Long-term impact of tillage on biogeochemical phosphorus cycle : analysis of the test of L'Acadie (Quebec, Canada) and modelling

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Impact à long terme du travail du sol sur le cycle biogéochimique du phosphore: Analyse de l'essai L'Acadie (Québec, Canada) et modélisation

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Titre : Impact à long terme du travail du sol sur le cycle biogéochimique du phosphore: Analyse de l'essai L'Acadie (Québec, Canada) et modélisation

Résumé :

La pratique du «sans labour» (NT) se développe dans le cadre de l'agriculture de conservation des sols. Cette pratique modifie nombre de propriétés du sol comme, par exemple, la répartition du phosphore (P) dans le profil du sol. L'objectif de cette thèse est d'analyser les impacts après plusieurs décennies du NT sur le cycle biogéochimique du P et d'intégrer ces effets dans un modèle de fonctionnement. Nous avons utilisé un essai au champ de longue durée sous maïs-soja (L'Acadie, Québec, Canada) implanté sur un sol argilo-limoneux. Le dispositif était un split-plot à 4 blocs avec mouldboard plough (MP) et sans labour (NT), subdivisés par 3 doses de fertilisation en P minéral [0 (0P), 17.5 (0.5P), 35 (1P) kg P ha-1] apportées sur le maïs et localisées à 5 cm de profondeur et à 5 cm du rang de maïs.

La concentration des ions phosphates du sol (Cp) test était relativement uniforme dans la couche labourée (0-20 cm) (0.08 mg P L-1), puis baissait légèrement dans 20-30 cm (0.05 mg P L-1) et davantage au-delà (0.01 mg P L-1). Sous les traitements [NT, 0.5P] et [NT, 1P] traitements, le Cp était plus élevé dans la couche 0-10 cm (0.28 et 0.19 mg P L-1) que dans la couche labourée mais baissait rapidement avec la profondeur. Cette stratification verticale sous NT était également observée pour les teneurs en P-Olsen, P-M3 et autres nutriments comme C, N et K. Après 23 et 24 années d'essai, il y avait tendanciellement moins de racines du maïs sous NT (-14%) que sous MP, probablement à cause de la présence plus importante d'adventices sous NT. Pour le soja, il y avait beaucoup plus de racines dans la couche 0-10 cm sous NT (44% de longueur total) que sous MP (21%) et inversement dans la couche 10-20 cm. Ces différences de distribution des racines sous NT et MP correspondent à la stratification de N, P, et K.

Cet ensemble de données sur la distribution des racines et du phosphore a été utilisé pour i) évaluer un modèle 1D décrivant la dynamique du P sur plusieurs décennies dans la couche labourée du sol, ii) proposer un mode d'estimation de la distribution du prélèvement dans le profil de sol, et iii) développer un modèle spatialisé 2D décrivant la dynamique du P pour le traitement sans labour. Ce modèle permet de simuler l'évolution de la disponibilité en P du sol sur le long terme quels que soient les modes de préparation du sol et le régime de fertilisation P. Même si le modèle surestime parfois la disponibilité en P à proximité de la zone fertilisée, il permet de prédire la stratification du P du sol en NT et ses conséquences sur le prélèvement de P en relation avec les propriétés du sol et le développement du système racinaire. Il pourra contribuer à améliorer le raisonnement de la fertilisation phosphatée dans le contexte du sans-labour.

Mots clés : labour, sans-labour, semis direct, agriculture de conservation, fertilisation phosphatée, agrosystèmes, stocks et flux, interception racinaire, sol, fertilité, bilan.

Title : Long-term impact of tillage on biogeochemical phosphorus cycle: Analysis of the test of L'Acadie (Quebec, Canada) and modelling

Abstract :

The no-till (NT) is gaining great attention for soil preparation. This practice modifies number of soil properties such as the distribution of phosphorus (P) in the soil profile. This work aims to analyze the impacts on the biogeochemical P cycle after decades of NT and incorporate those effects in an operational model. We used a long-term field experiment under corn-soybean rotation established on a clay loam soil (L'Acadie, Quebec, Canada). The design was a split-plot plan with 4 blocks under moldboard plough (MP) and NT, subdivided by 3 doses of P [0 (0P), 17.5 (0.5P), 35 (1P) kg P ha-1] applied in corn phase and localized to 5-cm deep and 5-cm from the corn row.

The phosphate ion concentration in MP was relatively constant (0.08 mg P L-1) in the tilled layer (0-20 cm), slightly lower in 20-30 cm (0.05 mg P L-1) and much lower below (0.01 mg P L-1). In [NT, 0.5P] and [NT, 1P] plots, Cp was higher (0.28 et 0.19 mg P L-1) in the 0-10 cm layer compared to the tilled layer in MP, but decreased sharply with depth. This vertical stratification in NT was also observed for P-Olsen, P-M3 and other nutrients as C, N, and K. After 23- and 24-year of experimentation, maize roots tended to be fewer (-14%) under NT than MP, probably because of increased weed infestation under NT. For soybean, more roots accumulated in the 0-10 cm layer under NT (44% of total length) than MP (21%) and vice versa for the 10-20 cm layer. Those differences in root distribution under NT and MP corresponded to the stratification of N, P, and K.

This set of data on the distribution of roots and phosphorus was used i) to develop a 1D model describing P dynamics over several decades in MP, ii) to test a method to assess the spatial P uptake distribution according local root length density and soil P availability, and iii) to develop a spatial 2D model describing P dynamic in NT. This model simulates the soil P availability dynamic on long term according soil properties and crop root distribution within soil profile for different soil preparation regime and different P fertilization rate. Although the model overestimates the P availability near the localized P fertilizer, it is able to predict the soil P stratification in NT treatment and its consequences on crop P uptake. This new model will be a useful tool to improve P fertilization management in context of no-till practices.

Keywords : tillage, no-till, direct drilling, conservation agriculture, phosphate fertilizer, agro-systems, stocks and flows, root interception, soil fertility, P budget.

Unité de recherche

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Résumé long:

Le travail du sol modifie la structure du sol et nombre de processus et facteurs de production impliqués dans le fonctionnement des agrosystèmes (teneur en eau, croissance et développement des cultures, transferts sol-plante, réactions physico-chimiques et microbiologiques, porosité du sol, population d'adventices, etc.). Le labour, qui mélange par retournement des couches superficielles de sol à l'aide d'une charrue, est toujours le mode de préparation du sol le plus couramment utilisé en France et au Québec. Mais les Techniques Culturales Sans Labour (TCSL) gagnent en importance depuis plusieurs décennies. En 2006, un tiers des cultures annuelles ont été implantées sans labour préalable du sol. Les TCSL permettent d'économiser du temps, de l'énergie, de limiter l'érosion des sols, d'améliorer la biologie du sol. Les TCSL modifient les propriétés biologiques, physiques et chimiques du sol. Il y a en particulier accumulation de certains éléments dans les premiers cm de sol ce qui crée des gradients de concentration vers les couches plus profondes. Cette stratification est très marquée pour le phosphore (P), un nutriment majeur peu mobile, susceptible de limiter la production agricole. Les TCSL pourraient affecter la disponibilité du P du sol, l'absorption par les racines, et le fonctionnement général du cycle biogéochimique sur le long terme ce qui pourrait finalement affecter la fertilité des sols. Dans un contexte de raréfaction des gisements de phosphate facilement accessibles à faible coût, de protection des eaux de surface, de recyclage, il convient de mieux comprendre, décrire et prévoir ces réactions afin de valoriser au mieux les stocks dans le sol et de concevoir des systèmes de cultures adaptés et parcimonieux.

Ce travail a pour objectif d'étudier et d'analyser les impacts à long terme du non-labour sur le cycle biogéochimique du P à l'échelle de la parcelle cultivée et d'intégrer ces effets dans un modèle dynamique. La démarche expérimentale mise en œuvre a été d'abord d'analyser la répartition spatiale, verticale et horizontale, des caractéristiques du système racinaire (biomasse, longueur, surface et diamètre) et du P disponible du sol pour les plantes. Ensuite, la part de P prélevé a été calculée pour chaque masse élémentaire de sol en considérant leur contribution relative au stock de P disponible dans le profil du sol et leur contribution relative à l'enracinement. Enfin, cet ensemble de données a été intégré dans les modèles 1D et 2D décrivant le fonctionnement à long terme du cycle biogéochimique du P à l'échelle de la parcelle cultivée avec et sans labour.

Cette recherche fut conduite à la station de recherche d'Agriculture et Agroalimentaire Canada située à L'Acadie, 40 km au sud-ouest de Montréal, Québec, Canada. Cet essai a été initié en 1992 sur un limon argileux en climat continental humide à forte amplitude thermique et cultivé selon une succession culturale maïs-soja. Le dispositif expérimental était de type split-plot avec 4 répétitions. Le facteur principal comprenait 2 modalités de préparation du sol [avec labour (MP) sur une profondeur de 20 cm et absence de labour (NT)], subdivisé par 3 doses de fertilisation phosphatée [0 (0P), 17.5 (0.5P), 35 (1P) kg P ha-1 apportées uniquement sur la phase de maïs]. L'engrais a été localisé à 5 cm de profondeur et à 5 cm du rang de maïs. Les résidus de récolte ont été restitués au sol. Le rendement en grains et leur teneur en P ont été mesurés chaque année dans chaque parcelle expérimentale. Le flux annuel de P exporté a été calculé en multipliant le rendement par la teneur en P. Les distributions spatiales 2D des racines dans le profil de sol ont été analysées en 2014 pour le maïs et en 2015 pour le soja de même que la disponibilité du P du sol pour les plantes en 2014. Des carottes (60 cm × Ø5 cm) de sols ont été prélevées au stade « floraison » à 5, 10 et 15 cm perpendiculairement au rang de culture. Elles ont ensuite été découpées pour obtenir 5 couches de sol (0-5, 5-10, 10-20, 20-30, 30-40

cm) chez le maïs et une 6ème couche (40-60 cm) chez le soja. Après séparation à l'eau, la longueur, la surface, la biomasse et le diamètre des racines ont été déterminées par analyse d'image pour chaque unité élémentaire de sol. En 2014, un second échantillonnage identique a été effectué pour collecter les échantillons de sol, séchés à 40 °C, broyés à 2 mm avant l'analyse de la disponibilité du P du sol pour les plantes. Deux approches ont été utilisées : i) une approche mécaniste qui consistait à mesurer dans des suspensions de terre, la concentration (Cp) des ions phosphates en solution et la quantité d'ions phosphate diffusibles à l'interface solide-solution ; ii) des extractions chimiques utilisées dans les laboratoires d'analyses de sol pour raisonner la fertilisation phosphatée. Il s'agit des extractions Mehlich3 (solution de 0.015M NH4F + 0.25M NH4NO3 + 0.2M CH3COOH + 0.013M HNO3 + 0.001M EDTA avec un pH de 2.5) et Olsen (solution de 0.5M NaHCO3 avec un pH de 8.5) respectivement utilisées au Québec et en France. D'autres propriétés qui pouvaient influencer le développement des racines (pH, teneur totale de C et N, teneur de K, Ca, Mg, Na, Fe, Al par l'extraction Mehlich3) ont également été déterminées.

En moyenne des 13 années de culture de maïs, le rendement-grains annuel du maïs était de 7.3 (±2.1) Mg ha-1. Sous NT, il y avait une réduction significative de rendement particulièrement nette en 2012 (-55% de MP) et 2014 (-45% de MP). L'effet de la fertilisation P n'était pas significatif même si le rendement de 1P était légèrement supérieur à ceux de 0P et 0.5P. En moyenne sur 11 années de culture de soja, le rendement annuel en grains était de 2.5 (±1.1) Mg ha-1. Il était significativement plus faible sous NT (2.3 Mg ha-1) que sous MP (2.7 Mg ha-1) et ne différait pas entre 0P, 0.5P et 1P. Les teneurs en P des grains de maïs (0.23 mg P kg MS-1) et de soja (0.55 mg P kg MS-1) étaient invariante entre NT et MP alors qu'elles étaient significativement plus faibles chez 0P (0.23 et 0.54 mg P kg MS-1 pour maïs et soja) par rapport à 0.5P et 1P (0.24 et 0.56 mg P kg MS-1 pour maïs et soja). La différence, cumulée sur les 24 années d'expérimentation, entre le P apporté par la fertilisation et le P exporté dans les récoltes, était de -391 (±31), -170 (±36) et de +29 (±43) kg P ha-1 pour, respectivement, 0P, 0.5P et 1P sous MP. Ces valeurs étaient supérieures d'environ 50 kg ha-1 en moyenne sous NT.

Quelle que soit l'approche analytique, mécaniste ou chimique, les informations sur le P disponible du sol étaient similaires puisque les résultats des différentes méthodes étaient corrélées. Le P disponible du sol en 2014 ne changeait pas significativement dans la couche labourée, puis baissait légèrement dans la couche sous-jacente (20-30 cm) et considérablement dans la couche 30-40 cm. Pour NT-0.5P et NT-1P, le P disponible dans la couche 0-10 cm était significativement plus élevé que dans la couche labourée de MP-0.5P et MP-1P mais diminuait rapidement avec la profondeur. Pour tous les régimes de fertilisation en P, il y avait significativement plus de P disponible dans la couche (20-30 cm) du MP que dans celle du NT. Cela indiquait qu'il y a eu un mélange de sol au-delà de la profondeur théorique de labour et qu'une fraction de l'apport de P a été diluée bien au-delà de 20 cm. Les valeurs de Cp étaient faibles dans ce sol qui a une capacité élevée à réagir avec les ions phosphatées, équivalente à celle d'un limon argileux en France. Pour le régime de fertilisation 1P, qui correspondait à un bilan proche de zéro, Cp était 0.093 (±0.026) mg P L-1, les quantités d'ions phosphatées diffusibles en équilibre à cette concentration étaient de 65, 180 et 385 kg P ha-1 après une journée, un mois et un an d'équilibre, respectivement, et les teneurs en P-Olsen et M3-P étaient respectivement de 23 et 75 mg P kg-1 sol. Cp diminuait pour 0.5P (0.077 mg P L-1) et 0P (0.041 mg P L-1) en relation directe avec les bilans de P de plus en plus négatifs.

La stratification verticale sous NT était également observée à des degrés divers pour d'autres éléments, comme le carbone, l'azote avec des teneurs 5 fois plus élevées dans la couche 0-5 cm que dans la couche 30-40 cm. Le potassium était près de deux fois plus concentré dans la couche 0-5 cm que dans la couche 5-10 cm.

Le maïs et le soja avaient un système racinaire comparable : la fréquence des racines baisse avec la profondeur du sol et la distance au rang. Après 23 et 24 années d'expérimentation, ni le mode de préparation du sol ni la fertilisation P n'ont affecté significativement les caractéristiques des racines de maïs ou du soja. La distribution des racines de maïs était similaire dans NT et MP mais systématiquement plus faible sous NT. Ainsi, la moyenne générale de la densité de longueur de racines (RLD) de maïs était de 1.48 et 1.28 cm cm-3 sous MP et NT, respectivement. Cette diminution sous NT touchait essentiellement les racines primaires (Ø > 0.8 mm) et secondaires (Ø compris entre 0.2 et 0.8 mm). Elle était probablement due à la présence dans NT d'une population plus importante d'adventices même si d'autres facteurs pouvaient contribuer à l'expliquer, comme la densité apparente du sol. La RLD tendait également à baisser avec un apport plus faible de fertilisation phosphatée (1.29, 1.23 et 1.69 cm cm-3 pour 0P, 0.5P et 1P, respectivement).

Pour le soja, la moyenne générale de la RLD était de 1.95 et 1.55 cm cm-3 sous NT et MP, respectivement. On observait des effets marqués et antagonistes sur la distribution des racines avec la profondeur du sol. Il y avait beaucoup plus de racines dans la couche 0-10 cm sous NT avec 44% de la longeur racinaire totale contre seulement 24% sous MP. C'est l'inverse dans la couche 10-20 cm avec 21% de la longeur racinaire totale sous NT et 36% sous MP. Ces effets suivaient la stratification verticale des nutriments majeurs (N, P, K) même si les écarts tendaient à s'atténuer lorsque le régime de fertilisation passait de 0P à 1P.

Cet ensemble de données sur le système racinaire et la disponibilité du P du sol a été utilisé pour tester un modèle 1D décrivant le cycle du P dans la couche labourée du sol et construire un modèle spatialisé 2D adapté au contexte des TCSL. Les deux modèles étaient basés sur une équation de conservation de la masse de P dans la masse de sol considéré. Cette équation reliait la dynamique du P disponible au bilan annuel des entrées et des sorties de P. Le modèle 1D permettait de prévoir les évolutions à long terme observées sous MP dans l'essai L'Acadie. Le modèle 2D simulait l'évolution à long terme des stratifications verticales en fonction du niveau de fertilisation phosphatée et des caractéristiques de sorption-désorption des ions phosphates avec la phase solide du sol. Mais la confrontation entre les simulations et les observations de terrain des valeurs de Cp montrait que le modèle 2D surestimait de beaucoup les valeurs de Cp dans les unités de sol recevant la fertilisation phosphatée. Plusieurs raisons pouvent expliquer ce décalage, comme par exemple :

- précision de la localisation de l'engrais d'une fois sur l'autre ;
- non prise en compte du transport de sol fertilisé par les vers de terre ;
- sous-estimation de la durée de diffusion du P de l'engrais dans la phase solide du sol ;
- précipitations locales du P au voisinage du granule d'engrais...

Le modèle 2D permettait néanmoins de simuler les effets à long terme du mode de travail du sol en interaction avec les pratiques de fertilisation sur le cycle du P. Il

peut être utilisé pour évaluer ces effets dans d'autres contextes pédoclimatiques et de tester des scénarios visant à accroître l'efficience d'acquisition et d'utilisation du P à l'échelle parcellaire. Mais ce travail contribue à une compréhension connaissance plus approfondie des conséquences de la stratification spatiale du P du sol sur le prélèvement de P en relation avec les propriétés du sol et le développement du système racinaire.

