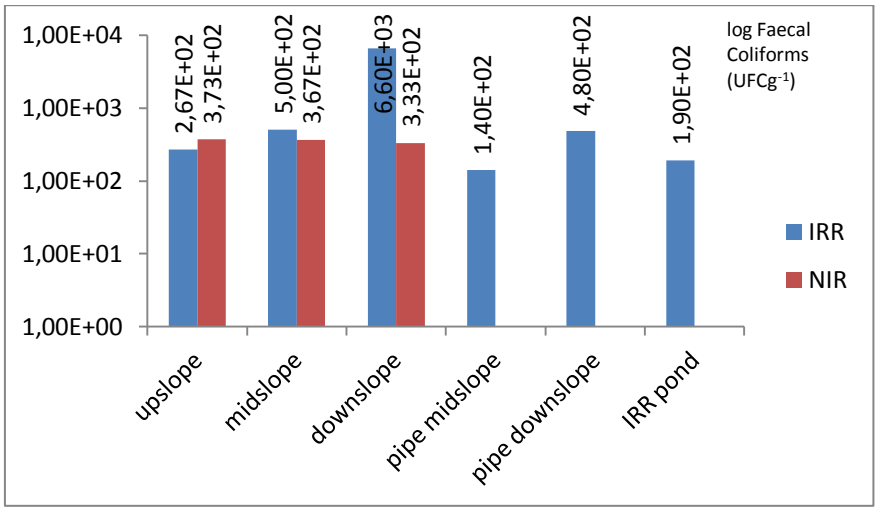


Fig. 5.6: Faecal coliforms enumeration in soil (0-5 cm) in Gačnik, July 2006.

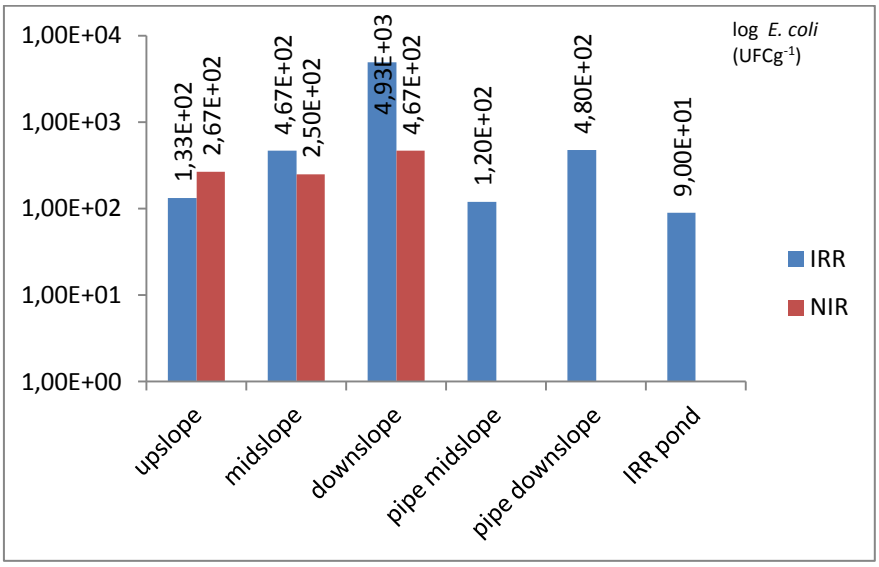


Fig. 5.7: *E. coli* enumeration in soil (0-5 cm) in Gačnik, July 2006.

I.2.3 *Presence of viruses*

Rota virus

All soils and water samples were free of Rotavirus (Fig. 5.8).

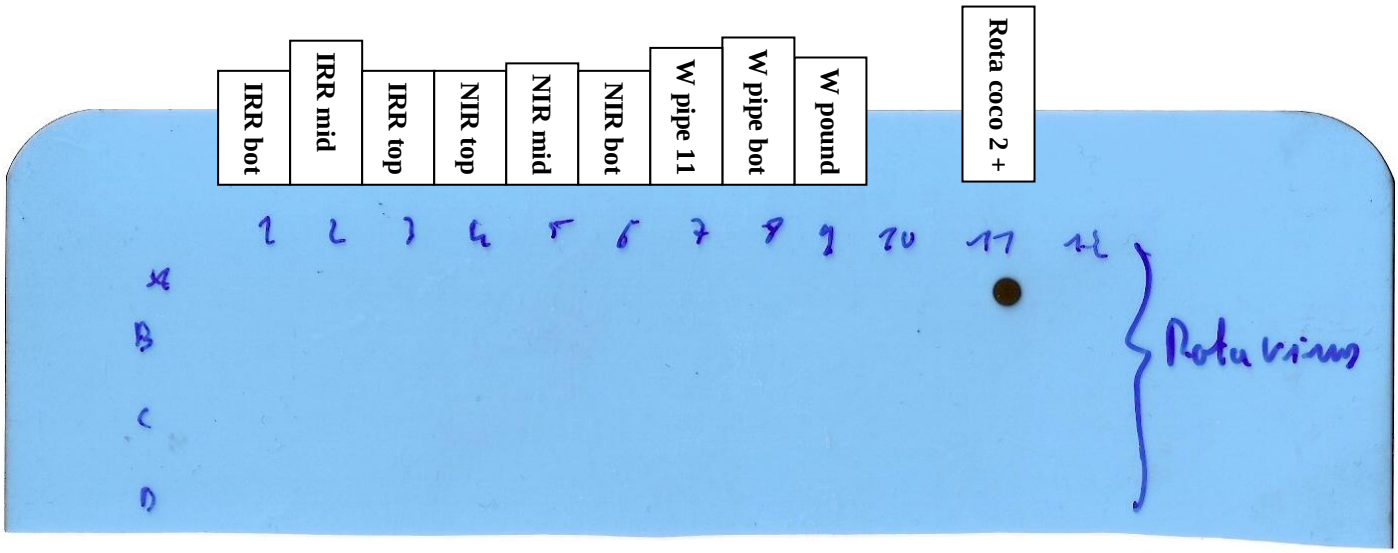


Fig. 5.8: Dot blot results for Rotavirus in water and soil samples (0-5 cm) in Gačnik, July 2006.

Hepatitis E virus (HEV)

HEV was present in all water samples (from irrigation pond and pipes) (Fig. 5.9a). In soil, HEV was detected only in IRR row at upslope position (Fig. 5. 9b).

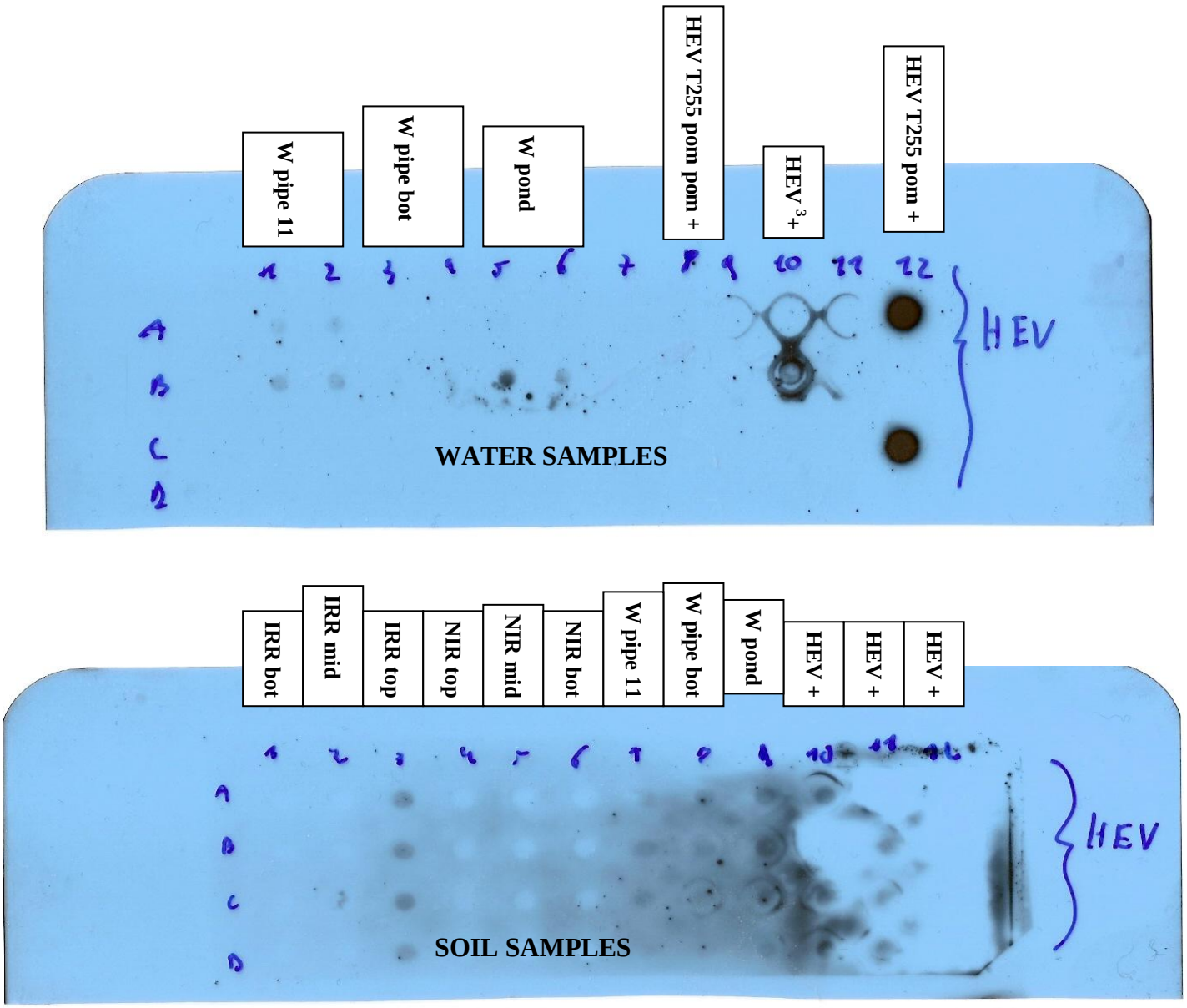


Fig. 5.9a & b: Dot blot results for HEV in water (a) and soil samples (b) in Gačnik, July 2006.

I.3 Discussion

Agriculture is the cause and the victim of water pollution at the same time (Ongley, 1996). Most farmers use surface water rather than ground water for irrigation, due to availability and lower operational costs. The inherent difficulty is that surface water resources are generally more susceptible to microbial contamination. Irrigation with microbiologically poor-quality water is a potential contaminating way for fruits and vegetables by pathogens and once on foodstuffs such as vegetables, pathogens may persist under normal storage conditions over the usual time between purchase and consumption. Waterborne pathogens may survive for days to months in the environment (in soil or on crops), according to abiotic conditions (especially temperature and sunlight exposure) (Gerba, 2006).

A number of documented outbreaks of human infections were associated with the consumption of raw fruits, vegetables, and non-pasteurised fruit juices. The bacteria most commonly found in polluted water are coliforms excreted by humans (Lawson *et al*. 1991; Beller *et al*. 1997; De Serres *et al*. 1999) and outbreaks with identified etiology were predominantly of bacterial origin, primarily with *Escherichia coli* O157:H7. *Escherichia coli* is an indicator organisms that is widely used to detect faecal contamination of water and the assumption is that if faecal coliform bacteria are present in a sample, then human pathogenic bacteria could also exist (FAO guideline, 1992). Infection has mainly been associated with consumption of lettuce, sprouts, and apple juice, but enterotoxigenic *E. coli* has also been linked to carrots (Buck *et al.*, 2003). Furthermore, a special care must be taken to emerging Hepatitis E virus.

In our study, the distribution of bacteria and fungi was positively related to soil water content and soil organic matter (SOM). Fungi and bacteria were numerically more present at downslope position, where soil moisture, organic matter and microbial biomass were higher. At downslope, bulk density was higher as well, but soil moisture was a more important factor for aerobic bacteria and aeration there was probably good enough. Also anaerobic bacteria were mostly present at downslope, which was expected according to higher bulk density. Upslope position was very poor with soil fungi. Total and faecal coliforms were mostly present at downslope position.

According to treatment, no significant differences in fungi and bacteria enumeration occurred, while higher numbers of total and faecal coliforms were noticed in irrigated row. More coliforms in IRR row could therefore be as possible remains of previous irrigations. Indeed, most bacteria and viruses are known to die in a few weeks to a few months, but much longer survival times have also been reported (FAO guideline, 1992). The Slovenian government published a guideline for irrigation water: 1000 MPN/l total coliforms for the crops which are consumed raw or cooked (except for drip irrigation). In our water samples, the range of total coliforms was at the above-mentioned level (Slovenian guideline), what can be considered as risky. Mitigating circumstances is that drip irrigation is an exception and irrigation water does not have a direct contact with fruits.

Fungi and anaerobic bacteria decreased in irrigation water from the pond towards water pipes. The opposite was observed for the aerobic bacteria and coliforms, probably due to higher oxygen concentration in pipes. Water from irrigation pond contained significantly less aerobic bacteria than from irrigation pipe. Pipes were not always filled with water and most of the time oxygen availability was high enough to support microbial growth from dissolved organic carbon. Faecal coliforms and *E. coli* were detected in water samples as well with the highest number in irrigation pipe at downslope position.

HEV viruses were detected in water from irrigation system and in soil from upslope, which could represent a serious human health risk. Many studies (Hamilton *et al.*, 2005 and Toze, 2006) have shown that viruses are capable of entering the plant through the root system (manuring) or when irrigation aerosols contaminate the plant surface. There is a defined potential for food borne transmission of Hepatitis E virus (HEV) and rotavirus but corroborating information is lacking and this type of virus has not been isolated directly from foods.

In our study, high pollution in irrigation pipe was noticed, where also COD and BOD levels were higher than in the pond. Previous bacterial results support this ascertainment. Irrigation

water was obviously the source of faecal coliforms and HEV virus. The question was what was the source of these pathogens in irrigation water. A possible explanation was faeces from the old field toilet, settled in the hill 50 m above the irrigation pond (15% slope). This toilet was not active anymore, but it could be used by seasonal workers occasionally. Additional source of pathogens could be the faeces of wild animals visiting the orchard.

II Pohorski dvor site: effect of organic fertiliser and lime

In Pohorski dvor, the effect of Compo guano (organic fertiliser) and liming on soil health was studied. The following treatments were included: no lime and no Compo guano (5), no lime with Compo guano (10), with Compo guano and no lime (1) and with Compo guano and lime (6). For this purpose, enumeration of specific bacteria (coliforms, *E.coli*), viruses (Rotavirus, HEV) and fungi was done.

II.1 Summary of previous results

Before presenting the specific microbes at this location, some basic previous results should be remembered:

- Initial soil pH at this location was very low (4.83 in KCl). Combination of lime and Compo guano increased soil pH, while Compo guano alone increased soil acidity.
- C/N ratio (10.9) indicates good microbial activity. The level of soil Corg is good (around 2 % in0-30 cm).
- Liming alone had a positive short-time effect on microbial biomass (BM), but on the cost of lower labile organic matter (LOM) after intensive respiration. In long-term period, liming alone decreased both BM and LOM. Compo guano contributed to more intensive microbial respiration and slight increase of active Corg. Combination of Compo guano and liming increased soil microbial biomass.
- Combination of lime and Compo guano contributes to higher soil nitrates values.
- During the study, liming slightly decreased gravimetric water content.

Parallel with analyses of specific microbes, soil pH, temperature and gravimetric water content were measured in the surface soil layer (0-5 cm). No significant difference was noticed among treatments regarding soil temperatures (5 cm depth) and soil pH. The average soil temperature (at the sampling date) was 12.5°C and soil pH (in KCl) was 4.83.

II.2 Specific microbes' characterization

Specific microbial analyses were done in October 2006 in the upper soil layer (0-5 cm).

II.2.1 *Fungi enumeration*

Compo guano with liming (Treatment 6) and pure control (treatment 5) showed significantly higher number of fungi compared to the other treatments (Fig. 5.10).

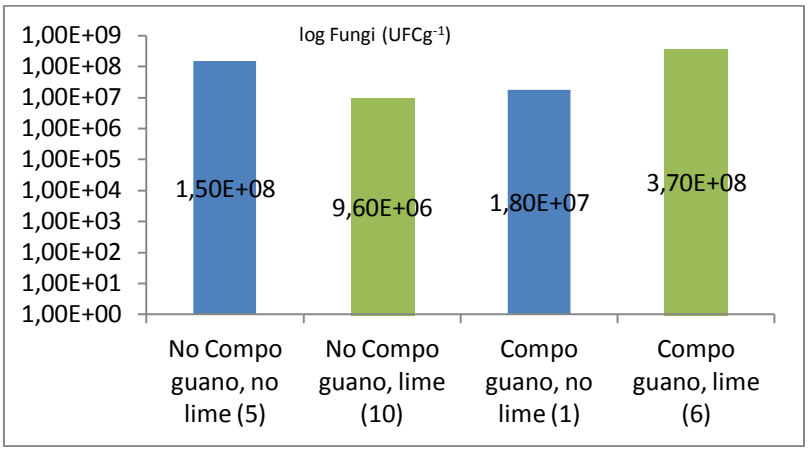


Fig. 5.10: Fungi enumeration in soil (0-5 cm) at Pohorski dvor, October 2006.

II.2.2 *Bacteria enumeration*

Aerobic bacteria

Aerobic bacteria are more numerous in case of liming, especially in combination with Compo guano (Fig. 5.11).