Mots clés : labour, sans-labour, semis direct, agriculture de conservation, fertilisation phosphatée, agrosystèmes, stocks et flux, interception racinaire, sol, fertilité, bilan, disponibilité du P du sol pour les plantes ; transfert d'ions phosphate à l'interface solide-solution ; sorption-désorption ; dilution isotopique

Long abstract:

Tillage alters soil structure and several processes and factors involved in the functioning of agro-ecosystems (water content, crop growth and development, soil-plant transfers, physio-chemical reactions and soil porosity, weed population, etc.). Plowing, that inverts soil surface, is still the tillage method most frequently used in France and Quebec. But no-till practice for soil preparation increased over the past decades. In 2006, one third of annual crops have been grown without prior soil tillage. No-till, as well as other simplified soil preparation techniques may save time and energy, reduce soil erosion and improve soil biodiversity. It also changes soil biogeochemical properties by accumulating nutrients in upper soil layers and building vertical concentration gradients. This stratification is evident for phosphorus (P), a major but little mobile element which may limit agricultural production. No-till could thus influence the long-term availability of soil P, root P uptake and the general function of soil biogeochemical cycle that could ultimately affect soil fertility. In the context of scarcity of easily accessible high-quality phosphate deposits, surface water protection and P resource recycling, it is necessary to better understand, describe and predict changes in the P cycle under different systems in order to maintain optimum P levels in the soil, increase P use efficiency and implement sustainable agricultural systems.

This work aims to study and analyze the impacts of no-till on the biogeochemical cycle of P across the cultivated land and elaborate operational models. The experimental approach was first implemented to analyze the spatial distribution of root traits (biomass, length, area and diameter) and the soil available P to plants. Then, the amount of P uptake for each unit mass of soil was calculated by considering the relative contribution of soil available P stocks and roots. Finally, this data set has been integrated into 1D and 2D models describing the long-term function of the biogeochemical cycle of P at field scale in moldboard plough (MP) and no-till (NT) systems, respectively.

The research was conducted on the research station of Agriculture and Agri-Food Canada located at L'Acadie, 40 km southwest of Montreal, Quebec, Canada. This trial has been initiated in 1992. A crop rotation of corn-soybean was established on a clay loam soil under a humid continental climate. The experimental design was a split-plot plan with 4 repetitions. The main factor included 2 tillage methods [moldboard plough (MP) to a depth of 20 cm and no-till (NT)] with three phosphate fertilizer doses [0 (0P), 17.5 (0.5P) 35 (1P) kg P ha-1 applied in the corn phase] randomly assigned as sub-plots. The fertilizer was located 5-cm deep and 5-cm from the corn row. Crop residues were returned to soil after harvest. Grain yields and P contents were measured every year in each sub-plot. The annual flow of P exportation was calculated by multiplying grain yield by grain P content. The 2D spatial root distributions in the soil were determined for corn in 2014 and soybean in 2015 as well as soil P availability was determined in 2014 only. Soil cores (Ø = 5.25 cm) were taken to a depth of 40 cm at 5, 10 and 15 cm intervals perpendicularly to crop row at "bloom" stage. Cores were cut into five soil layers (0-5, 5-10, 10-20, 20-30, 30-40 cm) for corn and also a sixth layer (40-60 cm) for soybean. After separating soil from roots, root length, surface, biomass and diameter were determined by image analysis. At the same time of corn root sampling, a second series of soil samples was conducted to determine soil P test. Soil samples were dried at 40 ° C then crushed to <2 mm before analysis. Two approaches were used: i) a mechanistic approach that consisted in measuring phosphate ion concentration (Cp) in soil suspensions and the amount of diffusive phosphate ions at the solid-solution interface; ii) routine chemical extractions

to manage P fertilization. Extraction methods were Mehlich3 (solution of 0.015M NH4F + 0.25M NH4NO3 + 0.2M CH3COOH + 0.013M HNO3 + 0.001M EDTA with pH at 2.5) and Olsen (0.5M NaHCO3 solution with pH at 8.5), used in Quebec and France, respectively. Other properties (pH, total contents of C and N, and contents of K, Ca, Mg, Na, Fe, Al by Mehlich3 extraction), that could also influence root development, were also determined.

Average corn yield was 7.3±2.1 Mg ha-1. The NT significant reduced yields, particularly in 2012 (-55% MP) and 2014 (-45% MP). The P fertilization effect had not significant effect although 1P yielded slightly higher than 0P and 0.5P. Average soybean yield was 2.5±1.1 Mg ha-1. Soybean yield was significantly lower under NT (2.3 t ha-1) than MP (2.7 t ha-1) and did not differ among 0P, 0.5P and 1P. The P contents in corn and soybean (0.24 and 0.55 mg P kg DM-1) were similar between NT and MP but were significantly lower in 0P (0.23 and 0.54 mg P kg DM-1 for corn and soybean, respectively) than 1P and 0.5P (0.24 and 0.56 mg P kg DM-1 for corn and soybean, respectively). Over 24 years of experimentation, the differences between P supplied by fertilization and P exportations by the crops, were -391±31, -170±36 and 29±43 kg P ha-1, respectively, for 0P, 0.5P and 1P under MP. Those values were about 50 kg P ha-1 greater under NT on average.

Soil tests were closely correlated. Under MP, soil available P did not differ significantly in the upper 20 cm, slightly declined in the underlying layer (20-30 cm) and declined much more in the bottom layer (30-40 cm). In [NT, 0.5P] and [NT, 1P] plots, soil available P was higher in the 0-10 cm layer compared to MP and decreased sharply with depth. Soil available P was systematically lower in the 20-30 cm layer under NT than MP, indicating soil mixture of beyond the theoretical depth of plowing (20 cm). The Cp values were low in the experimental soil hence showing high reactivity with the phosphate ions, equivalent to the clay loam soil in France. For 1P that led to a nearly zero P budget, the Cp value was 0.093±0.026 mg P L-1. The amount of diffusible phosphate ions at equilibrium at 0.093±0.026 mg P L-1 were 65, 180 and 385 kg P ha-1 after one day, one month and one year of equilibration, respectively. The P-Olsen and P-M3 values were 23 and 75 mg P kg-1, respectively, for 1P. The Cp decreased for 0.5P (0.077 mg P L-1) and 0P (0.041 mg P L-1), leading to more negative P budgets.

Vertical stratification in NT was also observed to varying degrees for other elements such as carbon, nitrogen, with contents five times higher in the 0-5 cm layer than in the 30-40 cm layer; potassium was almost two times more concentrated in the 0-5 cm layer than in the 5-10 cm layer.

Corn and soybean showed comparable root systems: root density decreased with depth and distance to crop row. After 23- and 24-year of experimentation, neither soil tillage nor P fertilization significantly affected the traits of corn and soybean roots. Corn roots were distributed similarly under NT and MP, but were consistently lower with depth under NT. Thus, the average root length densities (RLD) for corn roots were 1.48 and 1.28 cm cm-3 under MP and NT, respectively, due to the reduction in primary (Ø > 0.8 mm) and secondary (Ø between 0.2 and 0.8 mm) roots under NT attributable to a larger population of weeds and higher soil bulk density under NY. The RLD also tended to decrease with decreasing levels of P fertilization (1.29, 1.23 and 1.69 cm cm-3, respectively in 0P, 0.5P and 1P).

For soybean, the RLDs averaged 1.95 and 1.55 cm cm-3 under NT and MP, respectively. There were more roots in the 0-10 cm layer under NT with 44% of total

roots (in length) compared to 24% under MP. It was the inverse in the 10-20 cm layer with 21% of total roots (in length) under NT and 36% under MP. These different root distributions were consistent with the stratification of major nutrients (N, P, and K) while the difference tended to be mitigated as fertilizer rate increased from 0P to 1P.

Those data sets for root system and the soil available P were used to elaborate a 1D model on the P cycle in the tilled soil layer under MP and a 2D model for the P cycle in the NT system. Both models were based on a mass balance equation relating available P to the annual budget of P. The 1D model predicted the long-term evolution of soil available P under MP at L'Acadie. The 2D model simulated the long-term evolution of vertical soil P stratifications based on P fertilization. However, the 2D model overestimated the Cp values in soil units receiving fertilizer P. Several reasons may explain such overestimation, such as:

- fertilizer localization varying during the long-term experiment;
- not taking into account the movement of fertilized soil caused by bioturbation;
- underestimation of P fertilizer diffusion in the soil solid phase;
- local P precipitation in the vicinity of granule fertilizer, etc.

The 2D model, however, could simulate the long-term effects on soil P cycle of soil tillage interacting with P fertilization. It can be used to assess such effects under other soil and climatic conditions and run scenarios to increase P use efficiency at the field scale. This work contributes to a deeper understanding of the consequences of soil P stratification on P uptake in relation to soil properties and root development.

Keywords : tillage, no-till, direct drilling, conservation agriculture, phosphate fertilizer, agro-systems, stocks and flows, root interception, soil fertility, P budget, available soil P, phosphate ion transfer at the solid-solution interface, sorption-desorption, isotope dilution.

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Chapter I Phosphorus cycle in agroeco-systems and conservation agriculture: General Introduction

Phosphorus (P) is an essential element for plants, animals and humans. It is a key element in fundamental biochemical reactions involving genetic material (DNA and RNA) and energy transfer (ATP), and in the structural support for membranes (phospholipids) and bones (the biomineral hydroxyapatite) (Ruttenberg 2003). In agriculture, P is the second most important element for crop growth after nitrogen (N). Until P deficiency is corrected many crops do not respond to nitrogen (Kirkby and Johnston 2008). Most crops contain 0.2-0.5% of P in their dry matter where available P is in sufficient amount in the soil.

While P is the 11th most abundant element in the Earth's crust, it is unevenly distributed. According to the US Geological Survey DATABASE, the P resource (phosphate rocks) is concentrated in five countries (Morocco, China, Algeria, Syria and Jordan) with 85-90% of the world reserves. Many arable soils over the world (30-40%) are low in phosphorus (Kirkby and Johnston 2008). In intensive agriculture, soil P can be supplied by P fertilizers. Inorganic P fertilizers became available 160 years ago by producing superphosphate from phosphate rock (Kirkby and Johnston 2008). But phosphate rocks is a non-renewable resource while the demande for P fertilizers increases by 2.2% per year (FAO: World fertilizer trends and outlook to 2018). The limited reserve of phosphorus rock may be exhausted within the next 50-100 years (Cordell et al. 2009). It is necessary to improve P-use efficiency in agroecosystems.

The P cycle in agro-ecosystems comprises soil P stocks and various P fluxes (inputs and outputs). In soils, phosphorus could be mainly divided into inorganic and organic forms, which account for 35-70% and 30-65%, respectively, of total P depending on soil types (Shen et al. 2011). The P, directly used by plants, is the phosphate ions (HPO_4^{2-} and $H_2PO_4^-$); and it is strongly constrained by multiple interactions with soil constituents, such as precipitation and adsorption (Hinsinger et al. 2011). Phosphorus fertilization contributes to increase soil P status (Sims et al. 1998). Ulrich (2013) reported that in 2011, 45.89 Mt (in P_2O_5) of inorganic P fertilizers were consumed in the world. In addition to inorganic P fertilizers, animal manure, crushed animal bones, human and bird excreta, city waste composts and ash are other P sources (Van Vuuren et al. 2010). For example, Cordell et al. (2009) reported that manure, human excreta and food residues used as alternative P resources accounted to about 15, 3 and 1.2 Mt P year^{-1}, respectively. Crops take phosphate ions in soil solution by diffusion and convection through root system along soil profile. Because of small root interception (in contact with only 1-2% of soil) (Baligar 1985) and small diffusion coefficient of phosphate ions in soil (1 x 10^{-8} to 10^{-10} cm^2 s^{-1}) (Barber 1995), crop roots have to modify their morphology and structure or enhance biochemical activitity in the rhizosphere to explore and mobilize soil P under plant P deficiency (Hinsinger et al. 2011; Richardson et al. 2011).

On the other hand, over-fertilization or inapproriate fertilization increase the risk of P loss through erosion, runoff and leaching. Soil erosion takes place in ploughed fields as well as pastures. Jørgensen (2010) reported that the world soil erosion from agriculture areas (including cropland and pasture) reaches to 72.9 Gt yr^{-1} and gives P losses at 19.3 and 17.2 Mt P yr^{-1} for cropland and pasture, respectively. The world total phosphate fertilizer application can roughly lead to a loss of 0.5 Mt P yr^{-1} in surface runoff. Because of the high P-fixation capacity of many mineral soils, the losses caused by vertical P transport (leaching) are often assumed to be minor (Makris et al. 2006).

Soil tillage is another factor to affect P losses and soil P management. Conventional tillage contributes to suppress weeds, incorporate fertilizers and eliminate soil compaction, but also leads to soil structure degradation, soil erosion, moisture loss and disruption of soil lifecycle. Conventional tillage also requires high energy. For both environmental and economic benefits, the concept of conservation agriculture was introduced by Food and Agriculture Organization in 2001 and developed during the recent 15 years. Conservation agriculture is based on three principles: 1. Minimum mechanical soil disturbance to maintain

minerals within the soil, mitigating erosion and water loss; 2. Permanent soil cover to promote biological activities and improve the soil structure; 3. Crop rotations with more than two species to control disease and provide potential savings of nutrients, especially with legumes.

To implement the conservation agriculture, no-till or reduced tillage practices are conducted to achieve the first two principles, which reduce soil disturbance during planting operations and leave greater amounts of plant residues on the soil surface. According to Natural Resources Conservation Service of USDA (U.S. Department of Agriculture), reduced tillage systems comprise zone tillage, no-till, ridge-till, mulch-till, reduced-till and strip-till. Reduced (or simplified) tillage systems vary in working depth, soil turnover and mixture, working surface, and soil cover rate (Schubetzer et al. 2007).

No-till (NT) system is specific with no soil turnover or mixture. No-till system was adopted on 125M ha of arable soil over the world, about 9% of total arable soil (Pittelkow et al. 2015b). No-till covered 13.5 M ha of soil in Canada in 2007-2008, ranking the 4th in world (Derpsch and Friedrich 2009). In comparison, the proportion of the agricultural area in France in 2011 was 2% under NT and 33% under simplified tillage practices (Fiche Référence d'ADEME 2015). No-till systems reduce soil erosion and soil preparation labours and costs. Kisic et al. (2002) reported that no-till practice could reduce runoff and soil erosion loss from 171.7 mm and 114.4 t ha^{-1} to 60.7 mm and 12.2 t ha^{-1} in a 30-cm tilled soil under three crops [maize (Zea mays L.), soybean (Glycine hispida L.) and winter wheat (Triticum aestivum L.)] grown on a 9% slope soil in Croatia. In addition, in a cereal system, the time for traction of machines could be reduced from 7 h ha^{-1} with tillage to 4 h ha^{-1} using NT (Rieu 2001). Tebrügge (2001) reported that the implantation costs per 100 ha were 50 € ha^{-1} with NT method compared to 210 € ha^{-1} for tillage methods. However, NT could also reduce crop yields (Kumar et al. 2012; Guan et al. 2014). One of the reasons is the severer weed competition in NT. With less soil disturbance, NT system provides a relatively stable soil environment and greater soil biodiversity (Helgason et al. 2010; Scopel et al. 2013) including weed development (Melander et al. 2013; Nichols et al. 2015; Pakeman et al. 2015). This would, thus, increase the cost of weeding and pesticides in NT (Gilet 2001).

Moreover, NT system alters soil properties. Firstly, soil structure in NT is improved as shown by higher proportions of macro-aggregates (Messiga et al. 2011) and macro-pores (Dal Ferro et al. 2014). In the absence of soil loosening, soil compaction develops in NT leading to higher soil bulk density (Javeed et al. 2013) and penetration resistance (Fernández et al. 2015). These soil physical alterations limit crop root proliferation (Chassot et al. 2001; Qin et al. 2006), and consequently the water and nutrient uptake along soil profile. Secondly, nutrients are exported through harvest while nutrients from crop residues left on the soil surface could be recycled through decomposition, leading to preferential accumulation of nutrients in upper layers and nutrient stratification in NT (Bouthier and Labreuche, 2011). When the nutritive element is less mobile in soils, the stratification would be more evident, especially phosphorus. For example, Lupwayi et al. (2006) reported 3.5 times greater available P concentration (bicarbonate-extractable P) in the 0-5 cm soil layer than in the 5-15 cm layer in NT, similar to other studies (Franzluebbers and Hons 1996; Schwab et al. 2006; Costa et al. 2010; Messiga et al. 2010; Calegari et al. 2013). High heterogeneity of soil nutrients under NT influences rooting patterns because roots tend to explore high-nutrient zones (Li et al. 2012). Due to effects of NT system on soil properties and the distribution of P and roots, soil P cycle models for conventional tillage (Morel et al. 2014; Messiga et al. 2015) must be revisited

The general objective of our research is to identify and quantify the main factors affecting P cycle under conventional tillage and no-till systems. The approaches comprise the following steps:

1. A long-term (24-25 years) field experiments in a corn-soybean rotation system where the design comprises four blocks, two tillage practices (conventional tillage and no-till) and three P fertilization rates (0, 17.5 and 35 kg P ha^{-1} applied every two years on the corn phase). We test the effects of tillage practice and P fertilization on crop shoot and root growth;

2. Laboratory experiments to describe the transfer mechanism of phosphate ions at the interface between soil solution and solid phase of bulk soil where we examine the effects of time and the concentration gradient between the two phases on P sorption-desorption dynamic;

3. Literature and results of field and laboratory experiments to elaborate simulation models to simulate changes of P stock and P status along soil profile as influenced by the tillage practice and P fertilization rate.

The document is divided into six parts. Chapter II is a literature review on P cycling in agrosystems and the effects of NT on soil-plant interactions leading to the hypotheses on expected effects of no-till on P cycling tested in this thesis. Chapters III, IV, V, VI and VII (in the form of articles) present the experimental protocols and results of each investigation.

Chapter II reviews the studies on soil P species, soil available P and soil P cycle (including the relationship between soil P and crop root system) as well as the influences of conservation agriculture and no-till on soil properties and P cycle.

Chapters III and IV analyze the effects of tillage practice and P dosage on corn and soybean root biomass, morphology and spatial distribution in soil.

Chapter V introduces the simulation P cycle model (CycP) for the conventional tillage system which simulate changes in soil P stock and P status along the soil profile on the long run from P budget calculation and P transfer kinetics between soil solution and solid phase. We also test the performance of CycP with measured data at the experimental site of l'Acadie.

Chapter VI quantifies proportions of crop P uptake along the soil profile as influenced by root distribution and soil P distribution under tillage practices and P

Chapter VII integrates literature data and results from Chapters III, IV, V and VI into models to simulate changes in soil P stock and P status along the soil profile on the long run from P budgets under conventional tillage system (moldboard plough) using soil grids for the NT system. Simulations and observations are then compared.