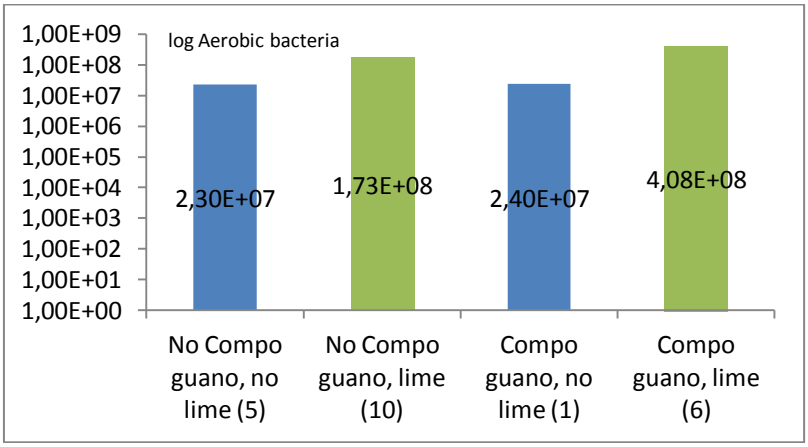


Fig. 5.11: Aerobic bacteria in soil (0-5 cm) at Pohorski dvor, October 2006.

Anaerobic bacteria

According to treatment, there were no significant differences in anaerobic bacteria enumeration (Fig.5.12).

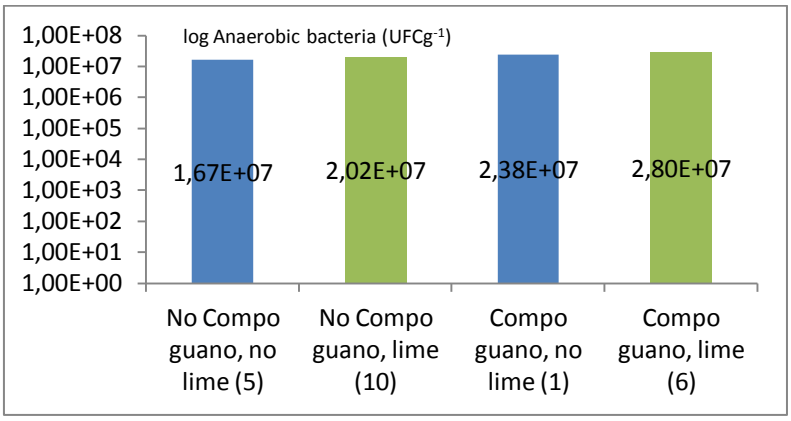


Fig. 5.12: Anaerobic bacteria enumeration in soil (0-5 cm) at Pohorski dvor, October 2006.

Total coliforms

Liming without Compo guano significantly increased number of total coliforms (Fig.5.13). Indeed, the enumeration medium is not really specific and a rather wide range of bacteria can grow in these conditions.

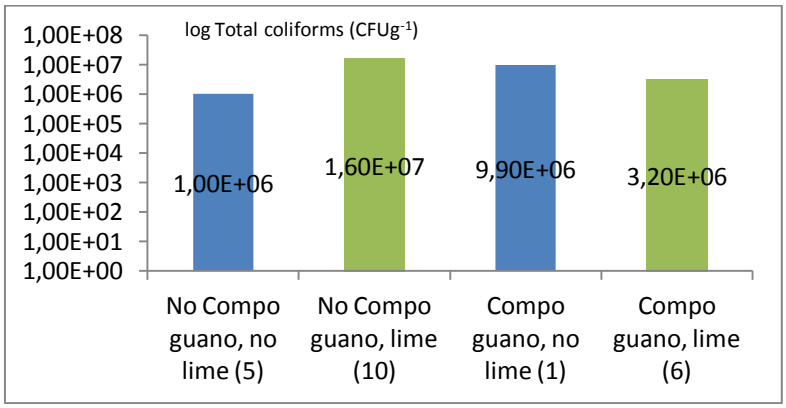


Fig. V.13: Total coliforms in soil (0-5 cm) at Pohorski dvor, October 2006.

Faecal coliforms and E. coli

Compo guano significantly contributed to higher numbers of faecal coliforms (Fig.5.14). *E. Coli* was only detected in Compo guano (treatment 1) at a density of $1.3x10^3$.

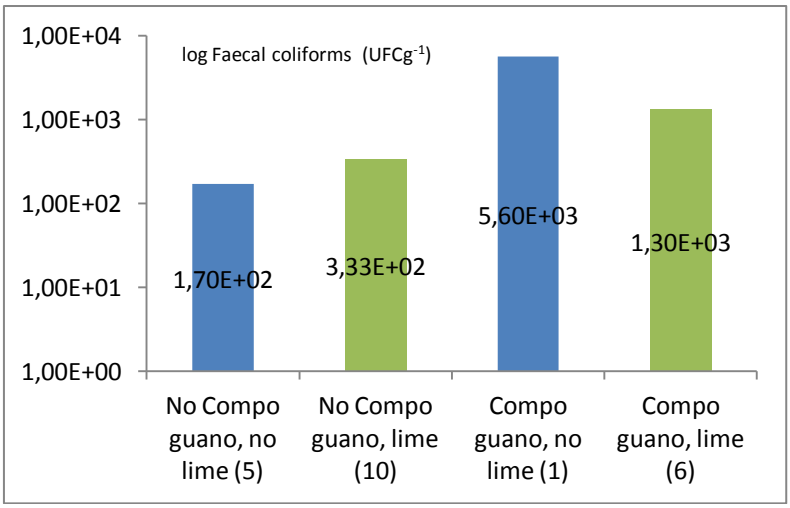


Fig. 5.14: Faecal coliforms enumeration in soil (0-5 cm) at Pohorski dvor, October 2006.

II.2.3 *Presence of viruses*

Rota virus was present in all soil samples, while no Hepatitis E virus was detected.

II.3 Discussion

Fungi were numerically more abundant in two very different situations: in the soils with lower pH (treatment 5; pH=5.3) and in the soils with higher pH (treatment 6; pH=6.6). Total bacteria (aerobic and anaerobic) were mostly present in the soils with higher pH (both treatments with liming). In general, fungi seem to dominate in acid soils. This has more to do with the inability of bacteria to compete with fungi at lower soil pH than to the better adaptability of fungi at these lower pH values. Fungi with their hyphae can grow into favourable micro sites in the soil, whereas bacterial may not be able to do so (Bezdicek *et al*., 2002).

Compo guano in combination with liming increased the number of fungi and aerobic bacteria. Liming alone contributed to a slightly higher number of total coliforms, probably due to higher pH. Compo guano alone significantly contributed to a higher number of faecal coliforms, especially *E. Coli*. Adding Compo guano alone is therefore more risky for pathogens (faecal coliforms) than in combination with liming. Beside that, this combination was the most useful from microbial activity aspect. Treatments did not affect the presence of viruses. Rota virus was present in all soil samples, therefore Compo guano was not its source.

III. Short conclusions for both locations

1. Drip irrigation with poor sanitary quality water can be a potential source of pathogens pollution.
2. It has to be underlined that pathogens may survive in the soil for longer periods of time, more than a year.
3. Organic amendments are a good solution for replacing mineral nitrogen fertilisers, but they can be risky according to some pathogens which can remain after incorrect or bad composting procedure.
4. Microbes are in close relations with physico-chemical soil characteristics, therefore a comprehensive approach to soil quality and health should be taken.

According to previous conclusions, some recommendations are proposed:

1. From sanitary aspects, irrigation water should be regularly monitored to avoid potential pathogens for human health
2. It is very important to emphasize that organic products, which are applied as amendments or fertilisers, should be properly prepared and checked for certain pathogens. The use of compost in soils requires that it achieves an adequate degree of maturity, which implies stable organic matter content and the absence of phytotoxic compounds and plant or animal pathogens (Bernal *et al*., 1998). Especially dangerous are organic amendments which originate from animal faeces.
3. Both studies are good examples for being cautious by working in agriculture. Producing yield in a correct manner can enable food safety.

General discussion and conclusions

General discussion and conclusions

In agricultural practices in Slovenia, the importance of soil condition is generally underestimated. Underestimation of soil properties and poor understanding of soil conditions can have many bad consequences, which results in quality or quantity of yield, in soil degradation or even environmental pollution.

Obligatory agricultural soil analyses which are performed in Slovene laboratories (ordered by farmers) are very basic (chemical only). Physical and even microbiological analyses are ordered exceptionally, for some scientific researches. The reason is also that Slovene soil science labs usually lack such equipment. Especially soil microbiology is a very young science in Slovenia and a lot should be done for its support and development. Especially in agriculture, there is an enormous lack of this knowledge in Slovenia.

In 2011, around 1.6% of all agriculture lands (7.275. ha) were irrigated in Slovenia. Slovenian National irrigation project was established in 1998, but we still do not have any serious studies how irrigation affects soil properties or even soil microbes. It is also necessary to stress that the conditions in Slovenia are extremely heterogeneous and it is therefore required to perform analyses on the basis of locally specific conditions for every single scheme, which makes studies more complicated. In north eastern Slovenia many orchards lay on hilly terrains, so also the technical measures on different slopes and the possibility of erosion should be take into consideration. It is very important to estimate the benefit of irrigation after an appropriate soil analysis and before building the irrigation system and also after to supervise the effects of irrigation on soil properties as long as irrigation is used. With improper irrigation a lot of damage to the environment could be done. Monitoring irrigation water quality in presence of pesticide, metals, salts or even pathogens, is more an exception than a rule.

In the last few years organic food became very popular in Slovenia and every year more farmers decide for organic production. Slovenia still has convenient natural conditions for organic farming, so small farmers see in this trend a good or even the only solution for surviving (mostly in combination with so called ‘eco-tourism’). In the year 2010 almost 3% of Slovene farmers are registrated as organic producers, which represents 30.696 ha of all agricultural lands. Some consumers doubt the food safety in organic production because soil with applied material (like some organic fertilizers in our case) can be a potential pathogen source. This new challenges about food safety and organic production are very interesting and require further studies because it can also help people to be acquainted with healthy nourishment. In this area not much research was done in Slovenia.

According to the importance of the mentioned agricultural practices, our study focused on their impact on soil quality and health. It was interesting, that investigation work was performed on the field study, which is useful to get valuable experiences in soil understanding according to all accompanging circumstances.

In the first part we were dealing with irrigation practice including slope and their effects on soil properties, especially on soil structure and soil microbes. The second part is dealing with the possibility of enhancing microbial biomass and nitrogen nutrition with different combinations of organic fertiliser and liming.
In the first part of the thesis, some aims were settled according to irrigation and slope effect.

To achieve the first aim of the project, which was 'to define the effect of irrigation and slope on soil physics, chemistry and soil microbiology', several physico-chemical and microbiological analyses were done. The main conclusions associated with irrigation and slope effect are:

- According to hilly terrain and land levelling, we were dealing with two groups of soil: one at upslope and another one at mid and downslope. Considering soil characteristics, slope effect was more expressed than irrigation effect.
- According to slope, water gravimetric content, organic matter, microbial biomass and respiration increased towards downslope; while total carbonates and structural stability increased towards upslope.
- Among chemical properties of irrigation water, only the amount of nitrates was high, which probably contributed to higher mineral nitrogen values in IRR soils.
- Lower gravimetric water content in irrigated row could be explained by modification in root distribution and developing due to drip irrigation.
- Irrigation leads to increasing of soil microbial biomass and its activity (as a short-time effect) and decreasing of total soil organic matter amount (as a long-term effect). Lower C/N ratio of soil organic matter and higher respiration (Cmin) are more intensive in IRR row with lower remaining SOM in this treatment.
- Irrigation affects soil structure in sense of degradation, what is associated with organic matter reduction.

To achieve the second aim of the project, which was 'to determine the connections between microbial indicators and (especially) physical properties of soils, with the interpretation of the results in the case of irrigation', some comparisons among important parameters were done. The main conclusions associated with connections are:

- Gravimetric water content is not always a major factor which governs microbial biomass and activity According to treatment, microbial biomass was higher in IRR row despite lower soil moisture. Too much water can also have a negative effect on soil microbes.
- Comparing microbial biomass and void ratio, both parameters decrease according to depth.
- According to treatment, microbial biomass was not in positive correlation with SOM, but according to slope and depth positive correlations between them were found. This is explained by higher C/N ratio and higher microbial respiration rate in IRR row.
- A major microbiological biomass and its activity (including mineralisation) occurred in IRR row and here TOC was lower. Microbes may attack sedimentary organic matter and this could additionally explain lower TOC value there. Organic matter, which is entrapped in geological substratum (marl) may be mineralised by irrigation process, which is very important according to the environmental point of view.
- Microbial biomass and its activity were not in positive correlation with structural stability, as expected. According to treatment, higher carbonate content was probably more important factor for higher structural stability than organic matter pool.

To achieve the third aim of the project, which was 'to propose microbial indicators which could be useful for assessing soil quality, health and sustainability in the case of irrigation', the following proposals are made:

- Soil microbial biomass, microbial respiration rate and labile organic matter pool quickly respond to environmental conditions as to irrigation.

- Monitoring irrigation water of some pathogens like faecal coliforms (including *E. coli*), HEV and Rota viruses enable us safe fruit production.

To achieve the forth aim of the project, which was 'to contribute to the knowledge of side-effects of irrigation on soil ecology', some conclusions were made. The main conclusions associated with side effect of irrigation:

- Irrigation causes some indirect consequences in soils like modifying of root system growth, what contributing to higher water demand and thus drier soils.
- Irrigation impacts soil structural stability, which can affect water movement through the soil profile, erosion processes with run-off water along the slope and crusting.
- Organic matter, which is entrapped in geological substratum (marl), may be mineralised by irrigation process, which is very important according to environmental point of view.

To achieve the fifth aim of the project, which was 'to enable a comprehensive approach, including soil physical and microbial properties ', some relationships among the important parameters are emphasized:

- Soil organic matter was not always positively related to structural stability: there is positive correlation according to treatment and negative correlation according to slope and depth.
- A high percentage of total carbonates in soil contributes to higher structural stability, what explaining higher structural stability with lower organic matter content.
- Seasonal variations of structural stability can not be explained only with soil water content, however, rainfall and microbial biomass information are helpful.
- According to relationships between microbial parameters and physico-chemical soil characteristics, some findings are presented in the second aim.

To achieve the sixth aim of the project, which was 'to control irrigation water and soil for possible water or soil-borne pathogens, some chemical and biological analyses were made. The main conclusions associated with irrigation water as a potential source of pathogens are:

- Irrigation water contains high nitrate concentration, increasing soil nitrates in IRR row.
- High chemical (COD) and biochemical oxygen demand (BOD) in irrigated water indicates water pollution.
- The presence of faecal coliforms and HEV virus in irrigated row indicates pollution from irrigated water, which was applied more than one year ago. This means that a virus can stay in soil for a longer period of time.

The following hypotheses were examined according to irrigation project:

1. **Irrigation and slope affect soil microbial biomass and activity.**
   This hypothesis is confirmed for irrigation and slope.
2. **Irrigation and slope affect on soil physical parameters like bulk density, structural stability, organic matter grain size fraction, gravimetric water content and available water content.**
   This hypothesis is confirmed for porosity, structural stability, composition of organic matter grain size fractions, gravimetric water content and available water content (the last is confirmed only according to treatment).

3. **The relationships between soil physical properties and soil microbial parameters are controlled by water supply and slope.**
   This hypothesis is confirmed for irrigation and slope, but it has to be studied in a complex manner.
4. **Microbial and physical soil indicators are appropriate for assessing soil quality in case of irrigation.**
   This hypothesis is confirmed for microbial biomass, respiration rate, labile organic matter pool and for structural stability.
5. **Irrigation water can represent a potential source of pathogens.**
   This hypothesis is confirmed for faecal coliforms (including *E. Coli*) and HEV.

In the second part of the thesis, some aims were settled according to organic fertilising and liming.

To achieve the first aim of the project, which was 'to elucidate the effect of applied organic matter on nutrient cycling, soil chemistry and soil microbiology (microbial biomass and microbial activity), some chemical and microbiological analyses were done. The main conclusions are:

- Compo guano contributes to a small increase in active Corg
- Compo guano in the combination with lime increases microbial biomass and activity.
- Compo guano contributed to the highest nitrate values when in combination with liming.

To achieve the second aim of the project, which was 'to elucidate the effect of organic mater (used as fertilizer) and liming on soil pH, mineralization processes', some microbiological analyses were done. The main conclusions are:

- Compo guano alone decreases soil pH, while in the combination with lime soil pH rises.
- Compo guano (alone) contributes to more intensive microbial mineralisation.
- Liming alone had a positive short-term effect on microbial biomass (MB), but on the cost of lower labile organic matter (LOM) after intensive respiration. During the longer period, liming alone decreased both MB and LOM.
- Liming increased soil pH (especially in upper soil layer).
- Liming alone decreased soil moisture and increased water potential probably due to effects on pore space, inducing better water drainage and better structure.
- Liming (without Compo guano) increased microbial biomass and decreased LOM as a short time effect. The long time effect was decreasing LOM and MB.
- The interaction of Compo guano and lime together was not clear, but in long term this is probably the best solution because it had positive consequences on both soil pH and available nitrogen, while preserving fair levels of MB and LOM.

To achieve the third aim of the project, which was 'to propose microbial indicators which could be useful for assessing soil quality and soil health in orchards', some proposals were made:

- Microbial biomass, labile organic matter pool and microbial mineralization can be used as a quick response to environmental conditions.

To achieve the fourth aim of the project, which was 'to compare behavior of microbes under organic fertilizing and liming in two different soil types according to water conditions', the following ascertainment was found:

- Difference in soil water regime, which occurred between blocks, doesn't affect soil microbes. Soil humidity is not such a limited factor for soil microbes as organic matter.

To achieve the fifth aim of the project, which was 'to control presence of some pathogens in soils, some biological analyses were done:

- Organic amendments are a good solution for replacing mineral nitrogen fertilisers, but they can be risky on account of some pathogens (faecal coliforms and Rota virus in our case), which can remain after incorrect or bad composting procedure.