Finally, we attempt to guide future research to improve:

1. The understanding of the long-term dynamics of available P in soil and the biogeochemical function of the P cycle in agroecosystems;

2. The simulation model;

3. The P fertilization strategy in NT agroecosystems.

Chapter II Phosphorus in agro-system

2.1. General situation of phosphorus resource in the world

Phosphorus (P), an essential element for plants, animals, and humans, plays a central role in soil fertilization and food security around the world. The P represents 0.12% of Earth crust composition (ranking the 11th). The lithosphere is thus the main source for the biosphere. Phosphorus cycles among lithosphere (bedrocks, sediments), pedosphere (soils), hydrosphere (river, lake and ocean), atmosphere and living matters (**Fig. 2-1**). However, the P cycle in biosphere is misbalanced because P tends to accumulate at a rate of 1 to1.55×10^{-7} t P per year in soils (Pédro 2012), mainly due to the fertilization to sustain crop yield.

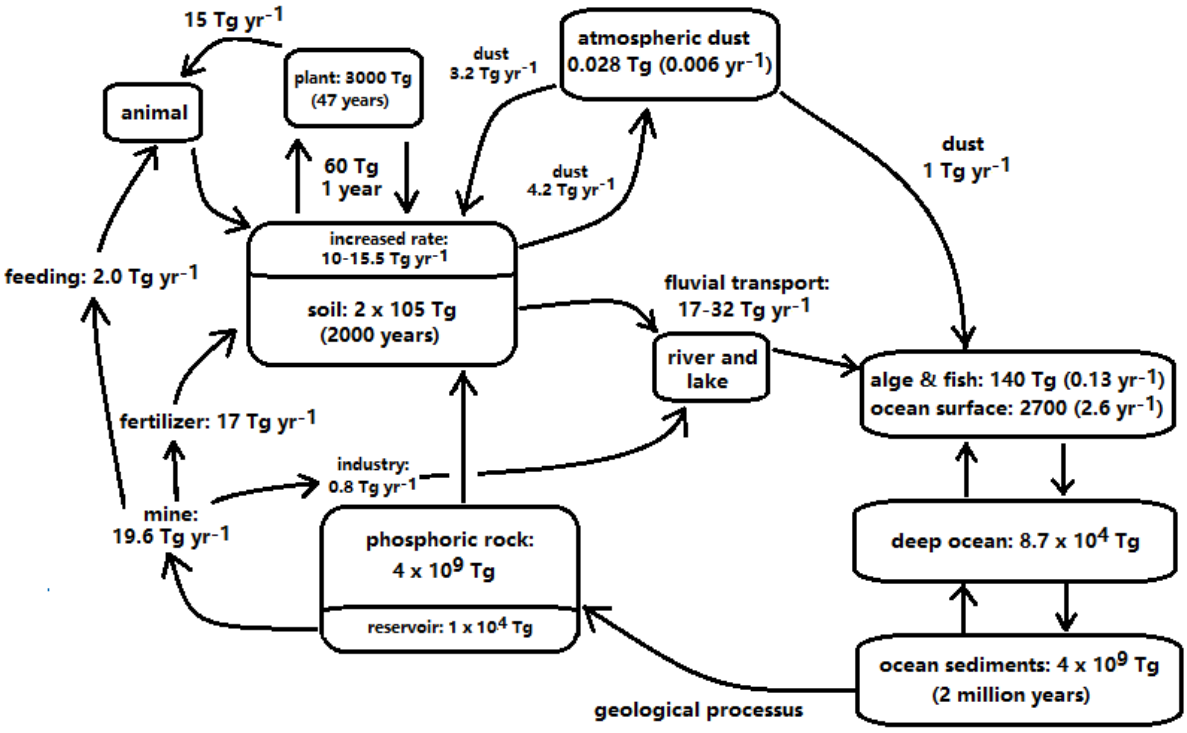


Fig. 2-1 The P cycle in the biosphere (Pédro 2012)

The main source of P for mineral P fertilizers is phosphate rock (PR) since the mid-to-late 19th century (**Fig. 2-2**). According to the International Fertilizer Industry Association, almost 53.5 M t of P_2O_5, viz. 175M t of PR averaging 30.7% P_2O_5, has been mined in 2008. In 2011, agriculture accounted for 89% of the PR consumed worldwide (55.96 Mt of P_2O_5 from 191 Mt PR), with 82% as fertilizers and 7% as feed additives (Ulrich 2013). It is widely agreed that over the last decades the use of P increased consistently (Van Vuuren et al. 2010) with the explosive growth of the world population. According to the estimation of FAO (Food and Agriculture Organization of the United Nations) in 2015, the use of P fertilizer could still be soaring during 2014-2018 period with growth rate from 0.1% to 6.3% in different regions of the world (**Table 2-1**) (FAO: World fertilizer trends and outlook to 2018). There is no alternative to the limited PR resources (Weikard and Seyhan 2009). The PR reserves are concentrated in a few countries such as Morocco, China and the United States (Dawson and Hilton 2011). Mineral P fertilizers could be partially replaced by the recycling of animal manure, crushed animal bones, human and bird excreta, city waste composts and ash (Van Vuuren et al. 2010) with a share of about 19.2 Mt P year^{-1} (Cordell et al. 2009). Nontheless, it is widely accepted that the existing high-value PR ores could be exhausted by the next 50-100 years (Cordell et al. 2009).

Besides, over-fertilization or inappropriate P fertilization could both increase P losses by runoff and lixiviation, enhancing the risk of eutrophication of surface water. Sustainable P management should recycle P efficiently through improved uptake and use efficiency, and reduce P contamination.

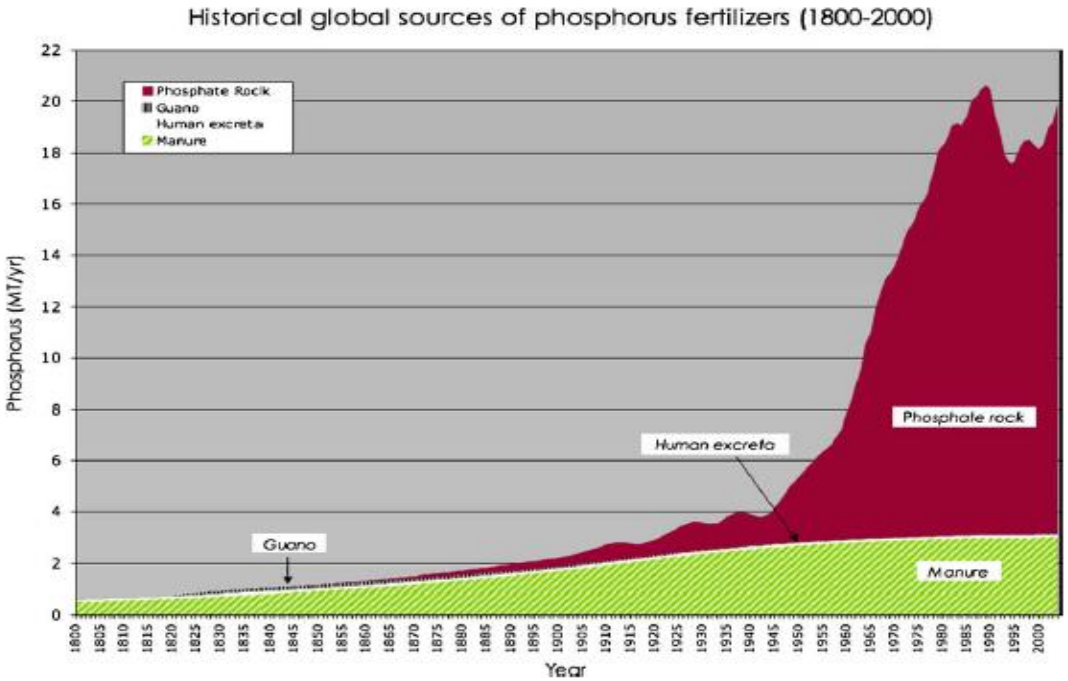


Fig. 2-2 Historical sources of phosphorus for use as fertilizers, including manure, human excreta, guano and phosphate rock (1800–2000) (Cordell et al. 2009)

Table 2-1 World and regional growth in P fertilizer demand, 2014 to 2018 (FAO: World fertilizer trends and outlook to 2018)

Region	P_2O_5 annual growth rate	Region	P_2O_5 annual growth rate
World	2.2%	**Asia**	2.2%
Africa	2.7%	West Asia	6.3%
North Africa	3.2%	South Asia	3.6%
Sub-Sahara Africa	2.3%	East Asia	1.2%
Americas	2.4%	**Europe**	2.3%
North America	0.5%	Central Europe	3.7%
Latin America & Caribbean	3.6%	East Europe & Central Asia	4.5%
Oceania	0.4%	West Europe	0.1%

2.2. Phosphorus in soil

2.2.1. Phosphorus species in soil

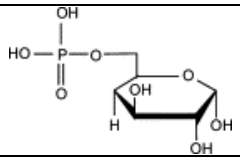
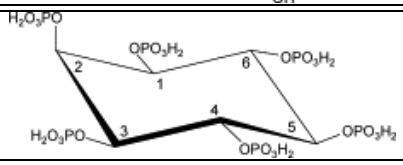
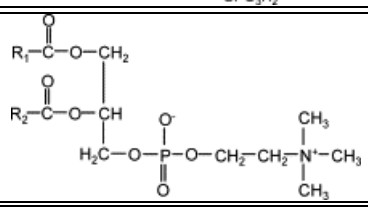
As the 11th most abundant element on earth, P is reasonably abundant in the Earth's crust (1.2 g P kg^{-1} on average). The only stable isotope of P in nature is the ^{31}P and under the natural conditions P is generally encountered in the oxides forms because of its high reactivity. In soils, phosphorus exists in the form of inorganic phosphorus (IP) and organic phosphorus (OP). Depending on soil types, IP accounts for 35% to 70% of total P in soil, while OP accounts for 30% to 65% of total P (Shen et al. 2011). In the long-term cultivated and

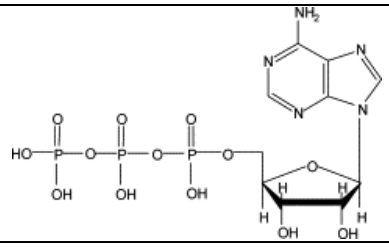
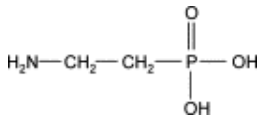
fertilized soil, IP might account for 75% of total P in soil, compared to 20% for OP and 5% for P in microorganisms (Grant et al. 2005).

Inorganic phosphorus in soil is mostly in the form of phosphate. The IP could be divided into P in minerals, adsorbed IP and soluble IP (Lin et al. 2009). Most IP is in the form of the little soluble P mineral compounds. In neutral and alkaline soils, P compounds are calcium phosphate, magnesium phosphate, octacalcium phosphate and various apatites ($Ca_3(PO_4)_2CaF_2$, $Ca_3(PO_4)_2CaF_2$, $Ca_3(PO_4)_2CaCl_2$, $Ca_5(PO_4)_3OH$, $Ca_{10}(PO_4)_6(CO_3)$ and etc.). Iron phosphate, aluminum phosphate and minerals as variscite ($AlPO_4 \cdot 2H_2O$) and strengite ($FePO_4 \cdot 2H_2O$) exist mainly in acid soils (Devau et al. 2011a). Adsorbed IP exists in several P-fixing minerals through the physical or chemical adsorption process. In calcareous soils, the clay content, calcium carbonate and iron oxide are the major factors for P sorption (Zhou and Li 2001; Shirvani et al. 2005). In non-calcareous soils, iron and/or aluminum oxy-hydroxides (Koopmans et al. 2004) and alumino-silicates (Turner et al. 2013) dominate the P sorption via ligand exchange. Some types of organic matter, such as fulvic acids in calcareous soils and humic acids in acid soils, could also sorb the P through cationic bridges (Soinne 2010). Adsorbed IP is generally in the forms of $H_2PO_4^-$ and HPO_4^{2-}, but rarely as PO_4^{3-} (Lin et al. 2009). Soluble IP could be used directly by plants as $H_2PO_4^-$ and HPO_4^{2-} ions in soil solution, depending on pH variation. However, the soluble P content in soil is only 0.003-0.3 mg P L-1 (Lin et al. 2009). In P fertilized soils, soluble P remains at a low level of 0.02-1.2 mg P L^{-1} (Kovar and Barber 1988).

Organic phosphorus in soil exists in soil organic matters and micro-organisms. The OP species are classified into phosphonates, phosphate esters (monoesters and diesters) and phosphoric acid anhydrides depending on C-P bond (Anderson 1980; Turner et al. 2005; Abdi et al. 2015). Some common OPs are listed in **Table 2-2**. Inositol phosphate account for a large proportion (about 50%) of OP while phospholipids, nucleic acids, nucleotides and sugar phosphates account for about 2% and nucleic acids including DNA and RNA for another 3% (Zhao et al. 2004). But about half of soil OP has not been identified yet. The OP could be converted into IP via mineralization to support plant growth (Harrison 1982; Oehl et al. 2004; Achat et al. 2009a). Stabilized forms of OP are inositol phosphates and phosphonates. Reactive forms are orthophosphate di-esters (nucleic acids, phospholipids, teichoic acid and aromatic compounds), orthophosphate monoesters and organic polyphosphates (Soinne, 2010).

Table 2-2 Common soil organic phosphorus compounds (Turner et al. 2005)

Functional class	Example compound	Structure
Phosphate monoester	d-Glucose 6-phosphate	
Phosphate monoester	*myo*-Inositol hexakisphosphate (phytic acid)	
Phosphate diester	l-α-Phosphatidyl choline (lecithin)	

Functional class	Example compound	Structure
Organic polyphosphate	Adenosine 5′-triphosphate	
Phosphonate	2-Aminoethyl phosphonic acid	

2.2.2 Soil phosphorus availability to plants

Soluble P is present at low concentration in the soil solution due to strong and multiple interactions with soil constituents (Hinsinger et al. 2011). On the one hand, the majority of added P will be also highly retained. Balemi and Negisho (2012) reported that more than 80% of added mineral P could be sorbed, as found elsewhere (Holford 1988; Zhou and Li 2001; Mehdi et al. 2007; Binner et al. 2015). On the other hand, the transport of phosphate ions in soil relies on mass flow and diffusion. Low concentration and small diffusion coefficient of phosphate ions (Krom and Berner 1980) in the soil solution would make it difficult for plants to acquire soil P. Hence, P is partitioned into several pools according to its mobility in soil (**Fig. 2-3**) (Barber 1995).

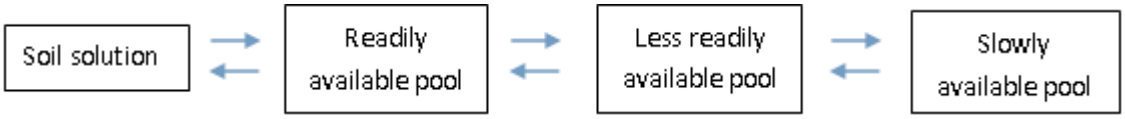


Fig. 2-3 Reversible relationships between P pools (Barber 1995)

Phosphorus in soil solution pool is in the form of di- and mono- hydrogen phosphate ions ($H_2PO_4^-$, $HPO4^{2-}$) depending on soil pH, and contributes directly to root absorption. However phosphate ions in the liquid phase could be easily drawn out into solid phase of soil constituents by physical and chemical adsorption, then be released as environmental conditions change. The dynamic process of P adsorption and desorption is affected by pH, the ionic force of soil solution and temperature. The P sorption can be could be modelled by the Langmuir isotherm equation (Hartikainen et al. 2010; van Rotterdam et al. 2012; Binner et al. 2015) (**Eq 2-1**) or the Freundlich isotherm equation (Idris and Ahmed 2012; Messiga et al. 2012a; Wolde and Haile 2015) (**Eq 2-2**) as follows:

$$\boldsymbol{Q} = \frac{\boldsymbol{Q_{max}KC}}{1+\boldsymbol{KC}} \qquad \textbf{Eq 2-1}$$

$\boldsymbol{Q}$ is the amount of P bound to the soil in equilibrium with a certain concentration in solution, $\boldsymbol{C}$ is the concentration in solution, $\boldsymbol{Q_{max}}$ is the adsorption maximum and $\boldsymbol{K}$ is an effective soil P affinity constant (van Rotterdam et al. 2012).

$$\boldsymbol{Q} = \boldsymbol{aC^n} \qquad \textbf{Eq 2-2}$$

$\boldsymbol{Q}$ is the amount of P sorbed per unit weight of soil, $\boldsymbol{C}$ is the P concentration in the equilibrium solution, $\boldsymbol{a}$ is a constant related to sorption capacity, $\boldsymbol{n}$ is phosphate sorption energy (Idris and Ahmed 2012).

Soil solution P could also be absorbed by micro-organisms and released after dying to make microbial P a highly dynamic soil P pool (Richardson and Simpson 2011). The P loosely tied onto the surface of the soil solid phase could be classified as readily available P.

The less readily available P refers to the P in the slightly soluble P pool, such as phosphates of Ca, Mg, Al and Fe (So and Lal 2006). But there is still little information on the relationship between P in the readily- and less readily-available pools (White and Hammond 2008). The P adsorbed inside soil aggregates should be another pool of less readily available P. The overall P sorption reaction in soil comprises a fast reversible adsorption of P at the surface of aggregates as reactive mass, and a slow diffusion through the solid phase or the micro-pores inside aggregates because internal water is immobile (Linquist et al. 1997; Koopmans et al. 2004). The fast sorption/desorption P at the surface of aggregate (reactive mass) acts as the readily available pool to replenish soil solution. In the study of Linquist et al. (1997) on an Hawaiian Ultisol, the volume of the reactive mass was evaluated to be within 0.188 mm from the aggregate surface. Wang et al. (2012) determined the reactive mass using the following equations:

$$\boldsymbol{W_1 = \sqrt[3]{\frac{1}{3}}R,\ \ W_2 = \left(\sqrt[3]{\frac{2}{3}} - \sqrt[3]{\frac{1}{3}}\right)R,\ \ W_3 = \left(1 - \sqrt[3]{\frac{2}{3}}\right)R} \qquad \textbf{Eq 2-3}$$

where $\boldsymbol{W_1}$, $\boldsymbol{W_2}$, and $\boldsymbol{W_3}$ are the radius of the interior, transitional, and exterior layers of aggregates, respectively; $\boldsymbol{R}$ is the distance between aggregate surface and its center.

However, the P adsorbed inside soil aggregates could only be available to replenish the soil solution via intra-aggregate P diffusion. Barber (1995) reported average soil P diffusion coefficient of 1 x 10^{-8} to 10^{-10} cm^2 s^{-1} in soil. In general, the coefficient of intra-aggregate diffusion is smaller than that of inter-aggregate diffusion by an order of magnitude. For example, Nye and Staunton (1994) estimated the intra-aggregate P diffusion coefficient for a sandy soil to be 1.5 x 10^{-12} cm^2 s^{-1}. It is thus reasonable to allocate intra-aggregate adsorbed P to the less readily available P pool. The last part of slowly available P pool is the P tightly fixed to the solid phase, i.e. the occluded P coated by the insoluble film of iron oxide (Crews et al. 1995).

In general, routine soil testing methods measure soluble P and P in the readily available pools to represent plant available P (Johnston and Syers 2009).

2.2.3. Soil phosphorus test

Total soil P could be tested by dissolving soil in concentrated sodium hydroxide (Smith and Bain 1982) or in hot concentrated acids (Bowman 1988) to convert all the P into the orthophosphate form. Then extracted P is analyzed by several methods such as colorimetry by the malachite green method (Van Veldhoven and Mannaerts 1987). Because only a part of P is available for plants, tests were developed to assess soil available P.

Most chemical extraction methods tend to test the amount of potentially available P for plant absorption, as the most common and practical approach to soil analysis. Water extraction only tests the soluble P. Chemical extraction methods were developed since the beginning of the 20th century. The Olsen-P method (Olsen et al. 1954) is world-widely used to estimate available P especially in calcareous soils (Saggar et al., 1992; Ziadi et al., 2013). Using a solution of bicarbonate (0.5 M $NaHCO_3$, pH=8.5), the Olsen method mainly releases the Ca bound-P by exchanging the Ca^{2+} with Na^+, but could over-estimate available P in acid soils. Another extraction widely used method is the Mehlich-3 extraction (Mehlich 1984) used in the mid-Atlantic States of United States and eastern Canada. With a mixed extraction

solution (0.2 M acetic acid, 0.25 M NH_4NO_3, 0.015 M NH_4F, 0.013 M HNO_3, 0.001M EDTA), Mehlich-3 extracts P and other micro-nutrients. The nitric acid extracts P from calcium phosphate, and the fluoride is for aluminum and iron phosphate, while the EDTA releases P by chelating P-fixing metals. Casson et al. (2006) pointed out that Mehlich-3 was not appropriate for use in calcareous soils because NH_4F reacts with $CaCO_3$ to form CaF_2 that might precipitate soluble phosphorus even after adding with acetic acid and EDTA. The Bray 1 extraction method (Bray and Kurtz 1945), appropriate for acid soils due to a dilute acid fluoride solution (0.025 M HCl, 0.03 M NH_4F), would lead to too low P values in calcareous soils by forming CaF_2 (Sawyer and Mallarino 1999).

The resin or anion exchange membrane (AEM) P test (Qian et al. 2008; Ziadi et al. 2013) is an approach of soil P analysis for the amount of P used by plant. Compared to chemical extraction methods, resin or AEM extracts P at soil pH rather than in an alkaline or acid environment after burying the exchange resin or membrane into soil. This *in situ* test is thought to simulate the process of P acquisition by plant roots under real conditions asssessing root available P *in situ* regardless of soil types. However, the resin method is time-consuming and specific to weather conditions.

The quantity of soil soluble P is controlled by sorption and desorption mechanism relating the solid and liquid phases. Several mechanistic methods have been developed using soil suspensions to describe P transfer at the interface between solid and liquid phases, hence to estimate the quantity of adsorbed P (Nemery et al. 2005). One approach is the sorption-desorption equilibration experiment, which results in the net change of P ion transfer at equilibrium upon addition of exogenous labile P. Another method is the isotopic dilution method using ^{32}P or ^{33}P that measures the gross quantity of phosphate ions transferred at the solid-liquid interface across time (Morel et al. 2014).

The isotopic dilution has the advantage of not disturbing the soil-solution system. One can estimate the potential amount of desorption of the P adsorbed onto the solid phase (gross amount of P transfer) at steady state (zero net transfer of P). The isotopic composition (i.e. $^{32}P/^{31}P$) is the same at equilbrium for P ions in solution and the P ions adsorbed on the solid phase participating in the exchange (Frossard et al. 1994; Nemery et al. 2005; Messiga et al. 2012a). By analyzing the isotopic abundance variation of ^{32}P (radioactivity variation) and the phosphate ion concentration (Cp) in the soil suspension, the gross amount of phosphate ions transferred at the solid-to-solution interface (Pr) and the E value (sum of Pr and phosphate ions in soil suspension) can be calculated. The relationship between Pr and Cp is described by the Freundlich kinetic function (Morel et al. 2000) as follows:

$$\boldsymbol{Pr = v * Cp^{w} * t^{p}} \quad \textbf{(}\boldsymbol{P_r}\textbf{<}\boldsymbol{P_r}\textbf{ limit)} \qquad \textbf{Eq 2-4}$$

where ***Pr*** is the diffusive phosphate ions in the solid phase in mg P kg-1 soil; ***Cp*** is the phosphate ion concentration in soil solution in mg P L^{-1}; ***t*** is the time in min; ***v***, ***w*** and ***p*** are the fitted parameters. The ***Pr*** value can be calculated at any time but is limited by an unknown value, the ***Pr*** limit , which is lower than the total inorganic P

Most soil P test methods are based on the reactions of dissolution, ion exchange and sorption-desorption and are deemed to extract soil available P, especially inorganic P. Such methods appear to be inadequate to interpret the evolution of P from different pools because the P fractions are not accurately set apart (Achat et al. 2011). Knowing the amounts of P in less readily available or slowly available pools as well as the part of organic P could help understanding soil P plant-availability. In order to test the amounts of different P fractions in soil, Hedley et al. (1982) elaborated a sequential extraction method that is proved to be useful in studies (Zubillaga and Camelo 1998; Gatiboni 2012). Using resin, $NaHCO_3$, NaOH, HCl, and H_2O_2, Hedley's method partitions the P into different inorganic, organic and microbial fractions (Gatiboni 2012).

Besides, spectroscopic methods have been developed to determine different soil fractions in soil such as the near-infrared reflectance spectroscopy (NIRS) (Ziadi et al. 2013;

Niederberger et al. 2015) and the nuclear magnetic resonance spectroscopy (NMR) (Turner et al. 1986; Cade-Menun and Preston 1996; Abdi et al. 2014; Abdi et al. 2015) by analyzing the different soil P forms or estimate P compound amount in soil according to functional groups (e.g. –OH) or the NMR spectral data of P containing compounds.

2.3. Phosphorus cycle in field

The soil phosphorus cycle illustrates the P turnover in cultivated soils (**Fig. 2-4**). External P added to soil is firstly retained by soil constituents via sorption and precipitation reactions. Reacted retained phosphorus will be made slowly available to plants and micro-organisms, lost by leaching, erosion and runoff, or converted to more stable forms. The P assimilated by living organisms could be recycled. At field scale (generally in the tilled soil layer in conventional agroeco-systems), the P cycle is simplified as several P input and output flows (Morel 2002). On the long run, P inputs comprise P fertilizers (mostly) and atmospheric depositions (mostly ignored). Phosphorus outputs are often P export through harvest, P leached below rooting zone and P runoff on soil surface. In addition, the transformation of P species (AlMaarofi et al. 2014) and the residual value of P application (Beck and Sanchez 1996) could be regarded as P flows considering seasonal changes in soil P stocks. To have a closer view of soil P cycle and soil P status, several factors must be addressed.

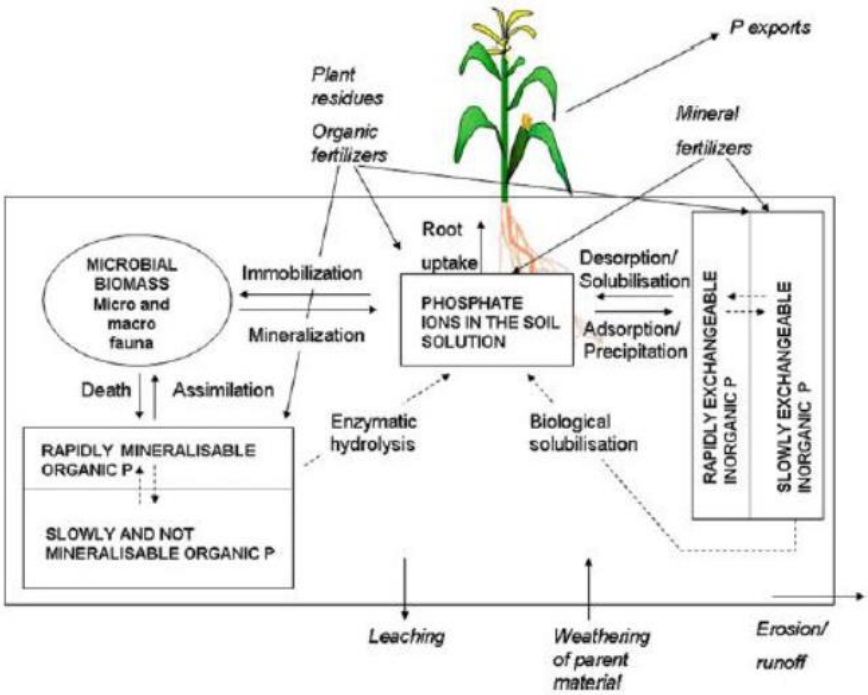


Fig. 2-4 Scheme of soil phosphorus cycle. (Tamburini et al. 2014)

2.3.1. Phosphorus fertilizer application

In general, P fertilizer could be divided into chemical and natural P fertilizers. The chemical P fertilizers refer to commercial phosphate fertilizers; animal manure, crushed animal bones, human and bird excreta, city waste composts and ash are regarded as natural fertilizers (Van Vuuren et al. 2010).

The common commercial-grade granular phosphate fertilizers are OSP or SSP (ordinary-superphosphate or single-superphosphate), TSP (triple-superphosphate), MAP (mono-ammonium phosphate), and DAP (di-ammonium phosphate) (Chien et al. 2011). The manufacturing of most commercial phosphate fertilizers starts with the phosphoric acid.

Fertilizer P content is measured as total phosphorus oxide (P_2O_5). The P content and the fertilizer solubility (in water and in ammonium citrate or citric acid solution) determines the efficiency of P fertilizers in soil (Kratz et al. 2010). Compared to the chemical mineral P fertilizers, the natural P fertilizers, especially the organic P fertilizers, are more soluble because of the competing reactions with organic acids and of the micro-organism activities. But very little difference of fertilizer solubility effect has been reported on crop yields (Prochnow et al. 1998; Prochnow et al. 2001) as soil interactions control phosphorus uptake more than the physical form of fertilizers (Roper et al. 2004).

The P fertilization increases soil P content (Sims et al. 1998b). Most added P is fixed by soil constituents. The effective volume of P fertilizers is thus constrained in a limited zone around fertilizer. Stecker et al. (2001) observed that the zone affected by fertilizer did not exceed 5-cm radical distance from fertilizer in one year (**Fig. 2-5**). High P fixation also explains why the seasonal use of added P fertilizers is only about 10-25%. The performance of P fertilizers also depends on soil test P. On ryegrass grown optimally in greenhouse pot experiments using the isotope labeling method, Linères and Morel (2004) found that effective P recovery (REC) and relative contribution of applied P to plant nutrition (Pdff) values were 14.0 and 17.8% in at high Olsen-P level (52 mg kg^{-1}) compared to 30.9% and 57.0% at low Olsen-P level (6.1 mg kg^{-1}) (**Table 2-3**). At high P status, plants mainly took up P from the soil and the P fertilizer mainly remained in the soil.

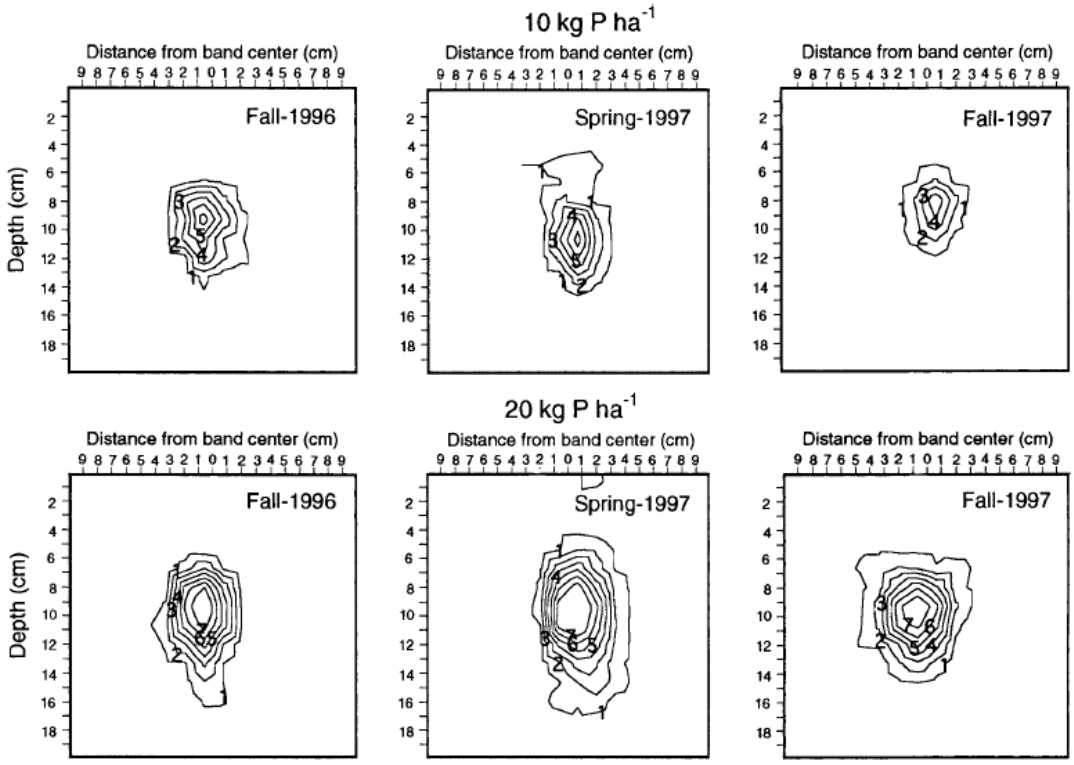


Fig. 2-5 Bray-1 P distribution from bands for fertilizer dosage of 10 and 20 kg P ha^{-1} in a Mexico soil. Values are relative to soil in adjacent nonband-affected soil and converted to log base 1.5. (Stecker et al. 2001)

Table 2-3 Dry matter production (DMW), total P (Pt=Ps+Pa), P taken up by crop from soil (Ps) and applied P (Pa), and bioavailability indicators, effective P recovery from applied P (REC) and relative contribution of applied P to plant nutrition (Pdff) (Linères and Morel 2004).

	DMW g kg^{-1}	Pt	Ps	Pa	REC %	Pdff %
		--------- mg kg^{-1} ---------				
Soil 1 with high content in Olsen-P						
Control	6.3	41.2	41.2			
KH_2PO_4	6.3	39.4	32.4	7.0	14.0	17.8
Sewage sludge	6.9	49.4	41.6	7.8	15.6	15.8
Soil 2 with low content in Olsen-P						
Control	6.2	5.51	5.51			
KH_2PO_4	12.5	27.1	11.7	15.5	30.9	57.0
MSW	7.7	8.8	4. 5	4.3	8.6	49.3
Cattle manure	12.3	18.3	11.0	7.3	14.6	39.7

2.3.2. Phosphorus losses by erosion, runoff and leaching

Intensive P fertilizer application often results in elevated P accumulation in soil. High soil P status increases the risk of P loss through wind and water erosion. Jørgensen (2010) mentioned the world soil erosion from agriculture areas (including cropland and pasture) amounts to 72.9 Gt yr^{-1} causing P losses at 19.3 and 17.2 Mt P yr^{-1} for cropland and pasture, respectively. There are two pathways of P transfer from agricultural soils to receiving waters: runoff and leaching (Zhang et al. 2003). Both pathways of P losses are quantified by the concentrations of various P forms (dissolved reactive P (DRP), dissolved un-reactive P (DURP), and particulate P (PP)) (Tan and Zhang 2011) and affected by a number of factors such as rainfall, field topography, soil types, soil preparation, fertilizer application, irrigation method and drainage system.

Roughly, the world total phosphate fertilizer application may lead to a loss of 0.5 Mt P yr^{-1} by surface runoff (Jørgensen 2010). Heavy rainfall (Simard et al. 2000) and steep field slope (Carroll et al. 2000) promote water flow which could increase soil water erosion and hence losses of PP. The concentration of DRP in runoff varies with different soil types (Wang et al. 2011). The DRP loss over a period of time is estimated based on the measurement of soil test P or degree of P saturation. The degree of P saturation (DPS) is defined as the ratio of sorbed phosphorus and phosphorus sorption capacity (number of sites available for phosphorus binding), and used as a potential phosphorus loss risk indicator (Casson et al. 2006). The P sorption capacity (PSC) of soils varies widely according to clay content, clay mineralogy, organic matter content, exchangeable aluminum, iron, and calcium concentrations, and pH. In non-calcareous soils, DSP, represented by the P saturation index (PSI), is defined as the ratio of extracted P to extracted Al and Fe. Various alternatives of PSI are presented in Beauchemin and Simard (1999).

Because of the high P-fixation capacity of many mineral soils, the vertical P transport (leaching) in relatively high clay content (>10%) soils is often assumed to be a minor contributor to surface-water P enrichment, compared to P loss by runoff and erosion (Makris et al. 2006). This assumption was challenged by Heckrath et al. (1995) and Zhang et al. (2003), pointing out that leaching is also a significant pathway for P loss in high P soils. Heckrath et al. (1995) reported that the concentration of total P in drainage water increased rapidly when soil P-Olsen exceeded a critical value (about 60 mg P kg^{-1}). Soils saturated with P increase the risk of P losses through leaching as well as runoff. Besides, soil tillage and the drainage system influence P leaching. The NT could reduce P loss by runoff and erosion but may increase P leaching through preferential flow (Tan and Zhang 2011). Simard et al. (2000) found a higher P transfer concentration in drained soils (232 μg L^{-1}) compared to undrained soils (152 μg L^{-1}), because rapid flow through the drainage pathway reduced the contact time between the percolating water and the subsoil, hence opportunities for P sorption.

2.3.3. Phosphorus uptake by plants

The largest P output in field is crop P exportation (generally P in the grain). The P uptake occurs mainly through root absorption whereby nearly all P is absorbed in form of phosphate ions. The dynamic process for absorption of dissolved inorganic phosphate ions through root surface unite has been described by Michaelis-Menten kinetics (Barber 1995) as follows:

$$I_r = \frac{I_{max}(C_l - C_{min})}{K_m + C_l - C_{min}} \qquad \textbf{Eq 2-5}$$

where I_r is P influx into the root, I_{max} is maximum influx, C_l is concentration of phosphate ions in the liquid phase, C_{min} is minimum concentration of phosphate ions in the liquid phase for net influx of zero, and K_m is concentration of phosphate ions in the liquid phase where influx is $1/2I_{max}$.