The following hypotheses are examined:

1. **Organic fertilisers and liming increase soil microbial biomass and its activity**
   This hypothesis is confirmed by the combination of both.
2. **Organic matter and liming affect soil pH and mineralization process.**
   This hypothesis is confirmed (see the second achieved aim).
3. **Organic fertilising and liming have different effect on soil microbes in different soil types (according to water conditions).**
   This hypothesis is rejected (see the fourth achieved aim).

New perspectives:

1. By analysing some physical and microbiological properties in one realistic field experiment with irrigation system or organic farming, it is possible to show some new perspectives to the producers and more attention might be paid to some important soil facts which were overlooked before. For this purpose, more educational training about the consequences of agricultural practises in the environment should be organised.

2. For improving the benefits of irrigation systems or organic farming, we need a more comprehensive approach for estimating soil quality and health. This evaluation requires focusing not only on chemical (fertility) considerations, but also on the dynamic soil conditions – a combination of physical, biological and chemical characteristics, which are directly affected by recent and current land use decisions and practices. It might be useful to find some quality indexes, which include the most important parameters and adjust them to special agricultural practices or land use.

3. Emphasis has to be placed on the quality of materials, which are used in agricultural practices and have a direct contact with soil. It does not matter if we deal with irrigation water or with organic fertiliser; we have to be sure about certain chemical or microbiological parameters (pathogens) according to environmental pollution policy or human health safety. For this reason, monitoring of irrigation water and preliminary analyses of organic fertilisers are required, but initially basic soil parameters which are the main limiting factors for soil microbial activity (pH, SOM, humidity), should be improved especially in organic farming.

4. For better estimation of the soil, we need to improve equipment and enlarge scientific groups according to work in this field. It would be convenient to settle at least one big (with few blocks) experiment without disturbing factors (like a slope), where the effect of irrigation/organic farming can be studied for a longer period of time.

5. It would be interesting to further investigate structural stability according to the participation of organic carbon from the bedrock, using isotopic methods of δC13 to distinguish different types of SOM. Further research on micro and macro-aggregates level can be made according to the amount of total and specific SOM types (fresh and sedimentary SOM). These analyses should be improved with microbial analyses as well to examine our hypothesis on microbial mineralisation of the sedimentary SOM.

6. It would be also interesting to study structural stability in organic farming according to different organic fertilisers in relationship with soil microbes.

7. According to soil health, some relations among polluted water or infected organic fertiliser, soils and yield (fruits) will be welcome to understand and ascertain the transport of certain pathogens.

According to previous perspective, enormous and interesting work in the future is waiting for us.

References

References

ABREU, X. - *Evaluación del efecto de las propiedades hidrológicas y sistema de manejo sobre la susceptibilidad a erosión superficial y en masa en suelos pedregosos con viña del Priorato (Cataluña, España)*.- PhD Thesis, University of Lleida, Lleida, Spain, 2005.

ACTON, D.F. and GREGORICH, L.J. - Understanding soil health. - In: ACTON, D.F. and GREGORICH, L.J., Eds., 1995. - *The Health of Our Soils: Toward Sustainable Agriculture*. - Canada, Centre for Land and Biological Resources Research, Research Branch, Agriculture and Agri-Food Canada, Ottawa, 1995, 5-10.

AFNOR. - *Qualité des sols - Méthodes physiques. Méthode de détermination du volume apparent et du contenu en eau des mottes* - 1992, NF X31-505, 373-384.

AFNOR. - *Analyse granulométrique par sédimentation - Méthode à la pipette*. - 1983, NF X31-107.

AFNOR. - *Qualité des sols - Détermination de la masse volumique des mottes - Méthode par enrobage à la paraffine - Indice de classement*. - 1993, NF X31-510.

AFNOR. - *Qualité des sols - Détermination des cations Ca^{++}, Mg^{++}, K^{+}, Na^{+} extractibles par l'acétate d'ammonium - Méthode par agitation*. - 2002, NF X31-108.

AFNOR. - *Qualité des sols - Détermination du calcaire actif*. - 2002, NF X31-106.

AFNOR. - *Qualité des sols - Détermination du phosphore soluble dans une solution d'oxalate d'ammonium à 0,1 moll $^{-1}$ - Méthode Joret-Hébert*. - 1999, NF X31-161.

AFNOR. - *Qualité des sols - Méthodes chimiques - Détermination de la capacité d'échange cationique (CEC) et des cations extractibles (Version corrigée)*. - 1999, NF X31-130.

AFNOR. - *Qualité des sols - Méthodes Chimiques - Détermination du Carbone Organique par oxydation sulfochromique*. - 1993, NF X31-109.

AFNOR. - Qualité des sols. - *Méthodes chimiques. Détermination du cuivre, du manganèse, du zinc et du fer. Extraction en présence d'EDTA*. - 1993, NF X31-120.

AGBENIN, J.O., TIESSEN, H. - Soil properties and their variations on two contiguous hillslopes in Northeast Brazil. - *Catena* , 1995, 24, 147-161.

ALBIACH, R., CANET, R., POMARES, F. and INGELMO, F. - Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. - *Bioresource Technology*, 2000, 75, 43-48.

ALBIACH, R., CANET, R., POMARES, F., INGELMO, F. - Organic matter components and aggregate stability after the application of different amendment to a horticultural soil. - *Bioresource Technology*, 2001, 76, 125-129.

AMELUNG, W., ZECH, W., ZHANG, X., FOLLETT, R.F., TIESSEN, H., KNOX, E., FLACH, K.W. - Carbon, nitrogen, and sulfur pools in particle-size fractions and influenced by climate. - *Soil Science Society of American Journal*, 1998, 62, 17-181.

ANDERSON, T.-H. - Microbial eco-physiological indicators to assess soil quality. - *Agriculture, Ecosystems & Environment*, 2003, 98(1-3), 285-293.

ANDERSON, T.-H. - The influence of acid irrigation and liming on the soil microbial biomass in a Norway spruce (Picea abies [L.] Karst.) stand. - *Plant and Soil*, 1998, 199, 117-122.

ANDREUX, F., BRUCKERT, S., CORREA, A., SOUCHIER, B. - Sur une méthode de fractionnement physique et chimique des agrégats des sols: origines possibles de la matière organique des fractions obtenues. - *CR Acad Sci Ser D*, 1980, 291, 381-385.

ANDREUX, F., CERRI, C., VOSE, P.B., VITORELLO, V.A. - Potential of stable isotope, 15N and 13C methods for determining input and turnover in soils. - In: HARRISON, A.F., INESON, P. (Eds.). - *Nutrient Cycling in Terrestrial Ecosystems*. - *Elsevier Applied Science*, London, 1990, 259-275.

ANDREWS, S.S. and CAROLL, C.R. - Designing a soil quality assessment for sustainable agroecosystem management. - *Ecological Applications*, 2002, 11(6), 1573-1585.

ANDREWS, S.S., KARLEN, D.L., MITCHELL, J.P. - A comparison of soil quality indexing methods for vegetable production system in Northern California. - *Agriculture, Ecosystems & Environment* 2002, 90, 25-45.

APHA (American Public Health Association). - *Standard Methods for the Examination of Water and Wastewater*. - 17th ed., Washington, D.C., American Public Health Association, 1989.

ATLAS, M.R. and BARTHA, R. 1997. - *Microbial ecology: fundamentals and application*. - Menlo Park, California, Benjamin/Cummings Science Publishing, 1989, 694 pp.

AYERS, R.S. and WESTCOTT, D.W. - Water quality for agriculture FAO. - *Irrigation and Drainage Paper*, 1985, 29, Rev.1, 174 p.

BACHMANN, J. and HARTGE, K.H. - Estimating soil stress distribution by using depth-dependent soil bulk density data. - *Journal of Plant Nutrition and Soil Science*, 2006, 169, 233-238.

BADALUCCO. L., GREGO, S., DELL'ORCO, S. and NANNIPIERI, P. - Effect of liming on some chemical, biochemical, and microbiological properties of acid soils under spruce (Picea abies L.). - *Biology and Fertility of Soils*, 1992, 14, 76-83.

BADR, M.A. - Spatial distribution of water and nutrients in root zone under surface and subsurface drip irrigation and Cantaloupe yield, - *World Journal of Agricultural Sciences*, 2007, 3(6), 747-756.

BALESDENT, J. - The significance of organic separates to carbon dynamics and its modelling in some cultivated soils. - *European Journal of Soil Science*, 1996, 47, 485-493.

BALESDENT, J., MARIOTTI, A. - Measurement of soil organic matter turnover using 13C natural abundance. - In: BOUTTON, T.W., YAMASAKI, S.I. (Eds.). - *Mass Spectrometry of Soils*. - Marcel Dekker, New York, 1996, 83-111.

BARTOLI, F., BURTIN, G., ROYER, J.J., GURY, M., GOMENDY, V., PHILIPPY, R., LEVIANDIER, Th., GAFREJ, R. - Spatial variability of topsoil characteristics within one silty soil type. Effects on clay migration. - *Geoderma*, 1995, 68, 279-300.

BARTOLI, F., PHILIPPY, R., DOIRISSE, M., NIQUET, S., and DUBUIT, M. - Structure and self- similarity in silty and sandy soils: the fractal approach. - *Journal of Soil Science*, 1991, 42, 167-185.

BELKACEM, S. and NYS, C. - Consequences of liming and gypsum topdressing on nitrogen and carbon dynamics in acid forest soils with different humus forms. - *Plant and Soil*, 1995, 173, 79-88.

BELLER, M., ELLIS, A., LEE, S.H., DREBOT, M.A., JENKERSON, S.A., FUNK, E., SOBSEY, M.D., SIMMONS, O.D., MONROE, S.S., ANDO, T., NOEL, J.S., PETRIC, M., MIDDAUGH, J.P. and SPIKA, J.S. - Outbreak of viral gastroenteritis due to a contaminated well. - *Journal of the American Medical Association*, 1997, 278, 563-568.

BERNAL, M.P., PAREDES, C., SANCHEZ-MONEDERO, M.A. - Maturity and stability parameters of composts prepared with a wide range of organic wastes. - *Bioresource Technology*, 1998, 63, 91-98.

BEZDUCEK, 2002.

BHARDWAJ, A.K., GOLDSTEIN, D., AZENKOT, A., LEVY, G.J. - Irrigation with treated wastewater under two different irrigation methods: Effects on hydraulic conductivity of a clay soil. - *Geoderma*, 2007, 140, 199-206.

BILGEHAM., G. - Structure characteristics of soil in Harran Plain before irrigation. - M. Şefik Yeşilsoy Int. S Menemen, Izmir, 1998, 208-214.

BLAKE, G.R. - Bulk density. - In: *Methods of soil analysis*. - American Society of Agronomy, Madison, 1965, 374-390.

BLAKE, G.R., HARTGE, K.H. - Bulk density. - In: KLUTE, A. (Ed.). - *Methods of Soil Analysis. Part 1*. - 2nd ed., American Society of Agronomy, Madison, 1986, WI, 363-382.

BLOEM, J., SCHOUTEN, T., SØRENSEN, S., BREURE, A.M. - Application of microbiological indicators in ecological approaches to monitor soil quality. - *Agriculture, Ecosystems & Environment*, 1998, 98(1-3), 255-262.

BLUME, E., BISCHOFF, M., REICHERT, J.M., MOORMAN, T., KONOPKA, A., TURKO R.F. - Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. - *Applied Soil Ecology*, 2002, 20(3), 171-181.

BORSELLI, L., TORRI, D., ØYGARDEN, L., DE ALBA, S., MARTÍNEZ-CASASNOVAS, J.A., BAZZOFFI, P., JAKAB, G. - Land levelling. - In: BOARDMAN, J., POESEN, J. (Eds.), - *Soil erosion in Europe*. - John Wiley and Sons, Inc., 2006.

BOUMA, J. - The land use system approach to planning sustainable land management at several scales. - *ITC Journal*, 1997, 3/4, 237-242.

BOUTTON, T.W. - Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. - In: BOUTTON, T.W., YAMASHI, S. (Eds.). - *Mass Spectrometry of Soils*. - *Marcel Dekker*, New York, USA, 1996, 47-82.

BRICCHI, E., FORMIA, F., ESPÓSITO, G., RIBERI, L., AQUINO, H. - The effect of topography, tillage and stubble grazing on soil structure and organic carbon levels. - *Spanish Journal of Agricultural Research*, 2004, 2, 409-418.

BRONICK, C.J. and LAL, R. - Soil structure and management: a review. - *Geoderma*, 2005, 124, 3-22.

BROWN, M.J., CARTER, D.L., LEHRSCH, G.A., SOJKA, R.E. - Seasonal trends in furrow irrigation erosion in southern Idaho. - *Soil Technology*, 1995, 8, 119-126.

BRULAND, K.W. *et al.* Dahlem. "*Flux to the Seafloor*", Group Report, eds., 1988, 210-213,

BUCHER, E. - *Soil quality characterisation and remediation in relation to soil management*. - PhD thesis, Department of Crop and Soil Sciences, Pennsylvania State University, USA, 2002.

BUCK, J.W, WALCOTT, R.R., BEUCHAT, L.R. - *Recent Trends in Microbiological Safety of Fruits and Vegetables*. - Plant Management Network, 2003.

BUCKLEY, D. H. and SCHMIDT, T. M. - The structure of microbial communities in soil: patterns of microbial distribution their dynamic nature and the lasting impact of cultivation. - *Environmental Microbiology*, 2003, 5, 441-452.

BULLOCK, M.S., KEMPER, W.D. and NELSON, S.D. - Soil cohesion as affected by freezing, water content, time and tillage. - *Soil Science Society of America Journal*, 1988, 52, 770-776.

BULLUCK, L.R., BROSIUS, M., EVANYLO, G.K., RISTAINO, J.B. - Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. - *Applied Soil Ecology*, 2002, 19, 147-160.

BURNS, R.G., NANNIPIERI, P, BENEDETTI, A., HOPKINS, D.W. - Defining soil quality. - In: BLOEM, J., HOPKINS, D.W., BENEDETTI, A. (Eds.). - *Microbiological Methods for Assessing Soil Quality*. - CAB International, Wallingford, 2006, 15-22.

BUSER, S. - Basic geological map of SFRY, sheet Maribor, 1:100.000. - L 33-56 and L 33-44, Federal Geological Survey, Belgrade, 1987.

BUYANOVSKY, G.A., ASLAM, M., Wagner, G.H. - Carbon turnover in soil physical fractions. - Soil *Science Society of America Journal*, 1994, 58, 1167-1173.

CANNELL, R.Q. and HAWES, J.D. - Trends in tillage practices in relation to sustainable crop production with special reference to temperate climates. - *Soil and Tillage Research*, 1994, 30(2-4), 245-282.

CANTÓN, Y., SOLÉ-BENET, A., ASENSIO, C., CHAMIZO, S, PUIGDEFÁBREGAS, J. - Aggregate stability in range sandy loam soils Relationships with runoff and erosion. - *Catena*, 2009, 77, 192- 199.

CAREY, P.L., BENGE, J.R., HAYNES, R.J. - Comparison of soil quality and nutrient budget between organic and conventional kiwifruit orchards. - *Agriculture, Ecosystems & environment*, 2009, 132: 7-15.

CARTER, M.R. - Long-term influence of compost on available water capacity of a fine sandy loam in a potato rotation. - *Canadian Journal of Soil Science*, 2007, 87, 535-539.

CARTER, M.R., GREGORICH, E.G., ANDERSON, D.W., DORAN, J.W., JANZEN, H.H., PIERCE, F.J. - Concepts of soil quality and their significance. - In: GREGORICH, E.G., CARTER, M.R. (Eds.). - *Soil Quality for Crop Product*. - Elsevier Science Publisher, Amsterdam, Netherlands, 1997, 1-19.

Centre for Sustainable Water Management, 2007-2008. *http://www.lec.lancs.ac.uk/cswm/EHH/po*

CERRI, C.C., FELLER, C., BALESDENT, J., VICTORIA, R., PLENECASSAGNE, A. - Application du traçage isotopique naturel en ^{13}C à l'étude de la dynamique de la matière organique dans les sols. - *Comptes Rendus de l'Académie des Sciences de Paris* t. 300, 1985, ser.II, 9, 423-428.

CETIN, B., OZER, H., KUSCU, H. - Economics of drip irrigation for apple (*Malus domestica*) orchards in Turkey. - *New Zealand journal of crop and horticultural science*, 2004, 32 (4), 349-354.

CHALLA, A. - *Impact of irrigation on morphological, physicochemical, and mineralogical properties of soils in trans-pecos, Texas*. - A thesis in soil science, submitted to the Graduate faculty of Texas tech university in partial fulfillment of the requirements for the degree of Master of science, 1987.

CHAN, K.Y. - Soil particulate organic carbon under different land use and management. - *Soil Use Management*, 2001, 17(4), 217-221.

CHAN, K.Y., HEENAN, D.P. and ASHLEY, R. - Seasonal changes in surface aggregate stability under different tillage and crops. - *Soil & Tillage Research*, 1994, 28, 301-314.

CHAN, K.Y., HEENAN, D.P., SO, H.B. - Sequestration of carbon and changes in soil quality under conservation tillage on light-textured soils in Australia: a review. - *Australian Journal of Experimental Agriculture*, 2003, 43, 325-334.

CHAUSSOD, R. - La qualité biologique des sols : des concepts aux applications. - *Comptes Rendus de l'Académie d'Agriculture de France*, 2002, 88, 2, 61-68.

CHAUSSOD, R. - La qualité biologique des sols : évaluation et implications. - *Etude et Gestion des Sols*, 1996, 3, 261-278.

CHAUSSOD, R. - Relations entre les caractéristiques physico-chimiques et microbiologiques de quelques sols cultivés. - *Science du Sol*, 1986, 86(2), 213-226.

CHAUSSOD, R., HOUOT, S., GUIRAUD, D., and HÉTIER, J.M. - Size and turnover of the microbial biomass in agricultural soils: laboratory and field measurements. - In: JENKINSON, D.S. and SMITH, K.A. (Eds). - *Nitrogen efficiency in agricultural soils*. -. Elsevier Applied Science Amsterdam, 1988, 312-326.