Therefore, the P absorption capacity of plant species (I_{max} and K_m), P (phosphate ion) concentration in soil solution (C_l) and plant root biomass (e.g. total root surface) are three important factors controlling P uptake by plants.

The P absorption capacity refers to P uptake rate at root surface. It mainly depends on how fast phosphate ions can be transported through root cells to reach xylem vessels. For most mineral salts, there are two ways to cross root cells: an apoplastic route between the cells and a symplastic route within the cells. Because phosphate ion concentration in root cell is much higher than that in the soil solution, phosphate ions enter plant mainly through the symplastic route using specialized transporters at the root/soil interface to extract phosphate ions from solution at micro-molar concentrations (generally less than 10 μM P), and other mechanisms allowing phosphate ions to move across membranes between intracellular compartments, where the concentrations of phosphate ion may be 1000-fold higher than in the external solution (Schachtman et al. 1998). This process is controlled by thermodynamic parameters and requires energy. And it varies for different crop species. In a hydroponic study, Bhadoria et al. (2004) observed that corn had a 6 times higher I_{max} value and a 2 times higher K_m value for phosphorus uptake compared to groundnut.

For a same plant species, the P uptake rate depends on root biomass that directly contacts the soil. In case of P deficiency, plants could adapt its root architecture with increased root/shoot ratio, root branching, root elongation, root hairs, cluster roots (Richardson et al. 2011; Balemi and Negisho 2012), and shallow growth angle (Lynch 2011) to enhance the root exploration volume and P absorption capacity (**Fig. 2-6**). Mollier and Pellerin (1999) found that P starvation led to higher corn (*Zea mays* L.) root mass and significantly higher root/shoot ratio and longer laterals. Yu et al. (2012) observed that corn without P fertilization formed more fine roots with diameter less than 0.6 mm compared to fertilized fields. According to Bates and Lynch (2000), *Arabidopsis thaliana* with a highly branched root system, could reduce primary root growth and increase the number and the length of lateral roots when sown in a low-P soil because the primary roots contribute little to P absorption. Its root hair density was 29% higher and three times longer after 16 days of P starvation. The P-efficient beans also maintained a higher root/shoot ratio during growth under P-deficiency filed conditions while root hair length and density of basal roots were significantly correlated with P acquisition (Ramaekers et al. 2010). Because P is applied near soil surface, root architecture is adapted to “topsoil foraging”. Rose et al. (2009) noted that lupine (*Lupinus angustifolius* L.) uses such strategy in deeper layers. In addition, most (probably 70%–80%) terrestrial plant species can interact with arbuscular mycorrhiza fungi (AMF). The AMF could produce abundant extramatrical mycelium, extending several cm from the infected root while ectomycorrhizal myceliums could potentially spread up to several meters (Atwell et al. 1999). The AMF increases the absorptive area of a root system several-fold and create an efficient P-collecting network beyond the P depletion zone. Compared with roots or root hairs, the AMF hyphae has much smaller diameter (1-5 μm) and could extend soil exploration by penetrating into smaller soil pores (Smith et al. 2011; Richardson et al. 2011). Besides, the P

absorbed by AMF can be transported more efficiently along the apoplastic route inside roots. Plants differ in their dependency on AMF (Menge and Johnson 1978).

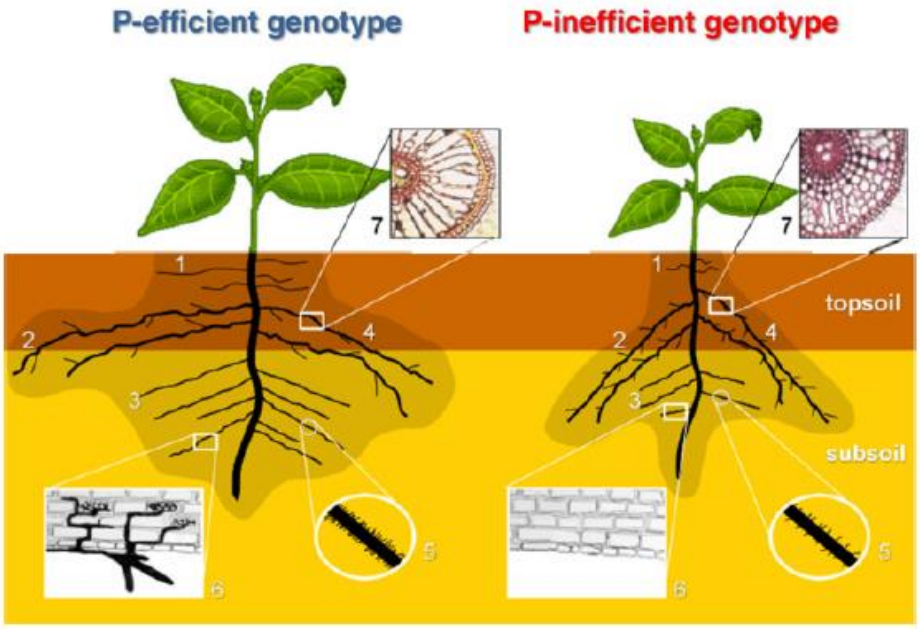


Fig. 2-6 Plant traits and mechanisms to improve P uptake efficiency. The P-efficient genotypes integrate different traits and mechanisms for adaptation to low P availability and increase tolerance to P deficiency compared to P-inefficient genotypes. Adaptations to low P availability include: (1) more abundant and longer adventitious roots, (2) more horizontally oriented basal roots, (3) more taproot laterals, (4) more dispersed higher order laterals, (5) increased root hair density and length (together with increased organic acid exudation and more high-affinity P transporters), (6) greater association with mycorrhizae, and (7) greater formation of aerenchyma. Consequently, the soil volume explored by P-efficient genotypes is much larger compared to P-inefficient genotypes. (Ramaekers et al. 2010)

The main factor influencing P uptake is phosphate ion concentration in solution. Although phosphate ion concentration is low in soil solution, plants can enhance P concentration in the rhizosphere (soil zone several mm around root surface) by rhizosphere acidification, carboxylates exudation and secretion of phosphatase (Li et al. 2008; Hinsinger et al. 2011). The decrease in rhizosphere pH has been reported for oilseed rape (*Brassica napus* L.) (Zhang et al. 2009) and bread wheat (*Triticum aestivum* L.) (Nuruzzaman et al. 2006; Solaiman et al. 2007). This root-induced pH shift could affect P adsorption (Devau et al. 2011) or increase the dissolution of P-contained minerals (Shen et al. 2011). While organic anions, especially carboxylates, mine soil inorganic P through chelation of metal cations through ligand exchange (Balemi and Negisho 2012). Pigeon pea (*Cajanus cajan* L.) exudes piscidic acid that chelates Fe^{3+} to release P from $FePO_4$ (Li et al. 2008). Also, acid phosphatases and phytases produced by plants in response to P stress can hydrolyze a range of organic-P forms thereby enhancing plant P uptake (Richardson et al. 2011). Additionally, rhizosphere microbial communities can alter soil pH, exude organic ligands and other P-solubilizing compounds in the rhizosphere that influence plant P uptake (Hinsinger et al. 2011).

2.3.4. Other phosphorus flows

Other worth-mentioning phosphorus flows at the field scale are atmospheric depositions, seeds, and crop residue restitution. Under temperate climate, the flow of atmospheric P deposition is only 0.5 kg P ha^{-1} $year^{-1}$ (Morel 2002) or down to 0.27 kg P ha^{-1} $year^{-1}$ (Tipping et al. 2014). Compared to P fertilization (about 32 kg P ha^{-1} $year^{-1}$) and P uptake (30 kg P ha^{-1}

year^{-1}), the amount of atmospheric deposition is so small that is often ignored. Seed P computed by multiplying seed P concentration by seed mass per hectare (Messiga et al. 2012b) could be ignored. The P uptake is the sum of P exportation by harvest and of P in crop residues. Crop residues left in the fields restitute the P to soil after residue decomposition. Thus, although the P restitution by crop residues could attain 10 kg P ha^{-1} year^{-1} (5 kg P for above-ground part and under-ground part, respectively) (Morel 2002), in practice only the amount of P exportation is estimated by multiplying grain P content by crop yield.

2.4. Phosphorus management

Studies on phosphorus cycle aim primary to estimate the amount of phosphorus that should be supplied to sustain high crop yields while protecting P resources and the environment.

2.4.1. Crop response to applied phosphorus

The Mitscherlich function is commonly used to relate P fertilization (or soil test P) to crop yield (Morel 2002; Pukhovskiy 2013) (**Eq 2-6**). The model provides a critical value of soil test P to obtaion maximum crop production. The model is defined as follows:

$$\boldsymbol{\delta Y/\delta x = c(A - Y)} \qquad \textbf{Eq 2-6}$$

where $\boldsymbol{Y}$ is crop yield at a given P dosage or soil test P, $\boldsymbol{A}$ is maximum crop yield, $\boldsymbol{x}$ is P fertilization or soil test P, $\boldsymbol{c}$ is a constant named efficiency factor related to crop growth rate.

The parameters of Mitscherlich function are site-specific and controlled by Liebscher's law of optimum (the relationship depends on how close to their optima are other factors) (Harmsen 2000). Bai et al. (2013) obtained different determination coefficients at three sites (Yangling: R^2=0.79–0.91, Chongqing: R^2=0.87–0.93, Qiyang: R^2=0.69–0.71) for the relationship between Olsen-P and relative crop yield using the Mitscherlich function. Pukhovskiy (2013) showed that the Mitscherlich function could be expressed as: 1.Mitscherlich-Spillman model; 2.Mitscherlich-Spillman modified by Boguslawski; 3. linear function with a plateau; 4. empirical quadratic model; 5. a linear approximation of the argument in logarithmic form with a plateau; 6. empirical quadratic model of in-transformed arguments; 7. empirical quadratic model of log-transformed argument. The relationship also depends on soil test P. Smethurst (2000) relating wheat yield to 8 concentrations of extracted P (Lactate, Truog, fluoride, Bray-1, Bray-2, Olsen, Colwell, and Mehlich) at 57 sites, reported different correlation coefficients. Thus, the curve fitting of Mitscherlich function should be ascertained by the proper choice of the underlying function form and soil test P.

2.4.2. Phosphorus budget

The annual P budget is generally described as the difference between P inputs and outputs at field scale as follows (Obour et al. 2011):

$$\boldsymbol{P\ \ budget = A_p + F_p + S_p - U_p - L_p - R_p} \qquad \textbf{Eq 2-7}$$

where $\boldsymbol{A_p}$ is atmospheric P deposition (always ignored), $\boldsymbol{F_p}$ is fertilizer P, $\boldsymbol{S_p}$ is soil test P, $\boldsymbol{U_p}$ is P exportation in crop grains by P uptake, $\boldsymbol{L_p}$ is leached P, $\boldsymbol{R_p}$ is runoff P.

Studies showed that the P budget (the difference between P inputs and P outputs) was closely related to soil test P. In a 13-year field trial, Beck and Sanchez (1996) observed that a positive P budget increased the Hedley NaOH extractable inorganic phosphorus from 61.3 kg

P ha^{-1} to 191.6 kg P ha^{-1}. In addition, in a 5-year experiment at 56 sites, Linquist and Ruark (2011) found a positive but weak relationship (R^2=0.27) between the average annual P budget and Olsen-P likely because change rate of Olsen-P widely strongly among soil types.

On the other hand, a strong relationship was obtained between P budgets and soil test P (by Messiga et al. (2012a) in a 7-year experiment established on a clay loam soil. The coefficients were R^2=0.67 for Mehlich3-P and R^2=0.70 for Olsen-P. Similar results were obtained in a luvic arenosol (Messiga et al. 2010). Such results (**Fig. 2-7**) indicated that the correlation between the cumulative P budget (especially positive P budget) and soil test P was linear and robust but site-specific. Several studies with similar results are listed in **Table 2-4**.

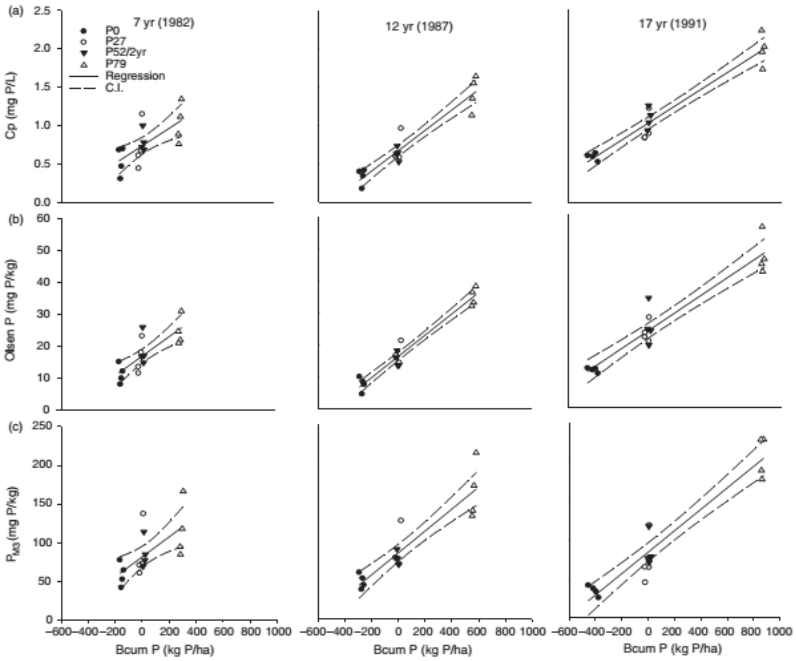


Fig. 2-7 Relationship between cumulative P budget (Bcum) and (a) concentration of P ions in solution (Cp, mg L^{-1}), (b) Olsen-P (mg kg^{-1}), and (c) Mehlich3-P (P_{M3}, mg kg^{-1}) after 7, 12, and 17-year periods of cultivation at four P applications rates. Symbols: individual plots. Sampling year is in parenthesis. C.I. = 95% confidence interval. (Messiga et al. 2010)

Table 2-4 Results of P budget and soil test P correlation in several sites.

Refernces	Regions	Soil types	Time	Soil test P	Soil depth	P budget	Coefficient (R^2)
Oehl et al. 2002	Therwil, Switzerland	Haplic Luvisol	21 years	Exchangeable P ions by isotopic dilution	20 cm	Average P budget=(P fertilization – P in grains)/years	R^2=0.53
Simpson et al. 2015	Hall, Australian Capital Territory	An acidic Alfisol	14 years	Exchangeable P ions by isotopic dilution	10, 20, 30 and 40 cm	Cumulative P budget=P fertilization + P feed – P in animal products	R^2=1.00, 0.97, 0.92 and 0.79
Messiga et al. 2015	Ercé, France	Alfisol	5 years	Olsen-P	5 cm	Cumulative P budget=P fertilizer applied – P removal in crop grains	R^2=0.72

Messiga et al. 2015	Les Verrières, Switzerland	Cambisol	5 years	Ammonium acetate, acetic acid and EDTA extracted P	10 cm	-	R^2=0.64
Messiga et al. 2015	Lévis, Canada	Fragihumod	6 years	Mehlich3-P	15 cm	-	R^2=0.71
Messiga et al. 2015	Maaninka, Finland	Entisol	8 years	Acidic ammonium acetate extracted P	20 cm	-	R^2=0.55
Messiga et al. 2015	Siikajoki, Finland	Sulfaquept	8 years	Acidic ammonium acetate extracted P	20 cm	-	R^2=0.33
Hua et al. 2016	Madian, North China Plain	Vertisol	9 years	Olsen-P	20 cm	Cumulative P budget=P fertilizer applied – P uptake (grain and straw)	R^2=0.61-0.97 depending on fertilization treatment
Ciampitti et al. 2011	La Marta, the Pampas region	Entic Haplustoll	6 years	Bray1-P	20 cm	Cumulative P budget=P fertilizer applied – P removal in crop grains	R^2=0.51
Ciampitti et al. 2011	Balducci, the Pampas region	Typic Hapludoll	6 years	Bray1-P	20 cm	-	R^2=0.52
Ciampitti et al. 2011	La Blanca, the Pampas region	Typic Hapludoll	6 years	Bray1-P	20 cm	-	R^2=0.68
Ciampitti et al. 2011	Santo Domingo, the Pampas region	Typic Argiudoll	6 years	Bray1-P	20 cm	-	R^2=0.74

Where the cumulative P budget is negative, a deflection point may occur in the correlation plot (**Fig. 2-8**) (Ciampitti et al. 2011; Messiga et al. 2015). Below the deflection point, there was a slow or negligible decline in soil test P without P fertilization likely due to the high buffering capacity (inertia) of the solid phase or plant roots acquiring P from below sampling depth.

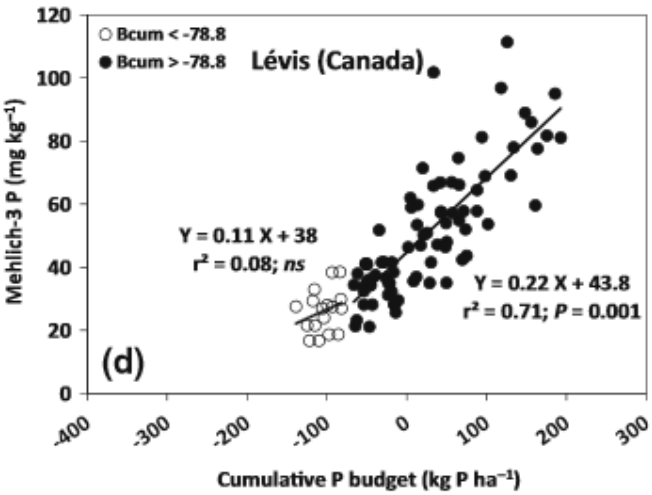


Fig. 2-8 Correlation between the cumulative P budget and soil test P (Mehlich3-P) for the 0-15 cm soil layer showing a deflection point of cumulative P budget at about -80 kg P ha^{-1}. (Messiga et al. 2015)

The close relationship between the cumulative P budget and soil test P can guide P fertilization to maintain soil test P for some optimum value for crop yield.

2.4.3. Phosphorus in nutrient balance

Current nutrient management strategies (Drinkwater and Snapp 2007) and nutrient models (Delgado-Baquerizo et al. 2013) use uncoupled nutrient cycles. It is assumed that other nutrients are controlled or of equal effect (a general assumption rarely met in practice). Both the Mitscherlich function and the cumulative P budget take only phosphorus into account, while crop response also depends on other nutrients. An optimum balance of nutrients is required to reach high yield levels (Nelson and Anderson 1984; de Wit 1992).

Elements expressed as proportions or concentrations add up to their measurement unit or 100% and are thus compositional. Compositional data are multivariate in nature and intrinsically related to each other (Aitchison 1982). Typical compositional data have three limitations because of the closure constraint (the unit or scale of measurement): 1. Compared to non-compositional data, compositional data have one degree of freedom less because their sum is closed, leading to redundant information in the last component added to the sum; 2. Compositional data only vary in the compositional range, for example [0, 1], rather than the real space [-∞, +∞]; 3. Interpretation of data analysis depends on measurement scale. The statistical analysis of such data requires tools of compositional data analysis to avoid methodological biases affecting the mean and the variance or leading to spurious correlations.

Compositional data should be log-ratio transformed into real-space data (Aitchison 2003). The most advanced data transformation technique is the isometric log-ratio (*ilr*) (**Eq 2-8**) (Egozcue et al. 2003) formulated according to a sequential binary partition (SBP) (Filzmoser and Hron 2009). An example of SBP is given in **Table 2-5**.

$$\mathbf{ilr_i} = \sqrt{\frac{\mathbf{rs}}{\mathbf{r+s}}}\,\mathbf{ln}\,\frac{(\prod_{+}\mathbf{x_j})^{\frac{1}{r}}}{(\prod_{-}\mathbf{x_k})^{\frac{1}{s}}} \qquad \mathbf{i = 1, 2, 3, \ldots, D-1; j+k=D} \qquad \textbf{Eq 2-8}$$

where the products $\mathbf{\Pi}_{+}$ and $\mathbf{\Pi}_{-}$ only include parts coded with + and -, and ***r*** and ***s*** are the numbers of the positive and negative signs (parts) in the ***i***-th order partition.

Table 2-5 Sequential orthogonal partition between six nutrients to compute five *ilr* orthonormal coordinates. (Parent 2011)

	N	P	K	Ca	Mg	Fv[2]	Interpretation	r[1]	s
1	1	1	1	1	1	-1	Nutrients vs. filling value	5	1
2	1	1	-1	-1	-1	0	Anions vs. cations	2	3
3	1	-1	0	0	0	0	N vs. P	1	1
4	0	0	1	-1	-1	0	K vs. Ca+Mg	1	2
5	0	0	0	1	-1	0	Ca vs. Mg	1	1

[1] r = number of positive signs and s = number of negative signs
[2] Filling value between unit of measurement and analytical results

With the *ilr* results, different nutrient amounts in soil could be adjusted compared to the reference nutrient balances for optimum crop yields. But this method demands a variety of soil nutrient measurements, which is time-consuming. Because the log-ratio transformation of compositional data only provides relative values, inverse conversion of log ratio data is needed to obtain absolute values is necessary (Wang et al. 2007).

2.4.4 Simulation models of phosphorus cycle in agroecosystems

Models have been developed to simulate soil P dynamics. Most models can predict soil P transformations affecting crop growth. A structure of different P pools (such as fertilizer P, labile P, root P and etc.) for up to ten soil layers is described in the Erosion-Productivity Impact Calculator (EPIC) P model of Jones et al. (1984) (**Fig. 2-9**). The P pools are related through four processes: 1. Rapid adsorption of inorganic P; 2. Slow adsorption of inorganic P; 3. Immobilization and mineralization of organic P; 4. Plant P uptake. The processes have been described empirically. Similar approach was used in Daroub et al. (2003).

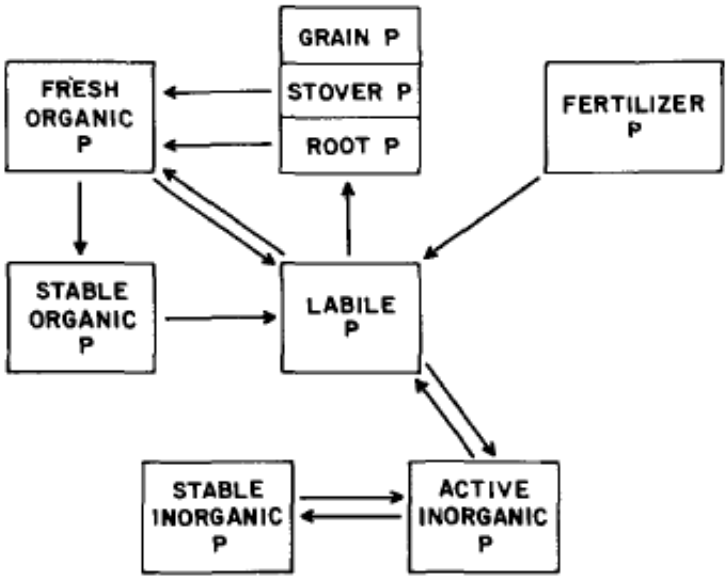


Fig. 2-9 Pools and flows of phosphorus in the EPIC P model (Jones et al. 1984)

However, fully described mechanisms are needed in an ideal model of soil P. Silberbush and Barber (1983) parametrized the Cushman mechanistic-mathematical model using 11 plant and soil variables (**Table 2-6**) (Silberbush and Barber 1983) to predict plant P uptake. They integrated root size changing over time, P flux into the root as related to P concentration in the soil solution at the root surface, and P supplied to the root by mass-flow and diffusion. The simulated P uptake showd different sensitivity to the variables and root surface area was the

most influential. Root architecture could also be taken into account. Ge et al. (2000) found that root architecture simulated by SimRoot impacted on P acquisition efficiency along the soil profile with heterogeneous P distribution. Heppell et al. (2015) used a similar approach.

Models such as the Agricultural Nutrient Model (ANIMO) (Groenendijk et al. 2005) use both mechanistic and empirical methods to describe processes. The model was simplified using directly soil test P (Schoumans and Groenendijk 2000).

Table 2-6 Plant and soil variables for simulation of P uptake by Cushman's mechanistic mathematical model, their definition, and values for Williams soybeans growing on Raub silt loam (Silberbush and Barber 1983)

Symbol	Parameter	Initial value
D_e	Effective diffusion coefficient	2.3×10^{-9} cm^2 s^{-1}
b	Buffer power	163
C_{l_i}	Initial conc. in soil soln.	0.0136 μmol cm^{-3}
v_o	Water influx to root	5.0×10^{-7} cm^{3}cm^{-2}s^{-1}
r_o	Root radius	0.015 cm
r_1	Half distance between roots	0.2 cm
Imax	Maximal influx rate	6.43×10^{-7} μmol cm^{-2}s^{-1}
Cmin	Minimal conc. where $I_n = 0$	2.0×10^{-4} μmol cm^{-3}
Km	Conc.-Cmin when $I_n = 1/2$ Imax	5.45×10^{-3} μmol cm^{-3}
L_o	Initial root length	250 cm
k	Root growth rate	0.03 cm s^{-1}

Process-based models usually run on a short-term, e.g. daily time step (Jones et al. 1984). The long-term models for soil fertility and crop production tend to use empirical and theoretical components, such as the QUantitative Evaluation of the Fertility of Tropical Soils (QUEFTS) model, which describes relations between (1) chemical soil tests, (2) potential NPK supply from soils and fertilizer, (3) actual NPK uptake, and (4) maize grain yield (Smaling and Janssen 1993).

Besides these complexe simulation models, some efforts have been made to elaborate simplified P cycle models. Morel et al. (2014) proposed to estimate phosphate ion concentration in soil solution by combining the transfer mechanism of phosphate ions at solid-liquid interface and soil P budget. The isotopic dilution exchangeable phosphate ions in soil for a long-term) represents soil P stocks varying with the cumulative P budget during a long period (i.e. one year). This P model was applied to conventional tillage where subsoil contribution was ignored.

Kautz et al. (2013) reported that subsoil could contribute significantly to crop P uptake, especially when the P level is low in the topsoil. Soil P accumulates mainly in the topsoil because of P fertilization and P released from crop residues (**Fig. 2-10**). The topsoil P could be transferred in part to the subsoil by P leaching with preferential flow, earthworm faeces, root exudates and P released from dead roots. However, the subsoil may show higher bulk density and lower porosity and water availability that may limit P uptake. There is higher soil P availability in the subsoil hotspots for P uptake such as the rhizosphere (soil zone several mm around root surface) and the drilosphere (about 2 mm wide zone around earthworm burrows) where biochemical activity is higher. Soil P cycle should thus consider the subsoil, especially under no or low P dosage.

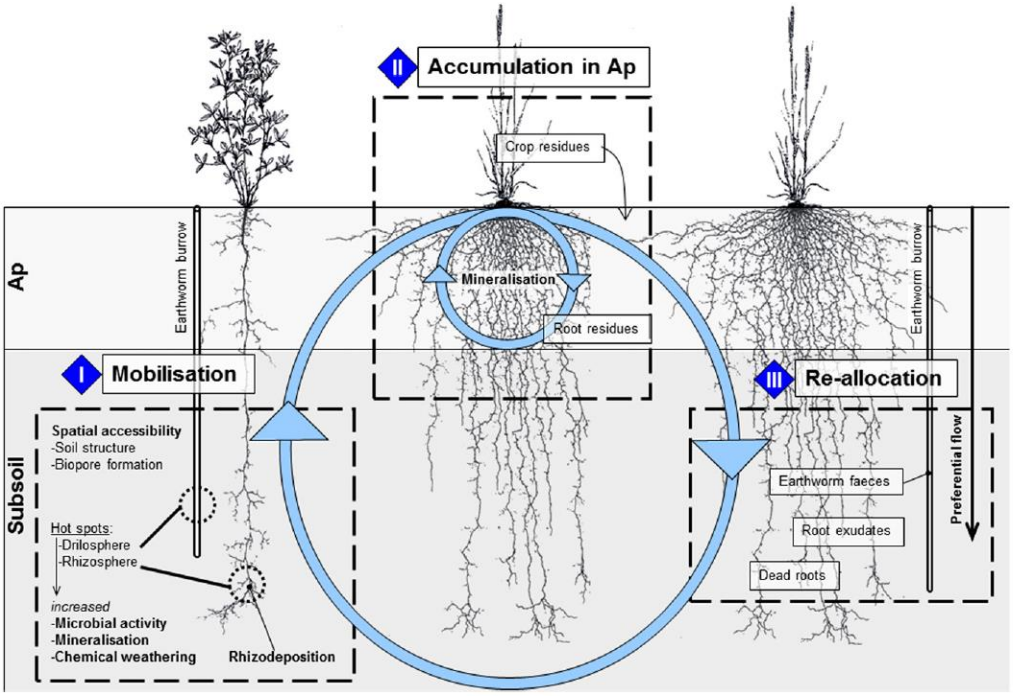


Fig. 2-10 Conceptual model of nutrient acquisition from the subsoil. (Kautz et al. 2013)

2.5 Conservation agriculture and no-till system

Most P cycle studies were conducted under conventional tillage systems. Conservation agriculture represents a new field for P research.

2.5.1. Conservation agriculture

In conventional agro-ecosystems, tillage aims to control the weeds, modify the soil characteristics (porosity and drainage) and promote plant growth (Schubetzer et al. 2007). As a result, soil structure is perturbed increasing the risk of soil erosion and nutrient losses. The concept of conservation agriculture (or sustainable agriculture) has been promoted by the Food and Agriculture Organization of the United Nations (FAO) to implement resource-saving agricultural crop production systems and to achieve acceptable economic and environmental benefits (Kassam et al. 2012; Kassam and Friedrich). The key actions are as follows (Chevrier and Barbier 2002):

1. Minimum mechanical soil disturbance to maintain minerals within the soil, mitigating erosion and water losses.
2. Permanent soil cover to promote biological activities within the soil structure.
3. Crop rotations with more than two species.

Scopel et al. (2013) also mentioned:

4. Production of biomass whenever possible.
5. Multifunctional cover crops.

Crop rotation is a means to control plant diseases and provides potential savings of resources. Cover crops are meant to increase farm incomes and reduce production costs. The no-till (NT) or simplified tillage technique meets the requirement of key 1 and 2.

2.5.2. No-till and simplified tillage methods

The NT is practiced on about 125 million hectares over the world, about 9% of total arable land (Pittelkow et al. 2015a). The NT system is mostly implemented in North and South America. According to the 2007-2008 survey conducted by Derpsch and Friedrich (2009), the United States, Brazil, Argentina, Canada and Australia are the leading countries, with by far the largest area under NT (**Table 2-7**).

Table 2-7 Extent of no-till adoption world-wide (countries with >100 000 ha) (Derpsch and Friedrich 2009)

Country	**Area under No-tillage (ha) 2007/2008**	**Country**	**Area under No-tillage (ha) 2007/2008**
USA	26 593 000	South Africa	368 000
Brazil	25 502 000	Venezuela	300 000
Argentina	19 719 000	France	200 000
Canada	13 481 000	Finland	200 000
Australia	12 000 000	Chile	180 000
Paraguay	2 400 000	New Zealand	162 000
China	1 330 000	Colombia	100 000
Kazakhstan	1 200 000	Ukraine	100 000
Bolivia	706 000	Russia	-
Uruguay	672 000	Others (Estimate)	1 000 000
Spain	650 000	Total	105 863 000

In comparison, only 2% of the agricultural area in France planted directly with no-till in 2011 (Fiche Référence d'ADEME 2015), but there was another 33% under simplified tillage practices such as shallow ploughing, strip tillage and etc. The definitions for working depth, soil mixture and working surface of all the soil preparation techniques are provided in **Table 2-8**.

Table 2-8 Simplified techniques for soil preparation (Schubetzer et al. 2007)

	Techniques	**Working depth**	**Soil turnover/mixture**	**Working surface**	**Soil cover**
Conventional tillage	Mouldboard plough based tillage	25 cm (15 to 40 cm)	Turnover and mixture	All field	Total incorporation
Simplified techniques	Non inversion tillage/Conventional tillage without mouldboard plough	15 to 40 cm	Mixture	All field	Strong incorporation
	Decompaction	0 to 15 cm	-	All field	Weak or medium incorporation
	Shallow tillage	0 to 15 cm	Mixture	All field	Weak or medium incorporation
	Direct drilling/No-till	-	-	Sowing row	No incorporation

2.5.3. Advantages and disadvantages of no-till

Among minimum tillage techniques, NT technique is a very special case because there is no soil mixture. With permanent soil cover, less soil fragmentation and reduced agricultural machinery, NT has economic and environmental advantages and disadvantages.

Firstly, the NT saves time and money during soil preparation. Yalcin et al. (2005) showed that NT reduced fuel consumption and saved time during soil preparation (**Table 2-9**). And Rieu (2001) reported that by taking NT management, the time of machine traction in a cereal field could be reduced to 4 h per ha compared to 7 h per ha with conventional tillage. Tebrügge (2001) reported that the implantation cost for 100 ha was only 50 € per ha for NT compared to 210 € per ha for conventional tillage in Europe.

Table 2-9 Tillage parameters of applications. 1. Conventional tillage with moldboard plough, 2. Minimum tillage with heavy-duty disk harrow and combination of spring tine harrow and spiral roller, 3. Minimum tillage with one pass of soil tillage combination of chisel, rotary tiller and spiral roller, 4. No-till for direct seeding. (Yalcin et al. 2005)

Methods	**Tillage type**	**Fuel consumption (L ha^{-1})**	**Slip (%)**	**Forward speed (km h^{-1})**	**Filed efficiency (ha h^{-1})**
1	Plough	19.6	21.57	6.17	0.27
	Disk Harrow-1 Pass	7.0	13.88	5.33	
	Disk Harrow-2 Passes	6.9	8.50	6.82	
	Float	16.0	12.16	5.45	
	Planting	8.9	8.5	5.00	
2	Heavy-Duty Disk Harrow	11.5	17.13	5.83	0.47
	Soil Combination	15.3	12.16	5.30	
	Planting	8.9	8.5	5.00	
3	Rotary Tiller	26.0	4.52	2.71	0.36
	Planting	8.9	8.5	5.00	
4	Direct seeding	8.9	8.5	5.00	1.25

Secondly, since crop residues are left on soil surface in NT, the soil is protected against dispersion by rain drops and from drying. So water and wind erosion could be reduced (Viaux 1999; Joannon 2004). Puustinen et al. (2005) found that the soil erosion with no-till was only 29% of that with ploughing in their study in Finland. Shipitalo et al. (2000) reported that surface mulch under NT could eliminate surface runoff during high intensity storms (**Fig. 2-11**).

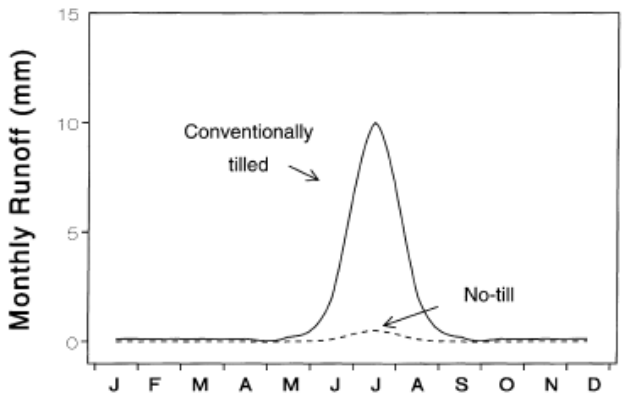


Fig. 2-11 Conceptualized effect of tillage on monthly surface runoff from NAEW watersheds with well-drained soils (Shipitalo et al. 2000).

Moreover, NT provides a relatively stable and triggering environment for biological activities. Scopel et al. (2013) observed greater mass of macro-fauna (gastropods, micro-mammals, beetles, spiders, nematodes, earthworms etc.) under tropical conditions using conservation agriculture, including NT. Linn and Doran (1984) observed that aerobic and anaerobic microorganisms in surface (0–75 mm) no-till soils were 1.35 to 1.41 and 1.27 to 1.31 times more abundant, respectively, than in surface-plowed soils. Whereas Feng et al. (2003) reported that microbial biomass C content under no-till was 60, 140, and 75% greater than under conventional tillage in February, May, and October, respectively. Biodiversity of soils is a very important driver for soil health (Soane et al. 2012).

Finally, NT contributes to carbon sequestration and greenhouse gas alleviation by maintaining higher organic matter concentration in the soil. Widespread adoption of conservation tillage within United States could sequester 24–40 Mt C per year (Lal et al. 2003). But there is also some arguments in this carbon sequestration function of NT, since the sequestered carbon under NT can also be minerlized and released into atmosphere during a long-term (Baker et al. 2007).

NT is still debated, because crop yields under NT could either decrease (Kumar et al. 2012; Guan et al. 2014) or increase (Himmelbauer et al. 2012; Islam et al. 2015) because advantages and disadvantages (**Table 2-10**) of NT are region-specific.