CHAUSSOD, R., NICOLARDOT, B., SOULAS, G., JOANNES, H. - Mesure de la biomasse microbienne dans les sols cultivés. II - Cinétique de minéralisation de matière organique microbienne marquée au carbone 14. - *Revue d écologie et de biologie du sol*, 1986, 23 (2), 183-196.

CHRISTENSEN, B.T. - Physical fractionation of soil and organic matter in primary particle size and density separates. - *Advances in Soil Science*, CRC Lewis Publishers, Boca Raton, 1992, 20, 1-90.

CHRISTENSEN, B.T. - Physical fractionation of soil and structural and functional complexity in organic matter turnover. - *European Journal of Soil Science*, 2001, 52, 345-353.

CLARK., G.A., ALBREGTS, E.E., STANLEY, C.D., SMAJSTRLA, A.G., ZAZUETA, F.S. - Water requirements and crop coefficients of drip-irrigated strawberry plants. - *Trans. ASAE*, 1996, 39(3), 905-913.

CLESCERI, L. S., GREENBERG, A. E. and TRUSSELL, R. R. - *Standard Methods for examination of Water and Waste Water*. - 17th edn., American Public Health Association-American Water Works Association-Water Pollution Control Federation, Washington, DC, 1989.

CLOUGH, A., SKJEMSTAD, J.O. - Physical and chemical protection of soil organic carbon in three agricultural soils with different contents of calcium carbonate. - *Australian Journal of Soil Research*, 2000, 38, 1005-1016.

COMITE INTERPROFESSIONNEL DES VINS DE CHAMPAGNE (CIVC). - *Viticulture raisonnée. Guide pratique*. - Ed Association Viticole Champenoise, 5 rue Henri-Martin, 51204 Epernay, 2001.

Convention on Biological Diversity, 2001. *http://www.enu./env/plec/cbd/Montreal/reports/FAO-soil.pdf* (sept., 2003).

COOTE, D.R., MALCOLM-MCGOVERN, C.A., WALL, G.J., DICKINSON, W.T. and RUNDRA, R.P. - Seasonal variation of erodibility indices based on shear strength and aggregate stability in some Ontario soils. - *Canadian Journal of Soil Science*, 1988, 68, 405-416.

CORWIN, D., LESH, S., OSTER, J. and KAFFKA, S. - Monitoring management induced spatial-temporal changes in soil quality through soil sampling directed by apparent electrical conductivity. - *Geoderma*, 2006, 131(3 & 4), 369-387.

COURTNEY, R.G., MULLEN, G.J. - Soil quality and barley growth as influenced by the land application of two compost types. - *Bioresource Technology*, 2008, 99, 2913-2918.

CRAIG, H. - Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. - *Geochimica et Cosmochimica Acta*, 1957, 12, 133-149.

CRANE, S.R., WESTERMAN, P.W. and OVERCASH, M.R. - Die-off of faecal indicator organisms following land application of poultry manure. - *Journal of Environmental Quality*, 1981, 9, 531-537.

ÇULLU, M. A. - Estimation of the Effect of Soil Salinity on Crop Yield Using Remote Sensing and Geographic Information System. - Journal of Agricultural and Food Chemistry, 2002, 27, 23-28.

CURRIE, D.R., GRANT, C.D., MURRAY, R.S. and MCCARTHY, M. - *Does Drip Irrigation Degrade Soil Structure in Vineyards?* - 18th World Congress of Soil Science, Philadelphia, Pennsylvania, USA, July 9-15, 2006.

DANIELSON, R.E., SUTHERLAND, P.L. - Porosizy. - In: KLUTE, A. (Eds.). - *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods*. - American Society of Agronomy and Soil Science Society of America, Madison, 1986, WI, 443-461.

DAVIDSON, D.A. - Soil quality assessment: recent advances and controversies. - *Progress in Environmental Science*, 2000, 2(4), 342-350.

DE BOER, W., HUNDSCHEID, M.P.J., SCHOTMAN, J.M.T., TROELSTRA, S.R. LAANBROEK, H.J. - In situ net N transformations in pine, fir, and oak stands of different ages on acid sandy soil, 3 years after liming. - *Biology and Fertility of Soils*, 1993, 15, 120-126.

DE HAAN, F.A.M., LEXMOND, Th.M., VAN RIEMSDIJK, W.H. Proc. Workshop. - *Indicators and indices for environmental impact assessment and risk analysis*. - ISPRA, A.G. COLOMBO, G. PREMAZZI (Eds.). EUR 13060-EN. EC, Luxemburg, 1990, 161-174.

DE SERRES, G., CROMEANS, T.L., LEVESQUE, B., BRASSARD, N., BARTHE, C., DIONNE, M., PRUD'HOMME, H., PARADIS, D., SHAPIRO, C.N., NAINAN, O.V. and MARGOLIS, H.S. - Molecular confirmation of hepatitis A virus from well water: epidemiology and public health. - *Journal of Infectious Diseases*, 1999, 179, 37-43.

DEGENS, B.P., SCHIPPER, L.A., SPARLING, G.P. and VOJVODIC-VUKOVIC, M. - Decreases in organic C reserves in soils can reduce microbial functional diversity. - *Soil Biology and Biochemistry*, 2000, 32, 189-196.

DEROME, J., KUKKOLA, M., MÄLKÖNEN, E. - *Forest living on Mineral Soils. Results of Finnish experiments*. - National Swedish Environmental Protection Board Report 3084:10107, 1986.

DESJARDINS, T., ANDREUX, F., VOLKOFF, B., CERRI, C.C. - Organic carbon and 13C contents in soils and soil size-fractions, and their changes due to deforestation and pasture installation in eastern Amazonia. - *Geoderma*, 1994, 61, 1-2.

DILLY, O., MUNCH, J.C. - Ratios between estimates of microbial biomass content and microbial activity in soil. - *Biology and Fertility of Soils*, 1998, 27, 374-379.

DORAN, J.W. - Soil health and global sustainability: translating science into practice. - *Agriculture, Ecosystems and Environment*, 2002, 88, 119-127.

DORAN, J.W. and PARKIN, T.B. - Defining and assessing soil quality. - In: DORAN, J.W., COLEMAN, D.C., BEZDICEK, D.F. and STEWART, B.A. (Eds). - *Defining Soil Quality for a sustainable Environment*. - Madison, Wisconsin, Soil Science Society of America Special Pub., 1994, 35, 3-21.

DORAN, J.W. and PARKIN, T.B. - Quantitative indicators of soil quality: a minimum data set. - In: DORAN, J.W., JONES, A.J. (Eds.). - *Methods for Assessing Soil quality*. - Soil Science Society of America, Special publication 49, Madison, 1996, WI, 25-37.

DORAN, J.W., SAFELY, M. - Defining and assessing soil health and sustainable productivity. - In: PANKHURST, C.E., DOUBE, B.M., GUPTA, V.V.S.R. (Eds.). - *Biological indicators of Soil Health*. - CAB International, New York, 1997, 1-28.

DORAN, J.W., SARRANTONIO, M., LIEBERG, M.A. - Soil health and sustainability. - *Advances in Agronomy*, 1996, 56, 1-54.

DORAN, J.W., ZEISS, M.R. - Soil health and sustainability: managing the biotic component of soil quality. - *Applied Soil Ecology*, 2000, 15, 3-11.

DRENOVSKY, R.E., VO, D., GRAHAM, K.J., SCOW, K.M. - Soil water content and organic carbon availability are major determinants of soil microbial community composition. - *Microbial Ecology*, 2004, 48, 424-430.

DUCAROIR, J., LAMY, I. - Evidence of trace metal association with soil organic matter using particle size fractionation after physical dispersion treatment. - *Analyst*, 1995, 120, 741-745.

ELLIOT, W.J.; PAGE-DUMROESE, D.; ROBICHAUD, P.R. - *The Effects of Forest Management on Erosion and Soil Productivity*. - An invited paper presented at the Symposium on Soil Quality and Erosion Interaction sponsored by The Soil and Water Conservation Society of America, July 7, 1996, Keystone, CO, 1996, 16.

ELLSWORTH, T.R., CLAPP, C.E. and BLAKE, G.R. - Temporal variations in soil structural properties under corn and soybean cropping. - *Soil Science*, 1991, 151, 405-416.

EMERSON, W.W., FOSTER, R.C., OADES, J.M. - Organo-mineral Complexes in Relation to Soil Aggregation and Structure. - In: *Interactions of soil minerals with natural organics and microbes.* Soil Science Society of America Journal Special Pub., Madison, Wisconsin, USA, 1986, 17, 521-548.

ENDELMAN, F.J., KEENEY, D.R., GILMOUR, T.J., SAF®GNA, P.G. - Nitrate and chloride movement in the plainfield loamy sand under extensive irrigation. - *Journal of Environmental Quality*, 1974, 3, 95-298.

ENEJE, R.C. and ADANMA, N. - Percent clay, aggregate size distribution and stability with depth along a toposequence formed on coastal plain sands. - *Journal of Earth Sciences*, 2007, 1, 108-112.

ENTRY, J.A., MILLS, D., MATHEE, K., JAYACHANDRAN, K., SOJKA, R.E., NARASIMHAN, G. - Influence of irrigated agriculture on soil microbial diversity. - *Applied soil ecology*, 2008, 40, 146-154.

ERKOSSA, T., ITANNA, F., STAHR, K. - Indexing soil quality: a new paradigm in soil science research. - *Australian Journal of Soil Research*, 2007, 45, 129-137.

EUROPEAN COMMISSION. - *Towards a Thematic Strategy for Soil Protection.* - Communication from the commission to the council, the European Parliament, the Economic and Social Committee and the Committee of the Regions, European Commission, Brussels, 2002, 35p.

FAO. - *Waste-water treatment and use in agriculture*. - Rome, Italy, FAO Soils Bulletin, 1992, 47.

FAO. - *World Reference Basis for Soil Resources*. - ISSS-ISRIC-FAO, Rome, 1998, 84.

FAUCI, M.F. and DICK, R.P. - Microbial biomass as an indicator of soil quality: effects of long-term management and recent soil amendments. - In: DORAN, J.W.; COLEMAN, D.C.; BEZDICEK, D.F. and STEWART, B.A., (Eds). - *Defining soil quality for a sustainable environment.* - Minneapolis, Soil Science Society of America Special Publication, 35, 1994. 17, 229-234.

FILIP, Z.K. - Soil quality assessment: an ecological attempt using microbiological and biochemical procedures. - *Adv. GeoEcol.*, 1998, 31, 21-27.

FLIEßBACH, A., OBERHOLZER, H.R., GUNST, L., MÄDER, P. - Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. - *Agriculture, Ecosystems and Environment*, 2007, 118, 273-284.

FLOREZ-VÉLEZ, L.M., DUCAROIR, J., JAUNET, A.M., ROBERT, M. - Study of the distribution of copper in an acid sandy vineyard soil by three different methods. - *European Journal of Soil Science*, 1996, 47, 523-532.

FONTAINE, S., BAROT, S. - Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. - *Ecology Letters*, 2005, 8, 1075-1087

FRANZLUEBBERS, A.J., Hons, F.M. and Zuberer, D.A. - Seasonal changes in soil microbial biomass and mineralizable C and N in wheat management systems. - *Soil Biology and Biochemistry*, 1994, 26, 1469-1475.

FRECKMAN, D. W. and. VIRGINIA, R. A. - Low-diversity Antarctic soil nematode communities: Distribution and response to disturbance. - *Ecology*, 1997, 78,363-369.

GARCÍA-RUIZ, J.M., LASANTA, T., RUIZ-FLAÑO, P., ORTIGOSA, L., WHITE, S., GONZÁLEZ, C., MARTI, C. - Land-use changes and sustainable development in mountain areas: a case study in the Spanish Pyrenees. - *Landscape Ecology*, 1996, 11, 267-277.

GARDNER, W. - Water content. - In: BLACK, C.A., EVANS, D.D., WHITE, J.L., ENSMINGER, L.E. and CLARK, F.E. (Eds). - *Methods for Soil Analysis, Part 1*. - American Society of Agronomy, Madison, Wisconsin, 1965, 82-125.

GERBA, C.P. - *Pathogens*. - Encyclopedia of Hydrological Sciences, Wiley Online Library, 2006.

GLANZ, J.T. - *Saving our Soil: Solutions for Sustaining Earth's Vital Resource*. - Johnson Books, Boulder, CO, 1995.

GOLCHIN, A., BALDOCK, J.A., OADES, J.M. - A model linking organic matter decomposition, chemistry, and aggregate dynamics. - In: LAL, R., KIMBLE, J.M., FOLLETT, R.F., STEWART, B.A. (Eds.). - *Soil Processes and Carbon Cycle*. - CRC Press, Boca Raton, 1997, 245-266.

GOLCHIN, A., CLARKE, P., OADES, J.M., SKJEMSTAD, J.O. - The effects of cultivation on the composition of organic matter and structural stability of soils. - *Australian Journal of Soil Research*, 1995, 33, 975-993.

GOLCHIN, A., OADES, J.M., SKJEMSTAD, J.O., CLARKE, P. - Study of free and occluded particulate organic matter in soils by solid state 13C CP/MAS NMR spectroscopy. - *Australian Journal of Soil Research*, 1994, 32, 285-309.

GOLLANY, H.T, SCHUMACHER, T.E., EVENSON, P.D., LINDSTROM, M.J., LEMME, G.D. - Aggregate stability on an eroded and desurfaced Typic Argiustoll. - *Soil Science Society of America Journal*, 1991, 55, 811-816.

GÓMEZ, E., FERRERAS, L., TORESANI, S., - Soil bacterial functional diversity as influenced by organic amendment application. - *Bioresource Technology* , 2006, 97, 1484-1489.

GOMEZ, E., FERRERAS, L., TORESANI, S., AUSILIO, A., BISARO, V. - Changes in some soil properties in a Vertic Argiudoll under shortterm conservation tillage. - *Soil Tillage Research*, 2001, 61, 179-186.

GONZÁLEZ, M., GOMEZ, E., COMESE, R., QUESADA, M., CONTI, M. - Influence of organic amendments on soil quality potential indicators in an urban horticultural system. - *Bioresource Technology*, 2010, 101, 8897-8901.

GOSHAL, N. and SINGH, K.P. - Effects of farmyard manure and inorganic fertilizer on Dynamics of soil microbial biomass in a tropical dryland agroecosystem. - *Biology and Fertility of Soils*, 1995, 19, 232-238.

GOULET, E., DOUSSET, S., CHAUSSOD, R., BARTOLI, F., DOLEDEC, A.F. and ANDREUX, A. - Water-stable aggregates and organic matter pools in a calcareous vineyard soil under four soil-surface management systems. - *Soil Use and Management*, 2004, 20, 318-324.

GREGORICH, E.G., CARTER, M.R., ANGERS, D.A., MONREAL, C.M. and ELLERT, B.H. - Towards a minimum data set to assess soil organic matter quality in agricultural soils. - *Canadian Journal of Soil Science*, 1994, 74, 367-385.

GUNAPALA, N., SCOW, K. - Dynamics of soil microbial biomass and activity in conventional and organic farming systems. - *Soil Biology and Biochemistry*, 1998, 30, 805-816.

GUPTA, V.V.S.R. and GERMIDA, J.J. - Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. - *Soil Biology and Biochemistry* 1988, 20, 777-787.

HAMBLIN, A. - *Environmental indicators for sustainable agriculture*. - Report of a national workshop, Publ. LWRRDC and GRDC, 1991, 96.

HAMILTON, A.J., BOLAND, A.M., STEVENS, D., KELLY, J. RADCLIFFE, J., ZIEHRL, A., DILLON, P.J. and PAULIN, R. - Position of the Australian horticultural industry with respect to the use of reclaimed water. - *Agricultural Water Management* 71, 2005, 181-209.

HAN, L., ZHANG, Y-L., JIN, S., WANG, J., WEI, Y.Y., CUI, N. and WEI, W. - Effect of Different Methods on Dissolved Organic Carbon and Microbial Biomass Carbon in the Greenhouse Soil. - *Agricultural Sciences in China*, 2010, 8(9), 1175-118.

HAO, Y., LAL, R., OWENS, L.B., IZAURRALDE, R.C., POST, W.M. and HOTHEM, D.L. - Effect of cropland management and slope position on soil organic carbon pool at the North application experimental watersheds. - *Soil and Tillage Research*, 2002, 68, 133-142.

HARRIS, R. In: PARR, J.F., G., W.R., ELLIOTT, L.F. (Eds.). - *Effect of Water Potential on Microbial Growth and Activity in Soils in Water Potential Relations in Soil Microbiology*. - Soil Science Society of America Journal, Madison, 1981, WI, 23-96.

HARRIS, R.F., CHESTERS, G., ALLEN, O.N. - Dynamics of soil aggregation, - *Advances in Agronomy*, 1966, 18, 108-169.

HAYNES, R. J. - Labile organic matter fractions as central components of the quality of agricultural soils: an overview, - *Advances in Agronomy*, 2005, 85, 221-268.

HELMISAARI, H.-S., HALLBÄCKEN, L. - Fine-root biomass and necromass in limed and fertilized Norway spruce (Picea abies (L.) Karst.) stands, - *Forest Ecology and Management*, 1999, 9, 99-110.

HENRY, J .L and HOGG, T. J. - *Evaluation of the Effects of Irrigation on Soil Chemical and Physical Properties. Irrigation sustainability* - Saskatchewan activity, Canada-Saskatchewan Irrigation Diversification Center, Canada, 2003.

HERMAWAN, B., CAMERON, K.C. - Structural changes in a silt loam under long-term conventional or minimum tillage. - *Soil Tillage Research*, 1993, 26, 139-150.