Table 2-10 Relative agronomic advantages and disadvantages of no-till in Europe, although not universally relevant (Soane et al. 2012)

Advantages	Disadvantages
Lack of compaction below plough furrow	Crop establishment problems during very wet or very dry spells
High work rates and area capability	Weed control problems
Increased bearing capacity and trafficability	Cost of herbicides, herbicide resistance
Reduced erosion, runoff and loss of particulate P	Risks of increased N_2O emissions and increased dissolved reactive P leaching
Opportunity to increase area of autumn-sown crops	Reduced reliability of crop yields, especially in wet seasons
Stones not brought to the surface	Unsuited to poorly structured sandy soils
Drilling phased to take advantage of favourable weather conditions	Unsuited to poorly drained soils
Increased area capability	Risk of soil compaction
Reduced overall costs (fuel and machinery)	Problems with residual plough pans
	Increased slug damage
	Unsuited for incorporation of solid animal manures

2.5.4. Soil properties and nutrient distribution under no-till

Most economic and environmental advantages and disadvantages of NT could be related to altered soil properties and nutrient distribution.

Soil physical properties

Soil compaction develops under NT due to traffic pressure (Peigne et al. 2013) leading to higher soil bulk density and resistance (Gantzer and Blake 1978; Kay and VandenBygaart 2002; Soane et al. 2012; Javeed et al. 2013; Guan et al. 2014) (**Fig. 2-12**).

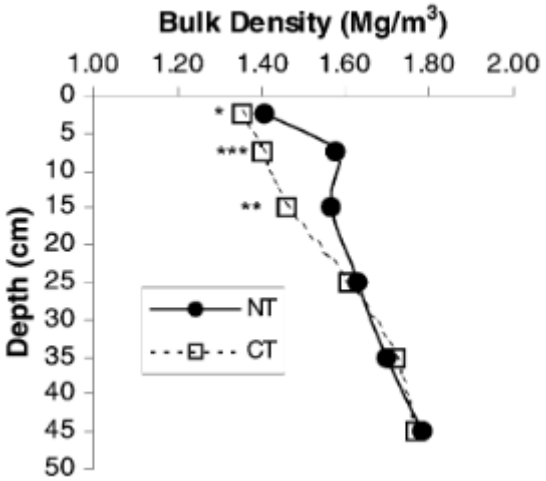


Fig. 2-12 Bulk densities as a function of soil depth at the end of 19 years of conventional tillage and no-till in southern Ontario (Kay and VandenBygaart 2002).

In general, an increase in soil bulk density indicates a decrease in total porosity under NT (Deubel et al. 2011). However, macro-porosity may increase under NT (Shipitalo et al. 2000; Kay and VandenBygaart 2002; Dal Ferro et al. 2014). It is attributed to the preservation of root and earthworm-formed macro-pores that are normally disrupted by tillage. And these progressively created macro-pores from root and faunal activity are usually vertically oriented and more persistent under traffic (Blackwell et al. 1990). On the other hand, NT does not disrupt soil aggregates mechanically and preserves roots and mycorrhizal hyphae, which are major binding agents for macro-aggregates; meanwhile, on the long run, NT has a reduced decomposition soil organic matter compounds, that may serve as aggregate binding agents (Jiao et al. 2006). As a consequence, the NT also promotes the formation of macro-aggregates (Jiao et al. 2006; Messiga et al. 2011).

Soils under NT tend to show higher water availability (**Fig. 2-13**) because of less evaporation caused by surface crop residues and soil mulching (Bonfil et al. 1999; Fabrizzi et al. 2005; De Vita et al. 2007; Scopel et al. 2013). Higher soil moisture makes the soil cooler under NT due to greater specific heat capacity of water (Chassot et al. 2001; Qin et al. 2006; Fernandez and White 2012).

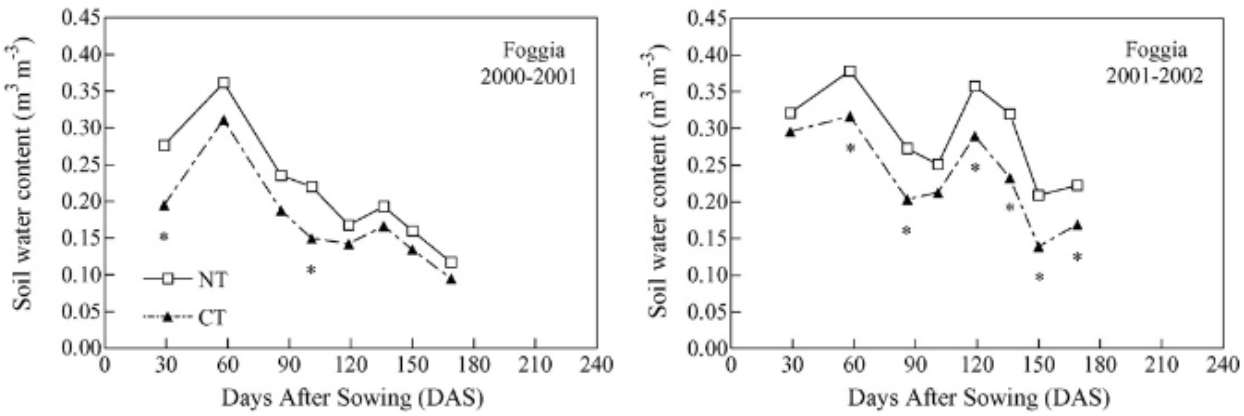


Fig. 2-13 Soil water content under conventional tillage (CT) and no-tillage (NT) during 2000–2001 and 2001–2002 growing seasons at Foggia, Italy. *Significant difference at P<0.05 level probability between tillage treatments. (De Vita et al. 2007)

These alterations of soil physical properties in NT have effects on crop growth. For instance, higher soil bulk density and penetration resistance could hinder crop root development (Qin et al. 2005; Nunes et al. 2015); cooler soil temperature could slow down seed germination (Javeed et al. 2013); higher macro-porosity over the long run may facilitate

water infiltration and availability in soil (Scopel et al. 2013); and higher water availability may increases crop yields in arid and semi-arid environments (Fabrizzi et al. 2005).

Soil chemical properties

The NT leads to the stratification of nutrient distribution along soil profile. Compared to conventional tillage (CT), where soil nutrients are distributed quite uniformly across the tilled layers, the NT tends to accumulate nutrients on top layers. Thomas et al. (2007) found 18% more organic C and 21% more total N under NT than under CT of the 0-10 cm depth for a semi-arid sub-tropic soil. Similar results were obtained by Arshad et al. (1990) and Alvarez et al. (1995). The NT reduces the contact between organic matter and bulk soil, organic matter decomposition, hence mineralization tends to take place at low rates and humification occurs through poly-condensation (Reicosky 2003). Exchangeable Ca and K could also accumulate in 0-10 cm layer under NT with more crop residue mulching than the plow practice (Juo and Lal 1979). Soil pH may stratify due to acidification by the acid rains, ammonium oxidation and the decay of organic materials (Grove et al. 2007).

Relatively immobile nutrients like phosphorus are strongly strafified (**Fig. 2-14**). Lupwayi et al. (2006) found more bicarbonate-extractable P in the top eight cm under NT than under CT and 3.5 times more available P in the 0-5 cm soil layer than in the 5-15 cm layer under NT, as also found in other studies (Franzluebbers and Hons 1996; Schwab et al. 2006; Costa et al. 2010; Messiga et al. 2010; Calegari et al. 2013).

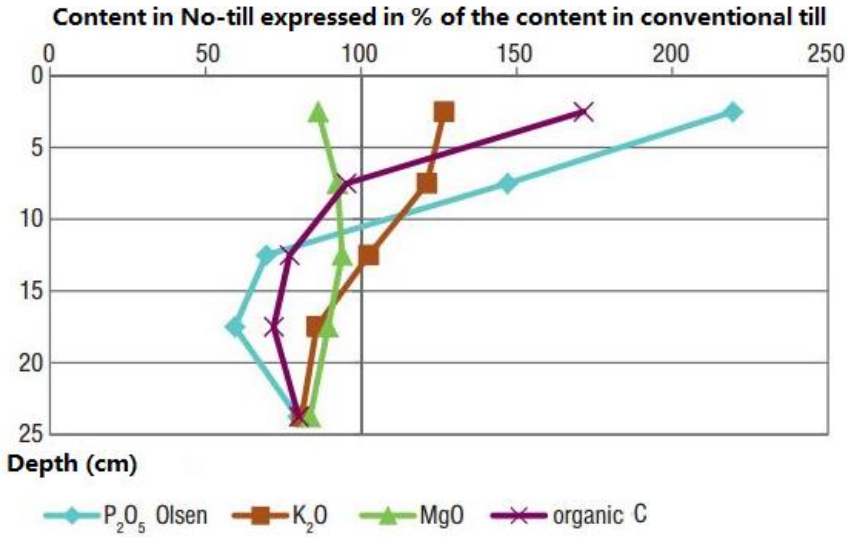


Fig. 2-14 Evolution of nutrient contents under no-till (Bouthier and Labreuche 2011)

The degree of P stratification increased with time under no-till. As shown in an Ontario survey on non-paired soils (Nutrient Stratification in Long-term No-till Fields in Ontario, article at http://www.gocorn.net/v2006/Nutrient/articles/mag_Nutrient2.htm), the P content in the 0-5 cm layer was 31%, 35% and 42% greater over that in the 0-20 cm layer in the fields with 5-7, 8-9 and >9 years under NT, respectively. The P stratification varied with soil types as also shown by Selles et al. (2002) for paired soils. Downward movement of surface nutrient should be faster on sands than clays but this depends on climate conditions such as precipitations. The degree of P stratification could be higher the higher the P dosage.

The different patterns of P distribution under NT might affect the performance of the P estimation methods mentioned in 1.4. For instance, NT tends to have more soil organic matter (SOM). In terms of Mitscherlich function based on P-Olsen, Johnston et al. (2014) found that the P-Olsen associated with the 95% yield was much smaller on the soil with higher SOM, which strongly suggested that the extra SOM improved soil structure and root ability to explore the soil for P so that a lower level of P-Olsen was required to achieve yield potential.

For P budget method, Messiga et al. (2012b) found a linear relationship between the cumulative P budget and soil P (Olsen-P and Mehlich3-P) in conventionally managed plots, but a cubic curve fitted for no-tilled plots (**Fig. 2-15**), indicating differential P availability between tillage systems.

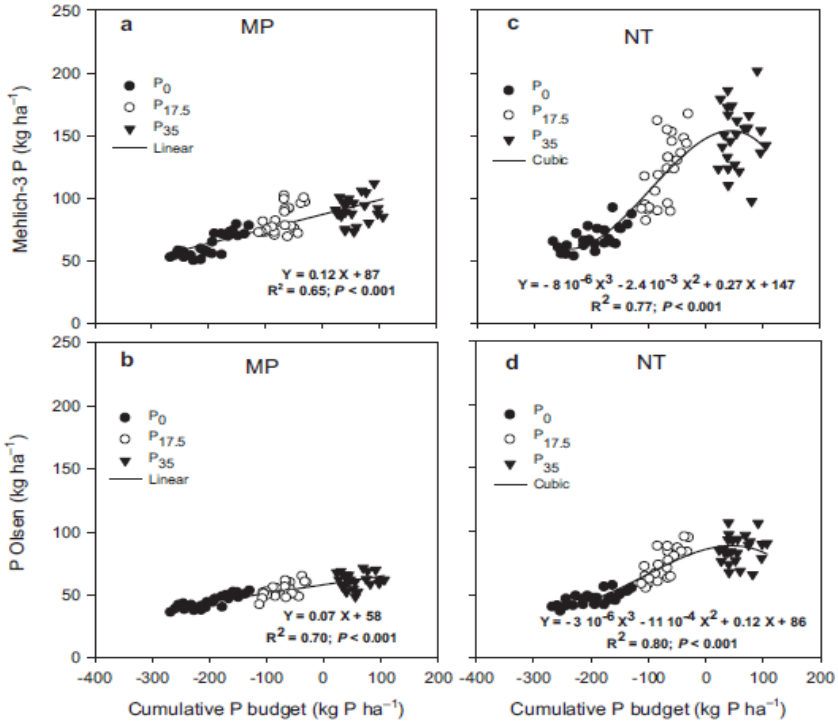


Fig. 2-15 Relationships between (a) Mehlich-3 P concentration (P_{M3}), (b) Olsen P concentration (P_{Ol}) and cumulative P budget (Bcum) for mouldboard plough (MP) and (c) Mehlich-3 P concentration (P_{M3}), (d) Olsen P concentration (P_{Ol}) and cumulative P budget (Bcum) for no-till (NT) fertilized with nine combinations of three P and three N additions in the maize phase of a two-year maize and soybean rotation. P0, P17.5, and P35 represent additions of 0, 17.5, and 35 kg P ha^{-1}. The three N addition rates were 0, 80, and 160 kg N ha^{-1} (Messiga et al. 2012b).

Soil biological properties

Compared to conventional tillage, NT increases soil biodiversity as macro- and micro-organism activities.

The casting activity of earthworms below soil surface contributes to aggregate stability in the 0-12 cm layer in NT soils (Soane et al. 2012). In a 25-year study, Helgason et al. (2010) found that NT increased the biomass of arbuscular mycorrhiza fungi (AMF) in the surface soil layer (0-5 cm) compared to CT. The NT would increase the rooting volume to acquire nutrients, especially relatively immobile P (see 2.3.3).

The stable soil environment under NT facilitates weed germination and growth. The problem of weed control is more serious under NT than CT (Soane et al. 2012; Melander et al. 2013; Nichols et al. 2015; Pakeman et al. 2015). More weed proliferation and diversity under NT has been reported in several studies (Stougaard et al. 1984; Kettler et al. 2000; Légère et al. 2008). High weed occurrence reduces crop yields by competition for light, water and nutrients (Afifi and Swanton 2011; Silva et al. 2011) and increases weeding costs (Gilet 2001).

2.6 Hypotheses and objectives

In summary of literature, two points should be noted for our study:

1. Soil P cycle studies mainly focused on the tilled soil layer in the CT agroecosystem while subsoil P, generally ignored, would play an important role.

2. The NT practice alters soil properties and nutrient distribution that influence soil P cycle, compared to CT systems.

We thus tested the following hypotheses:

1. Mineral P fertilization and tillage practices affect the P cycle in the long term by affecting the spatial distribution and morphology of roots and P availability in the soil.

2. It is necessary to take into account of spatial location in soil profile of both soil P availability and root surface area to accurately model the P biogeochemical cycle in context of soil conservation agriculture.

3. The contribution of subsoil P to crop P uptake is substantial in the soil P cycle under both CT and NT.

4. Models allow testing P fertilization strategies for soil tillage systems under different soil and climatic conditions.

The general objective of this study is to model P under CT and NT tillage systems. Specific objectives are as follows:

1. Qualify and quantify the effects of tillage practices and P dosage on root distribution and morphology of corn (*Zea mays* L.) and soybean (*Glycine max* L.) in the long run.

2. Combine the dynamic model of phosphate ion transfer at the soil solid and liquid interface and the P budget to evaluate soil P status time variations in the CT tilled zone.

3. Calculate the proportions of P uptake along soil profile including subsoil.

4. Simulate the dynamics of the P status along soil profile for CT and NT systems in the long run.

Chapter III The Long-term (23 years) Effects of Tillage Practice and Phosphorus Fertilization on the Distribution and Morphology of Corn Root

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***Running title*:** Corn roots and soil Tillage and P Fertilization

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REGULAR ARTICLE

The long-term effects of tillage practice and phosphorus fertilization on the distribution and morphology of corn root

Haixiao Li · Alain Mollier · Noura Ziadi · Yichao Shi · Leon-Étienne Parent · Christian Morel

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Abstract
Background and aims Relevant soil properties and nutrient distributions influencing crop root growth might be different under no-till (NT) and mouldboard plough (MP) management. The possible different root systems within different managements might have key impact on crop nutrient uptake and consequently crop production. Our objective was to assess the long-term combined effects of tillage and phosphorus (P) fertilization on corn (*Zea mays* L.) root distribution and morphology.
Methods Corn root and soil samples were collected during the silking stage at five depths (0–5, 5–10, 10–20, 20–30 and 30–40 cm) and three horizontal distances perpendicular to the corn row (5, 15 and 25 cm) under MP and NT with three P fertilizations (0, 17.5, and 35 kg P ha^{-1}) for a long-term (22 years) experiment in eastern Canada. Root morphology and soil properties were determined.
Results NT practice decreased corn root biomass by −26 % compared to MP, mainly by decreasing the primary and secondary roots. Additionally, corn roots in NT tend to be more expansive on the surface layer with higher root length and surface densities for the depth of 0–5 cm at two sampling distances of 15 and 25 cm. The 35 kg P ha^{-1} rate increased the root biomass by 26 and 41 % compared to the 0 and 17.5 kg P ha^{-1} rates.
Conclusions No-tillage practice and low rates of P fertilization reduce corn roots. This is probably caused by the weed competition in NT and the continued downward P status with low P rates over 22 years.

Keywords No-till · Mouldboard plough · Phosphorus · Field experiment · Conservation agriculture

Abbreviations

MP	Mouldboard plough
NT	No-till
0P, 0.5P and 1P	P fertilization rates of 0, 17.5 and 35 kg P ha^{-1} every two years
RMD	Root mass density
RSD	Root surface density
RLD	Root length density

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3.1. Résumé

Contexte: Les propriétés du sol et les distributions d'éléments nutritifs qui influent sur la croissance des racines peuvent être différents sous les systèmes de semis direct (no-till, NT) et labour conventionnel (moldboard plough, MP) et avoir un impact sur l'absorption des éléments nutritifs et, par conséquent, sur la production agricole. Notre objectif était d'évaluer les effets combinés à long terme du travail du sol et de la fertilisation en phosphore (P) sur la distribution et la morphologie des racines du maïs (*Zea mays* L.).
Méthodes: Le maïs a été cultivé sous MP et NT avec trois doses de P (0, 17,5 et 35 kg P ha^{-1}) dans un essai de longue durée (22 ans) dans l'Est du Canada. Les échantillons de racine de maïs et de sol ont été prélevés à cinq profondeurs (0-5, 5-10, 10-20, 20-30 et 30-40 cm) et trois distances horizontales perpendiculairement au rang de maïs (5, 15 et 25 cm) au stade d'apparition des soies. La morphologie de racines et les propriétés du sol ont été mesurées.
Résultats: Par rapport à MP, la pratique NT a diminué de 26% la biomasse des racines de maïs en affectant principalement la biomasse des racines primaires et secondaires. De plus, les racines de maïs sous NT ont eu tendance à se concentrer davantage dans la couche de surface avec les densités de longueur et de surface de racine plus élevées à la profondeur de 0-5 cm et à des distances de 15 et 25 cm du rang. La fertilisation de 35 kg P ha^{-1} a augmenté la biomasse des racines de 26% et 41% pour les niveaux de fertilisation 0 et 17,5 kg P ha^{-1}, respectivement.
Conclusions: La pratique NT et les doses de fertilisation du P moins élevées réduisent la densité des racines de maïs cela étant dû probablement à la compétition des mauvaises herbes en NT et la baisse de la disponibilité en P du sol associée aux faibles apports de P depuis plus de 22 ans.