HEWITT, A.E. and SPARLING, G. - *Setting soil quality standards for C contents of New Zealand soils*. - New Zealand Soil Science Society Conference, Gisborne, 16-19 November 1998, 135-136.

HILLEL, D. - *Fundamentals of Soil Physics*. - Academic Press, New York, 1980, 413p.

HILLEL, D. - *Out of the Earth: Civilization and the Life of the Soil*. - University of California Press, Berkeley, CA, 1991, 321 p.

HORNICK, S.B. - Factors affecting the nutritional quality of crops. - Am. J. Alter. Agric., 1992, 7, 63-68.

HOWARD, P.J.A. - Soil protection and soil quality assessment in the EC. - *Science of the Total Environment*, 1993, 129, 219-239.

http://www.herbgardeningguru.com/soil-ph.html

HUBER, S., SYED, B., FREUDENSCHUSS, A., ERNSTEN, V., LOVELAND, P. - *Proposal for a European soil monitoring and assessment framework.* - Technical report no.61, Europe Environmental Agency, Copenhagen, Denmark, 2001.

HUNT, H.W. and WALL, D.H. - Modelling the effects of loss of soil biodiversity on ecosystem function. - *Global Change Biology*, 2002, 8, 33-50.

ISBERIE, C. - *Interactions entre l'irrigation localisée et l'enracinement en arboriculture fruitière*. - Sémin. Groupe Étud. Arbre "La racine et le système racinaire", Orléans, 6-7 avril 1995, Cons. Rég.Centre, INRA, 1995, 47-53. Multigrade.

ISO 10390. - *Soil quality. Determination of pH.* - 2005.

ISO 10693. - *Soil quality. Chemical methods. Determination of carbonate content. Volumetric method.* - 1995.

ISO 10694. - *Soil quality. Determination of organic and total carbon after dry combustion (elementary analysis).* - 1995.

ISO 11261. - *Soil quality. Determination of total nitrogen: Modified Kjeldahl method*. - 1995.

ISO 13878. - *Soil quality. Determination of total nitrogen content by dry combustion ("elemental analysis").* - 1998.

JANEAU, J.L., BRICQUET, J.P., PLANCHON, O., VALENTIN, C. - Soil crusting and infiltration on steep slopes in northern Thailand. - *European Journal of Soil Science*, 2003, 54, 543-554.

JANZEN, H.H., CAMPBELL, C.A., BRANDT, S.A., LAFOND, G.P. and TOWNLEY-SMITH, L. - Light fraction organic matter in soils from long-term crop rotations. - *Soil Science of American Journal*, 1992, 56, 1799-1806.

JENKINSON, D.S. and LADD, J.N. - Microbial Biomass in Soil: Measurement and Turnover. - In: PAUL, E.A. and LADD, J.N. (Eds.). - *Soil Biochemistry*. - Marcel Dekker, New York, USA, 1981, 415-471.

JENKINSON, D.S. and POWLSON, D.S. - The effect of biocidal treatments on metabolism in soil. V) A method for measuring biomass. - *Soil Biology and Biochemistry*, 1976, 8, 209-213.

JOERGENSEN, R.G., MEYER, B.and MULLER, T. - Time-course of the soil microbial biomass Under wheat; A one year field study. - *Soil Biology and Biochemistry*, 1994, 26, 987-994.

KAISER, E.A. and HEINMEYER, O. - Seasonal variations of soil microbial biomass carbon Within plough layer. - *Soil Biology and Biochemistry*, 1993, 25, 1649-1655.

KANDELER, E., MARGESIN, R., OHLINGER. R *et.al*. - Microbial soil monitoring - proposal for an inventory of soils. - *Bodenkultur*, 1993, 44(4), 357-377.

KARLEN, D.L., MAUSCHBACH, M.J., DORAN, J.W., CLINE, R.G., HARRIS, R.F., and SCUMAN, G.E. - Soil Quality: A Concept, Definition and a Framework for Evaluation. - *Soil Science Society of America Journal*, 1997, 61, 4-10.

KARLEN, D.L., PARKIN, T.P., EASH, N.S. - Use of soil quality indicators to evaluate conservation reserve program sites in Iowa. - In: DORAN, J.W., JONES, A.J. (Eds.). - *Methods for assessing soil quality*. - *SSSA*, Madison, 1996, 345-355.

KAY, B.D. - Rates of change of soil structure under different cropping systems. - *Advances in Soil Sciences*, 1990, 12, 1-52.

KAY, B.D., GRANT, C.D. - Structural aspects of soil quality. - In: MACEWAN, R.J., CARTER, M.R. (Eds.). - *Soil quality is in the Hands of Land Manager*. - Centre for Environmental Management, University of Ballarat, Victoria, Australia, 1996, 37-41.

KEMMITT, S.J., LANYON, C.V., WAITE, I.S., WEN, Q., ADDISCOTT, T.M., BIRD, N.R.A., O'DONNELL, A.G., BROOKES, P.C. - Mineralization of native soil organic matter is not regulated by the size,activity or composition of the soil microbial biomass - a new perspective. - *Soil Biology & Biochemistry*, 2008, 40, 61-73.

KEMPER, W.D. and ROSENAU, R.C. - Aggregate stability and size distribution. - In A. KLUTE (Eds.). - *Methods of Soil Analysis. Part 1. Physical and mineralogical methods*. - 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, 1986, WI, 425-442.

KEMPER, W.D. and ROSENAU, R.C. - Soil cohesion as affected by time and water content. - *Soil Science Society of America Journal*, 1984, 84, 1001-1006.

KEMPF, B., BREMER, E. - Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. - *Archives of Microbiology*, 1998, 170, 319-330.

KLUTE, A. - Water retention: laboratory methods. - In: KLUTE, A. (Eds.). - *Methods for Soil Analysis.* - USA, *American Society of Agronomy*, 1986, 643-650.

KOCHSIEK, A.E., KNOPS, J.M., WALTERS, D.T. and ARKEBAUER, T.J. - Impact of managment on decomposition and the litter-carbon balance in irrigated and rainfed no-till agricultural systems. - *Agricultural and Forest Meteorology*, 2009, 149, 1983-1993.

KOKALIS-BURELLE, N., RODRIGUEZ-KABANA, R. - Changes in populations of soil microorganisms, nematodes, and enzyme activity associated with application of powdered pine bark. - *Plant Soil*, 1994, 162, 169-175.

KOSTOPOULOU S.K. and ZOTOS A. - The effect of soil water content and microbial activity on restoring the structure of a vertisol. - *Soil and Tillage research*, 2005, 82, 203-209.

KOUTIKA, L., BARTOLI, F., ANDREUX, F., CERRI, C.C., BURTIN, G., CHONE´, T., PHILIPPY, R. - Organic matter dynamics and aggregation in soils under rain forest and pastures of increasing age in the eastern Amazon Basin. - *Geoderma*, 1997, 76, 87-112.

KREUTZER, K. - Effects of forest liming on soil processes. - *Plant and Soil*, 1995, 168/169, 447-70.

KRISHNAKUMAR, S., SARAVANAN, A., NATARAJAN, S.K., VEERABADRAN, V., MANI, S. - Microbial population and enzymatic activity as influenced by organic farming. - *Research Journal of Agriculture and Biological Sciences*, 2005, 1, 85-88.

KUCERA, C. and D. KIRKHAM. - Soil respiration studies in tall-grass prairie in Missouri. - *Ecology*, 1971, 52, 912-915.

KUMAR, K. and GOH, K.M. - Crop residues and management practices: effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. - *Advances in Agronomy*, 2000, 68, 197-319.

LAL, R. - Soil quality and food security: the global perspective. - In: LAL, R. (Eds.). - *Soil Quality and Soil Erosion.* - CRC Press, Boca Raton, FL, 1999, 3-16.

LALFAKZUALA, R., KAYANG, H., DKHAR, M.S. - The effects of fertilizers on soil microbial components and chemical properties under leguminous cultivation. - *Am-Euras. J. Agric. Environ. Sci*., 2008, 3, 314-324.

LANGMAACK, M. - Earthworm communities in arable land influenced by tillage, compaction and soil. - *Zeitschrift für Ökologie und Naturschutz*, 1999, 8, 11-21.

LARSON, W.E. and PIERCE, F.J. - Conservation and enhancement of soil quality. - In: *Evaluation for Sustainable Land Management in the Developing World*. - Technical Papers. Bangkok, Thailand, IBSRAM Proceedings, International Board for Soil Research and Management, 1991, 12, 2.

LARSON, W.E. and PIERCE, F.J. - *The dynamic of soil quality as a measure of sustainable management. Defining Soil Quality for a Suitable Environment*. Soil Science Society of America Special Publication No.35. - Soil Science Society of America, Madison, USA1994, WI, 37-51.

LAWRENCE, G.P. - Measurement of pore size in fine textured soils: A review of existent techniques. - *Journal of Soil Science*, 1977, 28, 527-540.

LAWSON, H.W., BRAUN, M.M., GLASS, R.I., STINE, S.E., MONROE, S.S., ATRASH, H.K., LEE, L.E. AND ENGLENDER, S.J. - Waterborne outbreak of Norwalk virus gastroenteritis at a southwest US resort: role of geological formations in contamination of well water. - *Lancet*, 1991, 337, 1200-1204.

LE BISSONNAIS, Y. and ARROUAYS, D. - Aggregate stability and assessment of soil crustability and erodibility: II. Aplication to humic loamy soils with various organic carbon contents. - *European Journal of Soil Science*, 1997, 48, 39-48.

LE BISSONNAIS, Y., CROS-CAYOT., S., GASCUEL-ODOUX, C. - Topographic dependence of aggregate stability, overland flow and sediment transport. - *Agronomie*, 2002, 22, 489-501.

LEE, J. - Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production. - *Scientia Horticulturae*, 2010, 124, 299-305.

LEE, J.J., PARK, R.D., KIM, Y.W., SHIM, J.H., CHAE, D.H., RIM, Y.S., SOHN, B.K., KIM, T.H., KIM, K.Y. - Effect of food waste compost on microbial population, soil enzyme activity and lettuce growth. - *Bioresource Technology*, 2004, 93, 21-28.

LEHRSCH, G.A. and BROWN, M.J. - Furrow erosion and aggregate stability variation in a Portneuf silt loam. - *Soil Technology*, 1995, 7, 327-341.

LEHRSCH, G.A. and JOLLEY, P.M. - Temporal Changes in Wet Aggregate Stability. - *Transaction of the ASAE*, 1992, 35(2), 493-498.

LEIFELD, J., KÖGEL-KNABNER, I. - Soil organic matter fractions as early indicators for carbon stock changes under different land-use? - *Geoderma*, 2005, 124, 143-155.

LEMAÎTRE A., CHAUSSOD R., TAVANT Y. and BRUCKERT S. - An attempt to determine a pool of labile organic matter related to the soil microbial biomass. - *European Journal of Soil Biology*, 1995, 31 (3), 121-125.

LÉONARD, J., ANDRIEUX, P. - Infiltration characteristics of soils in Mediterranean vineyards in southern France. - *Catena*, 1998, 32, 209-223.

LICHTFOUSE, E. - Compound-specific isotope analysis. Application to archaelogy, biomedical sciences, biosynthesis, environment, extraterrestrial chemistry, food science, forensic science, humic substances, microbiology, organic geochemistry, soil science and sport. - *Rapid Communications in Mass Spectrometry*, 2000, 14, 1337-1344.

LICHTFOUSE, E., DOU, S., GIRARDIN, C., GRABLY, M., BALESDENT, J., BEHAR, F., VANDENBROUCKE, M. - Unexpected 13C-enrichment of organic components from wheat crop soils: evidence for the in situ origin of soil organic matter. - *Organic geochemistry*, 1995, 23, 865-868.

LOVELAND, P.J. and THOMPSON, T.R.E. - *Identification and development of a set of national indicators for soil quality*. - R&D Technical report P5-053/2/TR National Soil Resources Institute, Cranfield University, Silsoe, UK, 2001.

LUEKING, M.A. and SCHEPERS, J.S. - Changes in soil carbon and nitrogen due to irrigation development in Nebraska's sandhill soils. - *Soil Science Society American Journal*, 1985, 49, 626-630.

LUNDEKVAM, H.E., ROMSTAD, E., ØYGARDEN, L. - Agricultural policies in Norway and effects on soil erosion. - *Environmental Science & Policy*, 2003, 6, 57-67.

LUPWAYI, N.Z., ARSHAD, M.A., RICE, W.A., CLAYTON, G.W. - Bacterial diversity in water- stable aggregates of soils under conventional and zero tillage management. - *Applied Soil Ecology*, 2001, 16, 251-261.

LYNCH, J.M., BRAGG, E. - *Microorganisms and structural stability-Advances in soil sciences*. Springer Verlag, New York, 1985, 134-170.

MÄDER, P., PFIFFNER, L., FLIESSBACH, A., VON-LÜTZOW, M. and MUNCH, J.C. - *Soil ecology- Impact of organic and conventional agriculture on soil biota and its significance for soil fertility*. - In: International conference on Kyusei nature farming, 5. Proceedings. Bangkok, Asia Pacific Natural Agriculture Network, 1997, 24-40.

MADIGAN, M.T., MARTINKO, J.M., PARKER, J. - *Brock Biology of Micro organisms*. - 9th edition, Prentice Hall; Upper Saddle River. New Jersey, 2000.

MAGDOFF, F. - Concept, components and strategies of soil health in agroecosystems. - *Journal of Nematology*, 2000, 33, 169-172.

MALET, J.P., AUZET, A.V., MAQUAIRE, O., AMBROISE, B., DESCROIX, L., ESTEVES, M., VANDERVAERE, J.P., TRUCHET, E. - *Soil surface characteristics influence on infiltration in black marls: application to the Super-Sauze earthflow (southern Alps, France)*. - Earth Surface Processes and Landforms, 2003, 28, 547-564.

MANDAL, U.K., WARRINGTON, D.N., BHARDWAJ, A.K., BAR-TAL, A., KAUTSKY, L., MINZ D., LEVY, G.J. - Evaluating impact of irrigation water quality on a calcareous clay soil using principal component analysis. - *Geoderma*, 2008, 144, 189-197.

MARINARI, S., MASCIANDARO, G., CECCANTI, B., GREGO, S. - Influence of organic and mineral fertilisers on soil biological and physical properties. - *Bioresource Technology*, 2000, 72, 9-17.

MARSCHNER, B., KALBITZ, K. - Controls of bioavailability and biodegradability of dissolved organic matter in soils. - *Geoderma*, 2003, 113, 211-235.

MARSCHNER, B., WILCZYNSKI, A. - The effect of liming on quantity and chemical composition of soil organic matter in a pine forest in Berlin, Germany. - *Plant and Soil*, 1991, 137, 229-236.

MARTENS, R. - Current methods for measuring microbial biomass in soil: potentials and limitations. - *Biology and Fertility of Soils*, 1995, 19, 87-99.

MARTIN, A., MARIOTTI, A., BALESDENT, J., LAVELLE, P., VUATTOUX, R. - Estimate of organic matter turnover rate in a savanna soil by 13C natural abundance measurements. - *Soil Biology and Biochemistry*, 1990, 22, 517-523.

MARTINIELLO, P. - Biochemical parameters in a Mediterranean soil as affected by wheat-forage rotation and irrigation. - *European Journal of Agronomy*, 2007, 26, 198-208.

MÁTÉ, F. and TÓTH, G. - *Soil bonitation as a constituent of land evaluation* (Talajbonitáció mint a földértékelés egyik tényezõje) - Agrárökonómiai Tudományos Napok. GATE Mezõgazdasági Fõiskolai Kar. Gyöngyös, 1996.

MAZZARINO, M.J., L. SZOTT and JIMENEZ, M. - Dynamics of soil total C and N, microbial biomass, and water-soluble C in tropical agroecosystems. - *Soil Biology and Biochemistry*, 1993, 25, 205-214.

MCLEAN, E.O. - Potentially beneficial effects from liming: chemical and physical. Proceedings - *Soil & Crop Science Society of Florida*, 1971, 31, 189-196.

MELESTER, B.T., ACOSTA-MARTINEZ, V., KUMAR, R. AND ZAK, J. Texas Tech University, USDA-ARS, 94th ESA annual meeting, Alnuquerque, New mexico, 2.-7.8.2009, 2010.

MELI, S., PORTO, M., BELLIGNO, A., BUFO, S. A., MAZZATURA, A. AND SCOPA, A. - Influence of irrigation with lagooned urban wastewater on chemical and microbiological soil parameters in a citrus orchard under Mediterranean condition. - *Science of the Total Environment*, 2002, 285, 69-77.

MENDES, I.C., A.K. BANDICK, R.P. DICK AND P.J. BOTTOMLEY. - Microbial biomass and Activities in soil aggregates affected by winter cover crops. - *Soil Science Society of America Journal*, 1999, 63, 873-881.

METCAFF, O. and EDDY, J. - *Wastewater Engineering Treatment and Reuse*. - 4th Ed., McGraw-Hill, New York, 2003.

METZGER, L. and YARON, B. - Influence of sludge organic matter on soil physical properties. - In: Stewart, B.A (Eds.). - *Advances in soil science* - .Vol. 7. *Springer*, New York, 1987, 141-163.

MILLER, C. - Understanding the Carbon-Nitrogen Ratio. - *ACRES*, 2000, 30, 4-20.

MILLER, R., DONAHUE, R. - *Soils in Our Environment* - 7th edition. Prentice Hall (Eds), 1995, 649 p.

Ministry for Agriculture, Forestry and Food. Republic of Slovenia. - *Slovenian irrigation project*. - Feasibility reports No. 1-19, No H1-H5, AFC, Ljubljana, 1998.

MONDINI, C., CAYUELA, M.L., SINICCO, T., SANCHEZ-MONEDERO, M.A., BERTOLONE, E., BARDI, L. - Soil application of meat and bone meal. Short-term effects on mineralization dynamics and soil biochemical and microbiological properties. - *Soil Biology and Biochemistry*, 2008, 40, 462-474.

MOORE, J.M., S. KLOSE and TABATABAI, M.A. - Soil microbial biomass carbon and nitrogen as affected by cropping systems. - *Biology and Fertility of Soils*, 2000, 31, 200-210.

MORGAN, R.P.C. - *Soil Erosion and Conversation*. - 2nd Ed., Addison Wesley Longman Limited, Essex, U.K., 1996.

MORRIS, C.E., BARDIN, M., BERGE, O., FREY-KLETT, P., FROMIN, N., GIRARDIN, H., GUINEBRETIERE, M.H., LEBARON, P., THIÉRY, J.M., TROUSSELIER, M. - Microbial biodiversity: approaches to experimental design and hypothesis testing in primary scientific literature from 1975 to 1999. - *Microbiology and Molecular Biology Reviews*, 2002, 66, 592-616.

MUNYANZIZA, E., KEHRI, H.K., BAGYARAJ, D.J. - Agricultural intensification, soil biodiversity and agro-system function in the tropics: the role of mycorrhiza in crops and trees. - *Applied Soil Ecology*, 1997, 6, 77-85.

MURPHY, D.V., SPARLING, G.P., FILLERY, I.R.P. - Stratification of microbial biomass C and N and gross N mineralization with soil depth in two contrasting Western Australian agricultural soils. - *Australian Journal of Soil Research*, 1998, 36, 45-55.

MURRAY, R.S. - *A review of Dr Bruce Cockroft's work for Australian irrigated Horticulture*. - National Program for Sustainable Irrigation, 2006.

NANNIPIERI, P. - The potential use of enzymes as indicators of productivity, sustainability and pollution. - In: PANKHURST, C.E., DOUBE, B.M., GUPTA, V.V.S.R., GRACE, P.R. (Eds.). - *Soil Biota: management and sustainable farming systems*. - CSIRO, East Melbourne, 1994, 238-244.

National Research Council. - *Soil and Water Quality: an Agenda for Agriculture*. - National Academy Press, Washington, DC, 1993, 516 p.

NEALE, S.P., SHAH, Z., ADAMS, W.A. - Changes in microbial biomass and nitrogen turnover in acidic organic soils following liming. - *Soil Biology & Biochemistry*, 1997, 29, 1463- 1474.

NELSON, D.W., SOMMERS, L.E. - Total carbon, organic carbon and organic matter. - In: PAGE, A.L., MILLER, R.H., KEENEY, D.R. (Eds.). - *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties.* - 1982, 539-577.

NELSON, K.L., LYNCH, D.H., BOITEAU, G. - Assessment of changes in soil health throughout organic potato rotation sequences, - *Agriculture, Ecosystems & Environment*, 2009, 131, 220-228.

NUNES, J.M., LOPEZ, P., ALBARRAN, A. MUNOZ, A. and COELHO, J. - Changes in selected soil properties caused by 30 years of continuous irrigation under Mediterranean conditions, - *Geoderma*, 2007, 139, 321-328.

Official Gazette of Republic Slovenia No. 68. - *Decree on the boundary, warning and critical input values of hazardous compounds in the soil (in Slovene)*. - 1996.

OLA, S. - Geotechnical properties and behavior of some stabilized Nigerian lateritic soils. - *Quarterly Journal of Engineering Geology*, 1978, 11(2), 145-160.

OLK, D.C., GREGORICH, E.G. Overview of the symposium proceedings, - Meaningful pools in determining soil carbon and nitrogen dynamics. - *Soil Science Society of America Journal*, 2006, 70, 967-974.

ONGLEY, E.D. - *Control of water pollution from agriculture*. - FAO Irrigation and drainage paper 55, Rome, 1996, 101 p.

ÖNORM L. 1091. - *Chemical analysis of soils - Determination of inorganic nitrogen - Nmin-method.* - 1999.

ONWEREMADU, E.U., ONYIA, V.N., ANIKWE, M.A.N. - Carbon and nitrogen distribution in water-stable aggregates under two tillage techniques in Fluvisols of Owerri area, southeastern Nigeria. - *Soil Tillage Research*, 2007, 97, 195-206.

PANKHURST, C.E., DOUBE, B.M. and GUPTA, V.V.S.R. - *Biological indicators of soil health*. - Wallingford, UK, *CAB International*, 1997, 451 p.

PAPENDICK, R.I., CAMPBELL, G.S. - Theory and measurement of water potential. - In: PARR, J.F., GARDNET, W.R., ELLIOTT, L.F. (Eds.). - *Water Potential Relations in Soil Microbiology* - (Special Publications No. 9), Madison. Soil Science of Society of America, 1981, WI, 1 -22.

PARDO, A., AMATO, M., CHIARANDA, F.Q. - Relationships between soil structure, root distribution and water uptake of chick-pea (*Cicer arietinum*). Plant growth and water distribution. - *European Journal of Agronomy*, 2000, 13, 39-45.

PARKIN, T.B., DORAN, J.W., FRANCO-VIZCAÍNO, E. - Field and laboratory tests of soil respiration. - In: DORAN, J.W., JONES, A.J. (Eds.). - *Methods for Assessing Soil Quality*. - SSSA Special Publication No. 49, 1996, 231-245.

PAUL, E.A. and CLARK, F.E. - *Soil microbiology and biochemistry*. - London, UK, Academic Press, 1996, 12-33.

PEREIRA, L., GILLIES, J., JENSEN, M., FEDDES, R. and LESAFFRE, B. - Research Agenda on Sustainability of Water Resource Utilization in Agriculture. In: - *Sustainability of Irrigated Agriculture*. - NATO ASI series. Kluwer Academic Publishers, 1996, 1-16.

PEREZ-PIQUERES, A., EDEL-HERMANN,W., ALABOUVETTE, C., STEINBERG, C. - Response of soil microbial communities to compost amendments. - *Soil Biology and Biochemistry*, 2006, 38, 460-470.

PERFECT, E., KAY, B.D., VAN LOON, W.K.P, SHEARD, R.W., POJASOK, T. - Factors Influencing Soil Structural Stability within a Growing Seasons. - *Soil Science Society of America Journal*, 1990, 54, 173-179.

PERSSON, T. - Effects of liming on soil fauna in forests. A literature review. - *National Swedish Environment Protection Board*, 1988, 3418, 92.

PERSSON, T., RUDEBECK, A., WIREN, A. - Pools and fluxes of carbon and nitrogen in 40-year-old forest liming experiments in Southern Sweden. - *Water, Air, and Soil Pollution*, 1995, 85, 901-906.

PIAO, H.C., Y.T. HONG AND Z.Y. YUAN. - Seasonal changes of microbial carbon related to climatic factors in soils from karst areas of southwest China. - *Biology and Fertility of Soils*, 2000, 30, 294-297.

PICCOLO, A. - Humus and soil conservation. - In: PICCOLO, A. (Eds.). - *Humic Substances in Terrestrial Ecosystems*. - Elsevier Science B. V., Amsterdam, 1996, 225-264.

PICCOLO, G.A., GALANTINI, J.A., ROSELL, R.A. - Organic carbon fractions in a yerba mate plantation on a subtropical kandihumult of Argentina. - *Geoderma*, 2004, 123, 333-341.

PLANTE, A.F., CONANT, R.T., STEWART, C.E., PAUSTIAN, K., SIX, J. - Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. - Soil Science Society of America Journal, 2006, 70, 287-296.

POESEN, J.W., INGELMO-SÁNCHEZ, F., MÜCHER, H. - The hydrological response of soil surfaces to rainfall as affected by cover, position and rock fragments in the top layer. - *Earth Surfaces Processes and Landforms*, 1990, 16, 653-671.

PORTA, J., LÓPEZ-ACEVEDO,M., ROQUERO, C. - *Edafologia para la agricultura y el medio ambiente.* - Ediciones Mundi-Prensa (Eds.), Madrid, Espanha, 1994, 225-241.

POTTER, K.N., TOLBERT, H.A., JONES, O.R., MATOCHA, J.E., MORRISON, J.E., UNGER, P.W. - Distribution and amount of soil organic C in long-term management systems in Texas. - *Soil Tillage Research*, 1998, 47, 309-331.

POWLSON, D.S. - The soil microbial biomass: before, beyond and back. - In: RITZ, K., DIGHTON, J., GILLER, G.E. (Eds.). - *Beyond the biomass*. - *Willey*, Chichester, UK, 1994, 3-20.

PUGET, P., ANGERS D.A., CHENU C. - Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. - *Soil Biology and Biochemistry*, 1999, 31, 55-63.

RAJARAM, G. and ERBACH, D.C. - Effect of wetting and drying on soil physical properties. - *Journal of Terramechanics*, 1999, 36(1), 39-49.

RAMIREZ-FUENTES, E., LUCHO-CONSTANTINO, C., ESCAMILLA-SILVA, E., DENDOOVEN, L. - Characteristics and carbon and nitrogen dynamics in soil irrigated with wastewater for different lengths of time. - *Bioresource Technology*, 2002, 85, 179-187.

RAMOS, M.C., COTS-FOLCH, R., MARTÍNEZ-CASASNOVAS, J.A. - Effects of land terracing on soil properties in the Priorat region in Northeastern Spain: A multivariate analysis. - *Geoderma*, 2007, 142, 251-261

RAMPAZZO, N., BLUM, W.E.H., WIMMER, B. - Assessment of soil structure parameters and functions in agricultural soils. - *Bodenkultur*, 1998, 49, 69-84.

RASPE, S., HAUG, I. - Wurzelverteilung und Mykorrhizadynamik in Abhängigkeit vom Nährelementangebot im Boden. - In: RASPE, S., FEGER, K.H., ZOÖTTL, H.W. (Eds.). - *Ökosystemforschung im Schwarzwald*. - Ecomed-Verlagsgesellschaft, Landsberg, 1998, 223-237.

RAWLS, W.J., PACHEPSKY, Y.A., RITCHIE, J.C., SOBECKI, T.M., BLOODWORTH, H. - Effect of soil organic carbon on soil water retention. - *Geoderma*, 2003, 116, 61-76.

RICHARDS, B.N. - *The microbiology of Terrestrial ecosystems*. - Longman, Essex, 1987.

RICKARD, D.S. and. COSSENS, G.G. - Irrigation investigation in Otago, New Zealand, I. Description and physical properties of irrigated soils of the Ida Valley, - *New Zealand Journal of Agricultural Research*, 1966, 9, 197- 217.

RIETZ, D.N., HAYNES, R.J. - Effects of irrigation-induced salinity and sodicity on soil microbial activity. - *Soil Biology & Biochemistry*, 2003, 35, 845-854.

ROBERT, M., CHENU, C. - Interaction between soil minerals and microorganisms. - In: STOTZKY, G., BALLAG, J.M. (Eds.). *Soil Biochemistry*, 1992, 7, 307-403.

RODENKIRCHEN, H. - Evidence for a nutritional disorder of *Oxalis acetosella* L. on acid forest soils. I. Control situation and effects of dolomitic liming and acid irrigation. - *Plant and Soil*, 1998, 199, 141-152.

ROLDÁN, A., CARAVACA, F., HERNÁNDEZ, M.T., GARCÍA, C., SÁNCHEZ-BRITO, C., VELÁSQUEZ, M., TISCAREÑO, M. - No-tillage, crop residue additions, and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). - *Soil & Tillage Research*, 2003, 72, 65-73.

ROMIG, D.E., GARLYND, M.J., HARRIS, R.F., MCSWEENEY, K. - How farmers assess soil health and quality (Special Issue on Soil Quality). - *Journal of Soil and Water Conservation*, 1995, 50, 229-236.

ROSS D. J. - Soil microbial biomass estimated by the fumigation-incubation procedure: seasonal fluctuations and influence of soil moisture content. - *Soil Biology & Biochemistry*, 1987, 19, 397-404.

RUMPEL, C., EUSTERHUES, K., KÖGEL-KNABNER, I. - Location and chemical composition of stabilized organic carbon in topsoil and subsoil horizons of two acid forest soils. - *Soil Biology and Biochemistry*, 2004, 36, 177-190.

RUTJES, S.A.; ITALIANDER, R.; VAN DEN BERG, H.H.J.L.; LODDER, W.J. AND DE RODA HUSMAN, A.M. - Isolation and Detection of Enterovirus RNA from Large-volume Water samples by using Sequences-Based Amplification. - *Applied and Environment Microbiology*, 2005, 71, 3734-3740.

SAMUELSON, L., MATHEW, R., STOKES, T., FENG, Y., AUBREY, D. and COLEMAN, M. - Soil and microbial respiration in a loblolly pine plantation in response to seven years of irrigation and fertilization. - *Forest Ecology and Management*, 2009, 258 (11), 2431-2438.

SANCHEZ, P.A. - *Properties and Management of Soils in the Tropics*. - John Wiley & Sons Ltd., New York, 1976.

SASAL, C., ANDRIULO, A., ULLÉ, J., ABREGO, F., BUENO, M. - Efecto de diferentes enmiendas sobre algunas propiedades edáficas, en sistemas de producción hortícola del centro norte de la región pampeana. - *Ciencia del Suelo*, 2000, 18, 95-104.

SCHLOTER, M., DILLY, O., MUNCH, J.C. - Indicators for evaluating soil quality. - *Agriculture, Ecosystems and Environment*, 2003, 98, 255-262.

SCHLOTER, M., MUNCH, J.C., TITTARELLI, F. - Managing soil quality. - In: BLOEM, J., HOPKINS, D.W., BENEDETTI, A. (Eds.). - *Microbiological Methods for Assessing Soil Quality*. - CAB International, Wallingford, 2006, 50-62.

SCHOENHOLTZ, S.H., VAN MIEGROET, H. BURGER, J.A. - A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. - *Forest Ecology and Management*, 2000, 138, 335-356.

SEVINK, J., OBALE-EBANGA, F., MEIJER, H.A.J. - Land-use related organic matter dynamics in North Cameroon soils assessed by 13C analysis of soil organic matter fractions. - *European Journal of Soil Science*, 2005, 56, 103-111.

SEYBOLD, C.A., DICK, R.P., PIERCE, F.J. - USDA soil quality test kit: approaches for comparative assessments. - *Soil Survey Horizons*, 2001, 42, 43-52.

SHOCK, C.C., FEIBERT, E.B.G., ERIK, B.C., SAUNDERS, L.D. - Irrigation criteria for drip-irrigated onions. - *Hortscience*, 2000, 35 (1), 63-66.

SHRESTHA, B.M., SINGH, B.R., SITAULA, B.K., LAL, R., BAJRACHARYA, R.M. - Soil Aggregate and particle-associated organic carbon under different land uses in Nepal. - *Soil Soil Science Society of America Journal*, 2007, 71, 1194-1203.

SIKORA, L.J., YAKOVCHENKO, V., CAMBARDELLA, C.A. and DORAN, J.W. - Assessing soil quality by testing organic matter. - In: Soil Science Society of America *(*Eds.), 1996. - *Soil Organic Matter: Analysis and Interpretation*. - Special Publication no. 46, Madison, 1996, WI, 41-50.

SILVER M., EHRLICH, H.L., IVARSON, K.C. - Soil Mineral Transformation Mediated by Soil Microbes. - In: HUANG, P.M., SCHNITZER, M., 1986. - *Interactions of soil minerals with natural organics and microbes.* - Madison, Wisconsin, USA, Soil Science Society of America, 1996, 17, 497-520

SIST ISO. 5815. - *Water quality. Determination of Biochemical the Oxygen Demand after n days (BOD)*. - 1989.

SIST ISO. 6060. - Water quality. Determination of the Chemical Oxygen Demand (COD). 1996.

SIX, J., BOSSUYT, H., DEGRYZE, S., DENEF, K. - A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. - *Soil Tillage Research*, 2004, 79, 7-31.

SIX, J., ELLIOT, E.T., PAUSTIAN, K. - Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy, - *Soil Science Society of America Journal*, 2000, 64, 1042-1049.

SKJEMSTAD, J.O., CLARKE, P., TAYLOR, J.A., OADES, J.M., MCCLURE, S.G. - The chemistry and nature of protected carbon in soil, - *Australian Journal of Soil Research*, 1996, 34, 251-271.

SKOPP, J., JAWSON, M.D., DORAN, J.W. - Steady-state aerobic microbial activity as a function of soil water content, - *Soil Science Society of America Journal*, 1990, 54, 1619-1625.

SMITH, P., POWLSON, D.S., SMITH, J.U., FALLOON, P., COLEMAN, K. - Meeting Europe's climate change commitments: quantitative estimates of the potential for carbon mitigation by agriculture, - *Global Change Biology*, 2000, 6, 525-539.

SMOLANDER, A., MÄLKÖNEN, F. - Microbial biomass C and N in limed soil of Norway spruce stands. - *Soil Biology & Biochemistry*, 1994, 26, 503-509.

SNAPP, S.S., SWINTON, S.M., LABARTA, R., MUTCH, D., BLACK, J.R., LEEP, R., NYIRANEZA, J., O'NEIL, K. - Evaluating cover crops for benefits, costs and performance within cropping system niches. - *Agronomy Journal*, 2005, 97, 322-332.

SOJKA, R.E. and UPCHURCH, D.R. - Reservations regarding the soil quality concept. - *Soil Science of America Journal*, 1999, 63, 1039-1054.

SOLOMON, D., FRITZSCHE, F., LEHMANN, J., TEKALIGN, M., ZECH, W. - Soil organic matter dynamics in the subhumid agroecosystems of the Ethiopian highlands: evidence from natural C-13 abundance and particle-size fractionation. - *Soil Science Society of America Journal*, 2002, 66, 969-978.

SOULAS, G. and LORS, C. - Perspectives and limitations in a assessing side-effects of pesticides on the soil microflora. - In: - *Microbial Biosystems: New Frontiers*. - Proceedings of the 8th International Symposium on Microbial Ecology Bell CR, Brylinsky M, Johnson-Green P (eds). Atlantic Canada Society for Microbial Ecology, Halifax, Canada. 1999.

SPARLING G. and SCHIPPER, L. - *Soil quality and soil health*. - Proceedings of Manaaki Whenua Conference, Te Papa April 21, 1999.

SPARLING, G., VOJVODIC-VUKOVIC, M., SCHIPPER, L.A. - Hot-watersoluble C as a simple mesure of labile soil organic matter: the relationship with microbial biomass C. - *Soil Biology and Biochemistry*, 1998, 30, 1469-1472.

SPARLING, G.P. - Soil microbial biomass, activity and nutrient cycling as indicators of soil health. - In: PANKHURST, C.E., DOUBE, B.M., GUPTA, V.V.S.R. (Eds.). - *Biological Indicators of Soil Health. - CAB, Wallingford*, 1997, 97-119.

STAMATIADIS, S., WERNER, M., BUCHANAN,. FIELD, M. - Assessment of soil quality as affected by compost and fertilizer application in a broccoli field (San Benito County, California), - *Applied Soil Ecology*, 1999, 12, 217-225.

STATGRAPHICS® Centurion XV. User Manual. Herndon , VA : StatPoint, Inc., 2005, 287 p.

STEVENSON, F.J. - *Cycles of Soil Carbon Nitrogen Phosphorus Sulfur Micronutrients*. - *Wiley*, New York, 1986.

STEVENSON, F.J. and COLE, M.A. - *Cycles of Soil*. - 2nd ed., *Wiley*, NY, USA, 1999.

STEWART, B.A. - *Advances in Soil Science* - 20, 1 Spring-Verlag New York, NY, 1992.

SUN, B., ZHOU, S., ZHAU, Q. - Evaluation of spatial and temporal changes of soil quality based on geostatistical analysis in the hill region of subtropical China. - *Geoderma*, 2003, 115(1-2), 85-99.

SWARTS, D.J. - *Soil community structure and litter decomposition under irrigated Eucalyptus globulus in south western Australia*. - Doctoral thesis, School of Natural Science Centre for Ecosystem Management, Faculty of Communications, Health and Science Edith Cowan University, 2006.

SYLVIA, D.M., FUHRMANN, J.J., HARTEL, P.G., ZUBERER, D.A. - *Principles and Applications of Soil Microbiology*. - New Jersey, USA, *Prentice Hall*, 1999, 3-43.

TEDESCHI, A., DELL'AQUILLA, R. - Effects of irrigation with saline waters al different concentration, on soil physical and chemical characteristics. - *Agricultural Water Management*, 2005, 77(1-3), 308-322.

TEJADA, M., GONZALEZ, J.L. - Effects of the application of a compost originating from crushed cotton gin residues on wheat yield under dryland conditions. - *European Journal of Agronomy*, 2003, 19, 357-368.

TEJADA, M., GONZÁLEZ, J.L., GARCÍA-MARTÍNEZ, A.M., PARRADO, J. - Application of a green manure and green manure composted with beet vinasse on soil restoration: effects on soil properties. - *Bioresource Technology*, 2008, 99, 4949-4957.

TESAROVA, M. and GLOSER, J. - Total CO2 output from alluvial soils with two types of grassland communities. - *Pedobiologia*, 1976, 16, 364-372.

TORSVIK, V. J., GOKSØYR, F.L., DAAE, R. SØRHEIM, J. MICHALSEN AND SALTE, K. - Use of DNA analysis to determine the diversity of microbial communities. - In: K. RITZ, J. DIGHTON and K.E. GILLER (Eds.). - *Beyond the biomass: Composition and functional analysis of soil microbial communities.* - Chichester, U.K., John Wiley and Sons, 1994, 39-48.

TÓTH, G. E. - Soil Quality in the European Union. - In: TÓTH, G., MONTANARELLA, L. and RUSCO, E. (Eds.), JRC Scientific and Technical Reports. - *Threats to Soil Quality in Europe* - , 2008, 11-19.

TÓTH, G., STOLBOVOY, V. and MONTANARELLA L - *Soil Quality and Sustainability Evaluation -An integrated approach to support soil-related policies of the European Union*. - EUR 22721 EN. 40 p. Office for Official Publications of the European Communities, Luxemburg ISBN, 2007, 987-92-79-05250-7.

TOZE, S. - Reuse of effluent water-benefits and risks. - *Agricultural Water Management*, 2006, 80, 147-159.

TREONIS, A. M., AUSTIN, E.E., BUYER, J.S., MAUL, J.E., SPICER, L., ZASADA, I.A. - Effects of organic amendment and tillage on soil microorganisms and microfauna. - *Applied Soil Ecology*, 2010, 46, 103-110.

U.S. EPA. - Technical support document for land application of sewage sludge, - Vol. 1. EPA/822/R-93/001A. Washington, DC. 1992.

U.S.EPA (U.S. Environmental Protection Agency). - *Protocol for Developing Pathogen TMDLs*. - EPA 841-R-00-002. Office of Water (4503F), United States Environmental Protection Agency, Washington, D.C., 2001, 132p.

U.S.EPA. - Manual of Methods for Virology, - EPA Publication EPA/600/4-84/013(R-7), 1992.

ULRICH, E., LELONG, N., LANIER, M., SCHNEIDER, A. - *Interception des pluies en forêt: facteurs déterminants. Interprétation des mesures réalisées dans le sous-réseau* - CATAENAT de RENECOFOR. *O.N.F. Bulletin technique*, 1995, 30, 33-44.

UNGER, P.W. - Overwinter changes in physical properties of no-tillage soil. - *Soil Science Society of America Journal*, 1991, 55, 778-782.

VANCE E,.D., BROOKES, P.C. and JENKINSON, D.S. - An extraction method for measuring soil microbial biomass C. - *Soil Biology and Biochemistry*, 1987, 19, 703-707.

VIRTO., I. , IMAZ, M.J, BESCANSA, P. and ENRIQUE, A. - Pore size distribution in relation to soil physical properties in two irrigated semiarid Mediterranean soils as affected by management. - *Geophysical Research Abstracts*, 2005, 7, 03223.

VISSER, S. and PARKINSON, D. - Soil biological criteria as indicators of soil quality: soil microorganisms. - *American Journal of. Alternative Agriculture*, 1992, 7(1&2), 33-37.

WANG, F.-X., KANG, Y., LIU, S.-P., HOU, X.-Y. - Effects of soil matric potential on potato growth under drip irrigation in the North China Plain. - *Agricultural water management*, 2007, 88, 34-42.

WHO. - *Wastewater Quality Guidelines for Agricultural Use*. - Vol. 47, WHO, Geneva, pp. 26, WI, 1992, 425-442.

WILKINSON, S.C., ANDERSON, J.M., SCARDELIS, S.P., TISIAFOULI, M., TAYLOR, A., WOLTERS, V. - PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. - *Soil Biology & Biochemistry*, 2002, 34, 189-200.

WILSON, C.R., PEMBERTON, B.M., RANSOM, L.M. - The effect of irrigation strategies during tuber initiation on marketable yield and development of common scab disease of potato in Russet Baurbank in Tasmania. - *Potato Research*, 2001, 44, 243-251.

WOLTER, H. and KANDIAH, A. - Harnessing water to feed a hungry world. - *ICID Journal*, 1997, 46, 1-21.

XU, J.G. and N.G. JUMA. - Above and below ground transformation of Photosynthetically fixed carbon by two barley (*Hordium vulgare* L.) cultivars in a typic cryoboroll. - *Soil Biology and Biochemistry*, 1993, 25, 1263-1272.

YAKOVCHENKO, V.I., SIKORA, L.J., KAUFMAN, D.D. - A biologically based indicator soil quality. - *Biology and Fertility of Soils*, 1996, 21, 245-251.

YANCEY, P.H., CLARK, M.E., HAND, S.C., BOWLUS, R.D., SOMERO, G.N. - Living with water-stress evolution of osmolyte systems. - *Science*, 1982, 217, 1214-1222.

YANG J-C, NI P, ZU Y-G, *et al*. - Study on respiration rate of soil microorganism on *Aneurolepidium* Chinese grassland of northeast China. - *Acta Ecologica Sinica*, 1989, 9(6), 139-143.

YANG, X.-M., WANDER, M. - Temporal changes in dry aggregate size and stability: tillage and crop effects on a silty loam Mollisol in Illinois. - *Soil and Tillage Research*, 1998, 49 (3), 173-183.

YOUNG, I.M., RITZ, K. - Tillage, habitat space and function of soil microbes. - *Soil Tillage Research*, 2000, 53, 201-213.

ZALIDIS, G., STAMATIADIS, S., TAKAVAKOGLOU, V., ESKRIDGE, K., MISOPOLINOS, N. - Impacts of agricultural practices on soil and water quality in the Mediterranean region and proposed assessment methodology. - *Agriculture, Ecosystems and Environment*, 2002, 88, 137-146.

ZHANG, Q., ZAK, J.C. - Potential physiological activities of fungi and bacteria in relation to plant litter decomposition along a gap size gradient in a natural subtropical forest. - *Microbial Ecology*, 1998, 35, 172-179.

ZHAO, S., PENG, C., JIANG, H., TIAN, D., LEI, X. and ZHOU, X. - Land use change in Asia and the ecological consequences. - *Ecological Research*, 2006, 21, 890-896.

ZINN, Y.L., LAL, R., BIGHAM, J.M., RESCK, D.V.S. - Edaphic controls on soil organic carbon retention in the Brazilian Cerrado: Texture and Mineralogy. - *Soil Science Society of American Journal*, 2007, 71, 1204-1214.

Annexes

Annex 1 : Schedule of soil sampling in Gačnik in 2004.

May 2004								
Water treatment		**Slope position**			**Soil depth (cm)**			**Nb samples**
IRR	NIR			DOWN	0-10	20-30	-	4
Chemical analyses (total and active $CaCO_3$, C/N, Cu - in laboratories in Arras) *Microbial analyses* (biomass, C mineralization, labile organic mater pool, humidity (at INRA Dijon)								
IRR*	NIR*			DOWN	3-7	20-25	40-45	10
Physical analysis (total porosity* - at FKM)								
IRR*	NIR*			DOWN	20-30	40-50		8
Physical analysis (clod's porosity* - at ENESAD)								
September 2004								
IRR	NIR		MID (1 posit.)	DOWN (5 posit.)	0-15	15-30		24
Microbial analysis (biomass, C mineralization, labile organic mater pool, humidity (at INRA Dijon)								
IRR	NIR	UP	MID	DOWN	0-5	5-15	15-30	18
Physical analyses (water structural stability (after 2 and 6 h) and organic matter size fractionation analyses with Corg, Norg, δC13 – at UB)								

* lane also included

Annex 2 : Schedule of soil sampling in Gačnik in 2005.

May 2005								
Water treatment		**Slope position**			**Soil depth (cm)**			**Nb samples**
IRR	NIR	UP (2 posit.)	MID (1 posit.)	DOWN (5 posit.)	0-15	15-30	-	64
		sunny/ shady site	sunny/ shady site	sunny/ shady site				
Microbial analyses (biomass, C mineralization, labile organic mater pool , humidity (at INRA Dijon)								
IRR	NIR	UP	MID	DOWN	0-5	5-15	-	24
		sunny/ shady site	sunny/ shady site	sunny/ shady site				
Physical analyses (water structural stability (after 2h) and organic matter size fractionation analyses with Corg, Norg, δC^{13} - at UB)								
September 2005								
IRR	NIR	UP	MID	DOWN	0-15	15-30	-	24
		sunny/ shady site	sunny/ shady site	sunny/ shady site				
Microbial analysis (biomass, C mineralization, labile organic mater pool, humidity (at INRA Dijon)								
IRR	NIR	UP	MID	DOWN	0-5	0-15	15-30	18
Complete chemical analyses (at ENESAD and UB)								

Annex 3 : Schedule of soil sampling in Gačnik in 2006 and 2007.

June 2006								
IRR*	NIR*	UP	MID	DOWN	0-5	10-15	20-25	27
Physical analysis (bulk density at FKM)								
July 2006								
Soil profile: LANE (between IRR and NIR)		UP		DOWN	Profiles from surface to basic rock every 5 cm			10+17
Chemical analyses (C_{org}, $CaCO_3$ at UB)								
IRR*	NIR*	UP (6 posit.)	MID (6 posit.)	DOWN (6 posit.)	0-5	-	-	54
July 2007								
Water treatment		**Slope position**			**Soil depth (cm)**			**N° samples**
IRR	NIR	UP	MID	DOWN	0-5	-	-	6
Microbiological analyses (fungi, total coliforms, aerob. and anaerob. bacteria, HEV and Rotavirus)								
October 2007								
IRR	NIR	UP	MID	DOWN	0-5	-	-	6
Microbiological analyses (fungi, total coliforms, aerob. and anaerob. bacteria, HEV and Rotavirus)								

*lane also included

Annex 4 : Schedule of soil sampling in Pohorski dvor during 2004-2007.

	May 2004						$N°_{block}$	$N°_{sample}$
	no liming		liming		Depth (cm)			
Org. fertilizer	without org. fertil.	Compo guano	without org. fertil.	Compo guano	0-15	15-30	4	32
Chemical analyses (Corg, Norg, C/N, P_2O_5, CEC, Ca, Na, Mg, K, Al and H, exch. Cu, Fe and Mn + texture - in laboratories in Arras; pH at Facultly of agriculture in Maribor) *Microbial analyses* (biomass, C mineralization, humidity (at INRA Dijon)								
	September 2004						3	24
Microbial analyses (biomass, labile organic matter pool*, C mineralization, W and pH (at INRA Dijon)								
	May 2005						3	24
Microbial analyses (biomass, labile organic matter pool*, C mineralization, W and pH (at INRA Dijon)								
	September 2005						3	24
Microbial analyses (biomass, labile organic matter pool*, C mineralization, W and pH (at INRA Dijon)								
October 2007								
							3	12
Microbiological analyses (fungi, total coliforms, aerob. and anaerob. bacteria, HEV and Rotavirus) – samples from depth 0-5 cm.								

*just in depth 0-15 cm

Annex 5 : Measurements of water potential (hPa) in Gačnik in 2004 and 2005.

	IRR			NIR			Inter-row		
Depth (cm)	**20-25**	**40-45**	**80-85**	**20-25**	**40-45**	**80-85**	**20-25**	**40-45**	**80-85**
Date							**2004**		
15.6.2004	-34.7	-46.3	-15.4	-25.0	-66.7	-22.2	-15.0	-28.7	-78.7
22.6.2004	-89.0	-56.0	-33.0	-81.0	-65.0	-51.0	-47.0	-49.0	-34.0
28.6.2004	-33.0	-61.7	-20.6	-62.3	-62.7	-20.9	-54.0	-65.3	-81.3
5.7.2004	-33.3	-54.0	-18.0	-53.3	-64.3	-21.4	-78.0	-76.0	-96.0
12.7.2004	-68.3	-85.0	-28.3	-65.7	-76.7	-25.6	-134.7	-115.3	-212.0
19.7.2004	-289.7	-225.7	-75.2	-216.3	-122.3	-40.8	-286.0	-270.7	-595.3
26.7.2004	-52.3	-81.7	-27.2	-49.0	-66.7	-22.2	-379.3	-491.0	-657.3
2.8.2004	-71.7	-99.3	-33.1	-58.7	-77.7	-25.9	-365.7	-563.0	-477.3
16.8.2004	-383.3	-387.0	-129.0	-314.0	-308.0	-102.7	-373.0	-739.0	-749.0
23.8.2004	-62.0	-517.0	-172.3	-44.7	-435.0	-145.0	-351.0	-659.0	-550.0
30.8.2004	-47.3	-101.3	-33.8	-47.0	-76.3	-25.4	-359.3	-531.0	-215.0
7.9.2004	-68.3	-264.0	-88.0	-46.0	-95.3	-31.8	-332.7	-745.0	-309.3
14.9.2004	-203.0	-534.0	-854.0	-189.0	-217.0	-870.0	-352.0	-768.0	-216.0
21.9.2004	-323.0	-634.0	-844.0	-93.0	-290.0	-745.0	-353.0	-746.0	-367.0
Date							**2005**		
9.6.2005	-185.0	-275.0	-292.0	-56.0	-159.0	-78.0	-203.0	-167.0	-200.0
14.6.2005	-64.0	-288.0	-262.0	-144.0	-219.0	-132.0	-514.0	-348.0	-297.0
23.6.2005	-523.0	-718.0	-652.0	-552.0	-552.0	-450.0	-657.0	-566.0	-608.0
7.7.2005	-291.0	-64.0	-48.0	-61.0	-47.0	-55.0	-68.0	-53.0	-90.0
13.7.2005	-33.0	-31.0	-64.0	-32.0	-48.0	-53.0	-43.0	-41.0	-67.0
18.7.2005	-34.0	-31.0	-65.0	-36.0	-49.0	-50.0	-35.0	-53.0	-70.0
27.7.2005	-102.0	-112.0	-182.0	-93.0	-96.0	-160.0	-128.0	-108.0	-136.0
2.8.2005	-54	-67	-96	-49	-43	-105	-82	-65	-90
9.8.2005	-35.0	-36.0	-63.0	-35.0	-52.0	-104.0	-35.0	-45.0	-85
16.8.2005	-37.0	-52.0	-67.0	-35.0	-42.0	-79.0	-26.0	-44.0	-77.0
26.8.2005	-43.0	-51.0	-65.0	-30.0	-56.0	-48.0	-35.0	-59.0	-76
31.8.2005	-53.0	-68.0	-101.0	-50.0	-62.0	-102.0	-40.0	-57.0	-91.0
15.9.2005	-174.0	-128.7	-293.0	-215.0	-76.0	-68.0	-77.0	-108.0	-133.7
27.9.2005	-60.0	-76.0	-124.0	-55.0	-65.0	-122.0	-55.0	-69.0	-104.0

Annex 6 : Measurements of water potential (hPa) in Pohorski dvor in 2004 and 2005

		Treat. 5 (pure control)			**Treat. 10 (liming only)**			**Inter-row**		
depth (cm)	Day no.	**20-25**	**40-45**	**80-85**	**20-25**	**40-45**	**80-85**	**20-25**	**40-45**	**80-85**
Date								Block I		
15.6.2004	1	-52.0	-46.3	-34.7	-55.0	-39.0	-35.0	-55.0	-53.0	-47.0
22.6.2004	7	-41.0	-25.0	-33.0	-43	-29	-27	-52	-44	-36
28.6.2004	14	-15.0	-10.0	-11.0	-27.0	-24.0	-17.0	-55.0	-33.0	-23.0
5.7.2004	21	-165.0	-80.0	-100.0	-40.0	-37.0	-39.0	-38.0	-27.0	-29.0
12.7.2004	28	-150.0	-125.0	-121.0	-67.0	-61.0	-58.0	-61.0	-53.0	-54.0
19.7.2004	35	-315.0	-316.0	-166.0	-251.0	-103.0	-83.0	-204.0	-88.0	-80.0
26.7.2004	42	-370.0	-298.0	-249.0	-267.0	-144.0	-112.0	-205.0	-76.0	-77.0
2.8.2004	49	-463.0	-469.0	-350.0	-415.0	-302.0	-165.0	-357.0	-152.0	-112.0
9.8.2004	56	-169.0	-32.0	-22.0	-30.0	-30.0	-7.0	-28.0	-5.0	-15.0
16.8.2004	63	-47.0	-38.0	-32.0	-45.0	-37.0	-27.0	-92.0	-78.0	-77.0
23.8.2004	70	-45.0	-41.0	-36.0	-44.0	-39.0	-34.0	-28.0	-32.0	-15.0
30.8.2004	77	-122.0	-98.0	-72.0	-120.0	-100.0	-77.0	-42.0	-39.0	-38.0
7.9.2004	84	-78.0	-64.0	-55.0	-82.0	-67.0	-57.0	-40.0	-37.0	-28.0
14.9.2004	91	-72	-66.0	-42.0	-83	-68	-39	-35	-28	-22
Date								Block III		
		Treat. 5 (pure control)			**Treat. 10 (liming only)**			**Inter-row**		
depth (cm)	Day no.	20-25	40-45	80-85	20-25	40-45	80-85	20-25	40-45	80-85
15.6.2004	1	-57.0	-61.0	-84.0	-122.0	-111.0	-98.0	-125.0	-101.0	-82.0
22.6.2004	7	-75.0	-88.0	-108.0	-142	-123	-111	-99	-58	-41
28.6.2004	14	-45.0	-56.0	-87.0	-122	-89	-78.0	-65.0	-51.0	-38.0
5.7.2004	21	-74.0	-86.0	-104.0	-165.0	-80.0	-100.0	-98.0	-67.0	-71.0
12.7.2004	28	-163.0	-145.0	-162.0	-252.0	-125.0	-121.0	-187.0	-92.0	-110.0
19.7.2004	35	-657.0	-412.0	-257.0	-615.0	-316.0	-166.0	-315.0	-153.0	-130.0
26.7.2004	42	-670.0	-398.0	-290.0	-655.0	-398.0	-249.0	-402.0	-162.0	-135.0
2.8.2004	49	-806.0	-849.0	-742.0	-763.0	-769.0	-350.0	-574.0	-349.0	-165.0
9.8.2004	56	-680.0	-720.0	-698.0	-569.0	-532.0	-450.0	-101.0	-37.0	-77.0
16.8.2004	63	-369.0	-356.0	-382.0	-485.0	-395.0	-355.0	-132.0	-85.0	-62.0
23.8.2004	70	-73.0	-59.0	-88.0	-368.0	-306.0	-274.0	-125.0	-65.0	-55.0
30.8.2004	77	-88.0	-77.0	-105.0	-218.0	-168.0	-123.0	-144.0	-122.0	-101.0
7.9.2004	84	-52.0	-62.0	-70.0	-209.0	-165.0	-85.0	-136.0	-126.0	-96.0
14.9.2004	91	-48.0	-46	-65.0	-188	-177	-76	-109	-84	-79

Annex 7 : Physical organic matter fractionation and the results of Corg and C/N in each fraction in Gačnik, October 2004.

Treat.	Slope	Depth (cm)	Fract.	Fract. in soil %	CaCO3 in fract. %	Corg in fract. %	C/N
IRR	**downslope**	0-5	A	3.9	3.06	14.55	18.5
			B	8.7	7.13	5.05	12.5
			C	87.4	73.20	1.58	6.9
		5-15	A	3.4	2.51	4.00	12.8
			B	9.0	7.43	3.09	10.9
			C	87.6	71.62	1.32	6.5
		15-30	A	1.7	0.19	15.57	32.4
			B	7.1	5.38	3.86	12.4
			C	91.2	74.57	1.39	6.9
	midslope	0-5	A	3.5	1.36	19.44	21.8
			B	7.0	5.22	6.76	13.2
			C	89.5	74.53	1.6	6.8
		5-15	A	0.8	0.05	4.35	16.4
			B	7.2	5.69	3.17	11.9
			C	92.0	73.44	1.15	6.3
		15-30	A	1.3	0.05	7.72	20.8
			B	8.8	7.08	3.46	11.5
			C	89.9	73.56	1.52	7.0
	upslope	0-5	A	2.4	0.92	4.63	12.6
			B	6.0	4.30	2.55	10.5
			C	91.6	65.28	0.73	4.4
		5-15	A	1.2	2.84	8.08	26.1
			B	7.1	62.67	1.58	9.7
			C	91.8		0.59	4.1
		15-30	A	1.2	0.13	10.37	32.4
			B	6.5	4.49	1.38	9.5
			C	92.2	63.49	0.50	3.6
NIR	**downslope**	0-5	A	4.3	3.36	5.21	12.3
			B	10.2	8.84	3.73	11.8
			C	85.6	72.14	1.51	6.7
		5-15	A	2.2	0.94	4.93	17.1
			B	7.3	5.99	2.99	10.7
			C	90.5	73.25	1.49	7.6
		15-30	A	3.4	2.01	4.27	16.2
			B	7.2	5.80	3.29	10.9
			C	89.4	71.08	1.42	7.3
	midslope	0-5	A	2.5	1.08	7.99	15.4
			B	7.9	6.45	3.53	15.2
			C	89.7	76.05	1.36	6.5
		5-15	A	2.1	0.52	3.61	13.5
			B	8.5	6.92	2.90	10.5
			C	89.5	74.81	1.42	7.3
		15-30	A	2.2	0.58	3.78	13.2
			B	7.6	6.04	2.67	10.1
			C	90.2	74.19	1.19	6.7
	upslope	0-5	A	2.2	0.66	7.25	15.4
			B	7.9	5.79	1.99	9.4
			C	89.9	64.86	0.71	4.1
		5-15	A	2.3	0.59	7.03	17.4
			B	9.0	6.27	2.11	8.6
			C	88.8	63.33	0.86	5.0
		15-30	A	1.5	0.18	5.46	18.3
			B	11.5	8.35	0.82	5.0
			C	87.1	61.63	0.51	3.5

Annex 8 : Sedimentary and fresh organic carbon stocks (0-30 cm) according to size fraction, slope position and treatments in Gačnik, October 2004.

Fraction size	Slope position	Treatment	Sedimentary Organic Carbon Stock T.ha-1	Fresh Organic Carbon Stock T.ha-1	Total Organic Carbon Stock T.ha-1
A	Upslope	NIR	1.48	3.21	4.69
		IRR	1.69	2.47	4.16
	Midslope	NIR	0.57	3.27	3.84
		IRR	0.51	5.93	6.44
	Downslope	NIR	1.27	4.33	5.60
		IRR	5.92	4.09	10.01
B	Upslope	NIR	2.83	2.41	5.24
		IRR	2.44	1.53	3.97
	Midslope	NIR	2.27	6.55	8.82
		IRR	2.26	9.03	11.29
	Downslope	NIR	2.64	7.12	9.76
		IRR	3.58	7.56	11.14
C	Upslope	NIR	17.81	4.53	22.34
		IRR	17.45	1.75	19.20
	Midslope	NIR	23.72	20.87	44.59
		IRR	21.30	25.55	46.85
	Downslope	NIR	25.67	24.13	49.80
		IRR	24.54	21.38	45.92

Annex 9 : Microbial biomass , Cmin, LOM, pH and gravimetric water content (W) in Gačnik, 2004.

May 2004								
Depth (cm)	**Biomass** (mg Ckg^{-1})		**Cmin** (mg Ckg^{-1})		**LOM** (mg Ckg^{-1})		**W** (%)	
	IRR	**NIR**	**IRR**	**NIR**	**IRR**	**NIR**	**IRR**	**NIR**
0-10	459	371	415	356	1233	986	27.4	28.4
20-30	151	170	304	281	789	981	30.4	30.0

September 2004										
Depth (cm)	**Biomass** (mg Ckg^{-1})		**Cmin** (mg Ckg^{-1})		**LOM** (mg Ckg^{-1})		**W** (%)		**pH**	
	IRR	**NIR**	**IRR**	**NIR**	**IRR**	**NIR**	**IRR**	**NIR**	**IRR**	**NIR**
	Downslope									
0-15	394	343	335	303	3305	2813	28.2	33.2	7.60	7.74
15-30	292	317	328	316			27.8	31.8	7.64	7.69
	Position 2									
0-15	391	275	260	348	2244	2106	24.7	30.4	7.64	7.65
15-30	255	187	275	273			25.4	30.5	7.66	7.73
	Position 3									
0-15	349	314	258	324	2451	2308	25.7	31.3	7.67	7.63
15-30	352	195	266	263			23.5	29.8	7.57	7.71
	Position 4									
0-15	287	301	273	150	2221	2325	28.3	32.3	7.67	7.68
15-30	382	271	285	132			26.5	31.0	7.62	7.71
	Position 5									
0-15	284	265	249	130	2013	2222	27.0	31.0	7.67	7.75
15-30	315	218	257	119			23.7	29.1	7.65	7.73
	Midslope									
0-15	270	254	266	138	2182	2223	30.4	32.8	7.71	7.74
15-30	252	211	269	117			29.8	31.0	7.70	7.70

Annex 10 : Microbial biomass , Cmin, LOM, pH and gravimetric water content (W) in Gačnik, 2005.

May 2005																				
depth (cm)	Biomass (mg Ckg^{-1})				Cmin (mg Ckg^{-1})				LOM (mg Ckg^{-1})				W (%)				pH			
Treat.	**IRR**		**NIR**		**IRR**		**NIR**		**IRR**		**NIR**		**IRR**		**NIR**		**IRR**		**NIR**	
Sun e	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼
	Downslope																			
0-15	537	542	542	545	273	235			1528	1326	2830	2775	32.3	27.7	31.7	27.3	7.59	7.62	7.75	7.74
15-30	415	430	472	420	308	214							34.6	31.3	34	28.5	7.8	7.82	7.76	7.73
	Position 2																			
0-15	416	413	374	401	203	195			1220	1077	2023	2076	27.3	26.6	27.8	23.8	7.64	7.71	7.77	7.69
15-30	325	304	263	282	207	2218							28.2	27.9	31.3	26.4	7.94	7.87	7.73	7.67
	Position 3																			
0-15	477	519	377	479	206	220			1188	1088	2250	2356	29.6	27	28.3	24.8	7.69	7.66	7.87	7.81
15-30	348	408	293	306	199	210							29.7	29.4	30.5	27.4	7.81	7.82	7.73	7.69
	Position 4																			
0-15	479	475	469	477	225	194			1155	1282	2550	2434	27.8	29	32.4	27.6	7.63	7.75	7.89	7.84
15-30	345	344	397	404	225	226							30.1	30.6	32.2	30.2	7.88	7.87	7.78	7.76
	Position 5																			
0-15	429	419	457	383	192	215			1135	1192	2659	2212	29.6	30.3	30.8	27	7.76	7.72	7.9	7.88
15-30	291	347	395	359	214	203							30.8	30.7	33.2	29.1	7.91	7.89	7.82	7.78
	Midslope																			
0-15	369	365	379	389					1047	1219	2478	2318	30.2	31.6	31.6	28.5	7.73	7.78	7.9	7.84
15-30	334	355	323	350									30.1	31.7	32	29.7	7.93	7.96	7.79	7.82
	Position 7																			
0-15	291	290	233	297					421	485	1059	1120	25.3	23.6	26.7	24	7.76	7.74	7.96	7.9
15-30	186	206	177	160									28.3	27.2	28.8	26.4	7.97	8.01	7.9	7.86
	Upslope																			
0-15	391	300	387	400	86	82	288	286	507	468	1511	1506	25.1	22.3	26.5	24	7.82	7.77	7.95	7.89
15-30	223	154	183	217									26.3	23.6	28.9	24.6	8.04	7.96	7.9	7.89
# shady side									☼ sunny side											

September 2005																				
depth (cm)	**Biomass (mg Ckg^{-1})**				**Cmin (mg Ckg^{-1})**				**LOM (mg Ckg^{-1})**				**W (%)**				**pH**			
Treat.	IRR		NIR		IRR		NIR		IRR		NIR		IRR		NIR		IRR		NIR	
Sun e	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼	#	☼
	Downslope																			
0-15	189	237	223	219	2601	2813	2782	2522					33.6	31.4	38.1	31.4	7.7	7.68	7.78	7.72
15-30	205	227	242	162									34.7	31.1	39.2	31.8	7.73	7.78	7.83	7.81
	Midslope																			
0-15	156	165	216	196	2363	2398	2742	2452					36	32.4	27.2	31.1	7.8	7.81	7.76	7.74
15-30	206	221	262	201									35	32.8	34.7	31.5	7.81	7.81	7.78	7.77
	Upslope																			
0-15	153	137	252	192	1134	885	1843	1472					26.9	24.4	31.4	25.6	7.83	7.8	7.79	7.74
15-30	153	112	177	148									27	25.8	30	24.9	7.83	7.83	7.85	7.81
# shady side									☼ sunny side											

Annex 11 : Microbial biomass , Cmin, LOM, pH and gravimetric water content (W) in Pohorski dvor, 2004.

		Biomass (mg Ckg^{-1})		Cmin (mg Ckg^{-1})		W (%)	
	depth (cm)	**May**	**Sept.**	**May**	**Sept.**	**May**	**Sept.**
	Block I						
Treatment 1 COMPO GUANO	0-15	388	440	586	421	37.2	23.5
	15-30	219	285	258	222	34.5	21.3
	Block II						
	0-15	432	474	471	381	32.4	25.9
	15-30	270	319	305	213	31.9	23.3
	Block III						
	0-15	355	345	494	305	30	21.5
	15-30	192	252	246	199	27.4	18.2
Treatment 5 CONTROL	**Block I**						
	0-15	358	407	424	274	34.9	25.4
	15-30	225	303	271	223	34.5	23.7
	Block II						
	0-15	464	365	441	231	32.1	21.6
	15-30	286	326	299	238	32.7	20.9
	Block III						
	0-15	351	370	455	233	30.7	18.6
	15-30	260	312	275	94	30.6	17.5
Treatment 6 COMPO GUANO LIME	**Block I**						
	0-15	472	356	518	203	34.2	18.8
	15-30	262	304	293	254	33	18.7
	Block II						
	0-15	257	488	473	432	32.8	23.0
	15-30	247	298	327	304	32	20.5
	Block III						
	0-15	326	317	456	302	28.8	22.2
	15-30	196	247	255	101	28.3	20.3
Treatment 10 LIME	**Block I**						
	0-15	414	438	610	342	32.9	28.5
	15-30	241	292	280	225	34.1	25.9
	Block II						
	0-15	444	466	416	309	30.6	22
	15-30	275	366	284	302	31.7	20.6
	Block III						
	0-15	346	407	373	354	29.8	22.5
	15-30	234	275	271	100	29.4	20.6

Annex 12 : Microbial biomass , Cmin, LOM, pH and gravimetric water content (W) in Pohorski dvor, 2005.

		Biomass (mg Ckg^{-1})		Cmin (mg Ckg^{-1})		W (%)	
	depth (cm)	**May**	**Sept.**	**May**	**Sept.**	**May**	**Sept.**
	Block I						
Treatment 1 COMPO GUANO	0-15	435	451	274	274	37.1	34.7
	15-30	205	402	223	163	37.5	32.2
	Block II						
	0-15	475	508	342	342	33.5	33.8
	15-30	290	440	225	185	33.5	33.8
	Block III						
	0-15	475	421	203	203	29.5	27.2
	15-30	273	309	254	147	28.6	26.2
Treatment 5 CONTROL	**Block I**						
	0-15	467	492	679	421	37.7	33.8
	15-30	353	352	222	172	34.8	33.1
	Block II						
	0-15	524	353	783	432	33.7	30
	15-30	409	457	304	184	34.9	29
	Block III						
	0-15	502	438	710	231	30.6	24.5
	15-30	276	309	238	147	30.5	29.3
Treatment 6 COMPO GUANO LIME	**Block I**						
	0-15	588	567	381	381	34.6	31.4
	15-30	401	314	213	164	34.9	30.7
	Block II						
	0-15	622	585	309	309	33.4	30.3
	15-30	502	331	302	185	35.5	30
	Block III						
	0-15	477	304	305	305	28.3	29.7
	15-30	238	189	199	147	28.5	28.3
Treatment 10 LIME	**Block I**						
	0-15	617	518	534	309	33.8	32
	15-30	432	310	210	199	34.4	32.6
	Block II						
	0-15	559	535	521	354	32.2	31.6
	15-30	357	292	198	188	22	31
	Block III						
	0-15	439	353	506	233	29.6	29.1
	15-30	365	212	191	139	29.9	27.8