Mots-clés: pratique sans labour, charrue à soc, phosphore, expérience de longue durée, agriculture de conservation

3.2. Abstract

Background and aims: Relevant soil properties and nutrient distributions influencing crop root growth might be different under no-till (NT) and moldboard plough (MP) management. The possible different root systems within different managements might have key impact on crop nutrient uptake and consequently crop production. Our objective was to assess the long-term combined effects of tillage and phosphorous (P) fertilization on corn (*Zea mays* L.) root distribution and morphology.
Methods: Corn root and soil samples were collected during the silking stage at five depths (0-5, 5-10, 10-20, 20-30 and 30-40 cm) and three horizontal distances perpendicular to the corn row (5, 15 and 25 cm) under MP and NT with three P fertilizations (0, 17.5, and 35 kg P ha^{-1}) for a long-term (22 years) experiment in eastern Canada. Root morphology and soil properties were determined.
Results: NT practice decreased corn root biomass by 26% compared to MP, mainly by decreasing the primary and secondary roots. Additionally, corn roots in NT tend to be more expansive on the surface layer with higher root length and surface densities for the depth of 0-5 cm at two sampling distances of 15 and 25 cm. 35 kg P ha^{-1} rate increased the root biomass by 26% and 41% compared to 0 and 17.5 kg P ha^{-1} rates.
Conclusions: No-tillage practice and low rates of P fertilization reduce corn roots. This is probably caused by the weed competition in NT and the continued downward P status with low P rates over 23 years.

***Key-words*:** no-till, moldboard plough, phosphorous, field experiment, conservation agriculture

Abbreviations
MP - Mouldboard plough
NT - No-till
0P, 0.5P and 1P - P fertilization rates of 0, 17.5 and 35 kg P ha^{-1} every two years
RMD - Root mass density
RSD - Root surface density
RLD - Root length density
3^{rd}RLD, 2^{nd}RLD, 1^{st}RLD - Root length densities of tertiary, secondary and primary roots
3^{rd}RL%, 2^{nd}RL%, 1^{st}RL% - Root length proportions of tertiary, secondary and primary roots
RL*ilr1* - Isometric log ratio transformation of root length proportions (tertiary roots *vs.* secondary and primary roots)
RL*ilr2* - Isometric log ratio transformation of root length proportions (secondary roots *vs.* primary roots)

3.3. Introduction

No-till (NT) practice is currently recommended for various cropping systems in terms of the conservation agriculture, defined in Kassam et al. (2012) and Kassam and Friedrich (2011). NT management has several economic and environmental benefits, including reduction of soil erosion, sequestration of soil organic carbon (Soane et al. 2012) and reduction of agricultural machinery. Long-term NT practice results in the alteration of soil properties (Madejón et al. 2007) and the stratification of nutrients along the soil profile with the addition of fertilizer (Messiga et al. 2011). Moreover, long-term NT also results in either decreased (Kumar et al. 2012; Guan et al. 2014) or increased (Himmelbauer et al. 2012; Islam et al. 2015) crop yields. The above-ground growth and biomass yield of crops is heavily dependent on the root system (Guan et al. 2014). Thus, one key to the proper use of the NT management technique is understanding its complex interactions among soil physical properties, nutrient (e.g. phosphorus) heterogeneity and root development (Benjamin et al. 2013).

Long-term NT practice can change the soil physical properties, leading to greater soil bulk density and soil resistance (Gantzer and Blake 1978; Soane et al. 2012; Javeed et al. 2013; Guan et al. 2014). In addition, increased aggregate stability (Jiao et al. 2006; Messiga et al. 2011) and macro-porosity (Dal Ferro et al. 2014) are observed in NT systems, with less soil perturbation and higher organic matter content from crop residues (Scopel et al. 2013). These soil physical property modifications could be either positive or negative for corn root growth. For example, higher soil bulk density could hinder root penetration and reduce root length density (Qin et al. 2005; Nunes et al. 2015); the enhance of soil resistance could result in increased average root diameter (Qin et al. 2005), whereas increased vertical macro-pores encourage roots to proliferate into deeper layers (Chassot et al. 2001; Costa et al. 2010).

In term of nutrient distribution in NT, the heterogeneity of P seems to be more common and important, as P is not only poorly mobile in soil (Hinsinger et al. 2011) but also limits crop growth (Plenet et al. 2000a; 2000b). The stratification of phosphorus in NT maintains higher P concentration in the topsoil. Lupwayi et al. (2006) found more bicarbonate-extractable P in the top eight cm of soil with NT than with conventional tillage and 3.5 times greater available P concentration in the 0-5 cm soil layer than in the 5-15 cm layer in NT, similar to other studies (Franzluebbers and Hons 1996; Schwab et al. 2006; Costa et al. 2010; Messiga et al. 2010; Calegari et al. 2013). Corn roots could respond to this P heterogeneity in both root biomass (Barber 1971; Zhang et al. 2012; Xia et al. 2013; Deng et al. 2014) and root morphology (Mollier and Pellerin 1999; Zobel et al. 2007; Zhang et al. 2012) variations.

In addition, NT tends to maintain higher soil moisture because of reduced soil evaporation (Scopel et al. 2013). It could influence crop roots via both water availability (Gardner 1964; Van Vuuren et al. 2010; Dass et al. 2016) and soil temperature (Chassot et al. 2001; Javeed et al. 2013). Moreover, since there is no soil inversion, weed germination and growth are promoted in NT. Higher weed proliferation and diversity in NT have been reported in several studies (Stougaard et al. 1984; Kettler et al. 2000; Légère et al. 2008). This makes weed control as one of the main problems with NT (Soane et al. 2012; Melander et al. 2013; Nichols et al. 2015). And weed competition would adversely affect crop root growth (Norris 1992; Rajcan and Swanton 2001; Silva et al. 2011; Rizzardi and Wandscheer 2014).

Accordingly, corn root distribution and morphology could be different in NT and conventional tillage systems with the possible interactions of various P fertilization rates. However, the tillage practice and P fertilization effect on root development are often studied separately, and their interactions on root remain unclear.

This study aims to evaluate the effects of tillage practice and P fertilization on corn root distribution and morphology with both vertical and horizontal coordinates. We used a long-term field experiment to evaluate the combined effects of tillage [mouldboard plough (MP) vs. no-till (NT)], and mineral P fertilization (0, 17.5 and 35 kg P ha^{-1} applied every two

years as triple-superphosphate) in a long-term (23 years) soybean-corn rotation in Eastern Canada. We hypothesized that tillage practice and P fertilization could modify the soil physical properties and P distribution, and therefore modify the corn root distribution and morphology.

3.4. Materials and Methods

3.4.1. Site description

The long-term crop rotation experiment was established in 1992 at the L'Acadie Experimental Farm (45°18' N; 73°21' W) of Agriculture and Agri-Food Canada. Details on this field experiment have been recently reported in Ziadi et al. (2014). Briefly, the soil is a deep clay loam soil (364 g kg^{-1} of clay and 204 g kg^{-1} of sand in the Ap horizon) of Humic Orthic Gleysol, Typic Haplaquept. This deep soil originates from a fluvial deposit that evolved from fine-textured greyish-to-brown parent material. The soil was tile-drained with a slope of less than 1% and was cropped with alfalfa (*Medicago sativa* L.) before 1992. From 1992 to 1994, the site was planted with corn and the corn (*Zea mays* L.) and soybean (*Glycine max* L.) rotation was initiated in 1995. Chemical characteristics of the topsoil (0-15 cm) at the onset of the experiment were, on average, as follows: organic matter content=38 g kg^{-1}; Mehlich-3 P=135 kg P ha^{-1}; P saturation index=4.3%; and pH water=6.3 (Légère et al. 2008). The experimental set-up was a split-plot plan replicated four times with two tillage practices (MP and NT) randomized into main plots and nine combinations of nitrogen (N) and P levels randomized into subplots including three N (0, 80, 160 kg N ha^{-1} every year) and three P (0, 17.5, and 35 kg P ha^{-1}) applied, referred to as 0P, 0.5P and 1P. The 0P, 0.5P, and 1P corresponded to approximately 0, 0.5 and 1 time(s) the P annually exported at harvest (Messiga et al. 2010). For the purpose of this study, we only considered the optimal level of 160 kg N ha^{-1} fertilization, the two tillage practices (MP and NT) and the three rates of P applications. We therefore considered a total of 24 field-plots measuring 25-m in length by 4.5-m in width. The MP operation occurred every fall after the crop harvest to a depth of 20 cm. Every April, soil is then tilled by disking and harrowing to 10 cm before seeding. In the NT treatment, plots were ridge-tilled from 1992 to 1997 and flat direct-seeded from 1998. In all treatments, crop residues were left on the soil surface at harvest. The fertilizers were only banded-applied (5-cm perpendicular to the seeding rows) to the corn phase every two years, using a disk opener (3-4 cm deep) (CRAAQ 2003). The P fertilizers were applied as commercial triple-superphosphate (($Ca(H_2PO_4)_2$, H_2O), 45% P_2O_5) in a single operation at seeding. The rate of 160 kg ha^{-1} N was applied first at seeding as urea by 48 kg N ha^{-1}, and then was followed by adding 112 kg N ha^{-1} side-dressed as ammonium nitrate at approximately the 8-leaf stage of corn growth. All of the plots received 50 kg K_2O ha^{-1} band-applied at planting on 1992 and 2007 based on soil analysis and local recommendations (CRAAQ 2003). Herbicides were applied based on provincial recommendations. In corn years, glyphosate was applied to all plots at 0.89 kg ae ha^{-1} prior to seedling in late April or early May. Corn plots were treated pre-emergence with a tank mix of metolachlor (1.32 kg ai ha^{-1}) and cyanazine (2.25 kg ai ha^{-1}) (Légère et al. 2008). On 2 June, 2014, after 22 years of tillage and P fertilization treatments, Pioneer P9623AM corn (2850 corn degrees centigrade) was sown at a rate of 83×10^3 plants ha^{-1} with six rows (with a row space of 75 cm) in each field-plot.

3.4.2. Root sampling and analysis

Root samples were collected on 19-20 August, 2014 (80 days after seeding, approximately at the reproductive stage of corn silking (Ritchie et al. 1993). For each field-plot, five

consecutive corn plants were randomly selected in the 3rd or 4th row to avoid side effects. The root samples were taken using the soil core method (Böhm 1979), with a hand-held power sampler (8-cm inner diameter). In each field-plot, soil cores were sampled perpendicularly on one side of one chosen plant. Cores were taken up to a depth of 40 cm at three perpendicular horizontal distances to the corn row. The axis positions of hand-held power sampler were at 5, 15 and 25 cm perpendicularly to the corn row. Each core was then cut out at 5 depths: 0-5, 5-10, 10-20, 20-30 and 30-40 cm, for a total of 360 root samples. 24 supplementary root samples were taken to the layer of 40-60 cm in the subplots receiving 35 kg P ha^{-1} to evaluate root distribution in deep layers. Root samples were put in plastic bags and stored at 4 °C for a few days before soil-root separation. Root samples were first soaked in a 1 M solution of NaCl (1 L for 5-cm soil core) for 16 h to disperse the soil aggregates. Then, the samples were transferred to a hydro-pneumatic elutriation washing machine (Smucker et al. 1982). The cleaned roots were collected first on a primary sieve of 760 microns and second on a secondary sieve of 400 microns (Bolinder et al. 2002). The roots recovered on the secondary sieve were selected by hand to remove the remaining mineral particles and organic debris. Next, the roots were rinsed and placed on a transparent tray with distilled water, dispersed with tweezers and scanned in black and white (400 dpi, tagged image file format [TIF], white background) using the Imagery Scan Screen (EPSON Expression 10000XL) (Sheng et al. 2012). The root length, surface area and average diameter were automatically analyzed using the professional image analysis software “WinRhizo” (2009a, 2009b) (Regent Instruments Inc., Quebec). Root length was classified into three diameter classes (0-0.2 mm [tertiary roots], 0.2-0.8 mm [secondary roots] and >0.8 mm [primary roots]) (Drouet and Pagès 2003; Sheng et al. 2012). After scanning, the roots were dried with paper towels for fresh weight. Then, the roots were oven dried at 55 °C to a constant weight and weighted for dry weight. Root length density (RLD), surface area density (RSD) and mass density (RMD) were calculated as root length, surface area and dry mass divided by soil core volume. The root length proportions of tertiary, secondary and primary roots (3rdRL%, 2ndRL% and 1stRL%) were calculated as the root lengths of each root order divided by the total root length in each sample. The content of P in roots was analyzed by the method described in (Isaac and Johnson 1976).

3.4.3. Soil sampling and analysis

First general soil sampling was conducted on 3 July 2014 at the moment of the second nitrogen application in 18 selected sub-plots (within three blocks). Soil samples from each plot represented a composite of five 5.25 cm-diameter soil cores collected at five depths (0-5, 5-10, 10-20, 20-30 and 30-40 cm). Soil samples were used for soil bulk density analysis with the method described by Hao et al. (2008).

A second soil sampling was conducted at the same moment with root sampling, following the same manners by using a hydraulic power sampler (5.25-cm inner diameter). Soil samples were air-dried, sieved through a 2-mm sieve and stoked for several analyses. Soil pH was measured in distilled water using a 1:2 soil/solution ratio (Hendershot et al. 1993). Total carbon (C) and N were quantified by dry combustion with a LECO CNS-1000 analyser (LECO Corp., St. Joseph, MI). Olsen extracted P (P-Olsen) was measured by shaking 1.0 g of soil with 20 mL of 0.5 mol L^{-1} sodium bicarbonate solution (pH 8.5) for 30 min (Olsen et al. 1954). The concentrations were determined by the Murphy Riley method (Murphy and Riley 1962) using a spectrophotometer (Jenway 6320D) (at 882 nm) with a 1-cm long optical cell.

3.4.4. Crop harvest

The crop-yield data were collected from 1992 to 2014. Crops were harvested at maturity, generally in October for corn. Corn grain yield was determined by harvesting plants on 10 m of the two middle rows of each plot. Grain was threshed and weighed, and yields were adjusted to 15% moisture content. The grain P content was measured by mineralizing 0.1 g grain ground (0.2 mm) with a mixture of sulphuric and selenious acids, as described by Isaac and Johnson (1976). The P concentrations were measured on a QuikChem 8000 Lachat autoanalyzer (Zellweger Analytic Inc., Lachat Instruments Division, Milwaukee, WI) using the Lachat method 13-107-06-2-E (Ziadi et al. 2007). Annual P export was calculated by multiplying grain P content by grain yield. The annual P budget was calculated as the difference between P applied as fertilizer and annual P exported by the grains.

3.4.5. Statistical analysis

All of the root data were first subjected to a screening to find the outliers (five outliers were found by comparing the root diameter distributions with the scanned images), which were related to the misuse of sieve during the root separation on 28 August. Next, the screened data subjected to a test with GLIMMIX procedure and transformed (if necessary) using *transreg* procedure (SAS Institute Inc., 2010) to achieve normality of distribution and homogeneity of variance before ANOVA. Additionally, the root length proportions were treated as the compositional data and transformed into the isometric log ration (*ilr*) according to the sequential binary repartitions (RLilr_1: tertiary roots|(secondary and primary roots); RLilr_2: secondary roots|primary roots) and the equation (**Eq 3-1**) described in Egozcue et al. (2003).

$$\boldsymbol{ilr_i = \sqrt{\frac{rs}{r+s}}\, ln \frac{(\prod_+ x_j)^{\frac{1}{r}}}{(\prod_- x_k)^{\frac{1}{s}}} \quad i = 1, 2, 3, \ldots, D-1; j + k = D} \qquad \textbf{Eq 3-1}$$

where the products $\boldsymbol{\Pi_+}$ and $\boldsymbol{\Pi_-}$ only include parts coded with **+** and **-**, and $\boldsymbol{r}$ and $\boldsymbol{s}$ are the numbers of the positive and negative signs (parts) in the $\boldsymbol{i}$-th order partition.

According to the experimental design, ANOVA was performed for all of the treatments using MIXED procedure to evaluate the fixed effects of tillage, P fertilization, sampling depth and perpendicular distance to the corn row and their interactions on root distribution and morphology along with soil bulk density, pH, soil C and N and soil P status (P-Olsen); the random effects were set as the block and its interaction with tillage practice. In addition, the sampling depth and perpendicular distance to the corn row were repeated-measure factors in the design. Similarly, the crop-yield data were subjected to the ANOVA using MIXED procedure (SAS Institute Inc., 2010) to evaluate the effects of tillage practice, P fertilization, and year along with their interactions; the random effects were set as the block and its interaction with tillage practice. Treatment effects were deemed significant when $P<0.05$; differences among least square means for all of the treatment pairs were identified using LSMEANS (/diff) statement (*t*-test) (SAS Institute Inc., 2010). A Pearson correlation test between corn yields and corn roots (RLD, RSD and RMD) was calculated with CORR procedure (SAS Institute Inc., 2010).

3.5. Results

3.5.1. Root mass and its distribution in soil profile

Across all of the treatments, the average root mass density (RMD) was 0.2 mg cm^{-3}, viz. 800 kg ha^{-1} considering the 40-cm soil profile. The sampling depth and perpendicular distance to the row had significant effects on root mass density (**Table 3-1**). RMD significantly

decreased with increasing soil depth from 0.39 mg cm^{-3} to 0.05 mg cm^{-3}, whereas horizontally, significantly higher RMD was observed at 5-cm distance to the row (**Table 3-2**). This led to high accumulation of root biomass at 0-5 and 5-10 cm depths with, respectively, 30% and 24% of the total root biomass over the whole 40-cm soil profile. Horizontally, 64% of the total biomass was at 5-cm distance, whereas 16% and 20% of the total biomass was at 15-cm and 25-cm distances, respectively. The interaction of sampling depth and perpendicular distance to the row was noted on RMD (**Table 3-1**). This showed that the decrease of RMD along the soil profile was more obvious at 5-cm distance because of the higher values than those at 15-cm and 25-cm distances for each depth (**Fig. 3-1-I**).

On average, the RMD was -26% lower in the NT treatment compared to in the MP treatment (P>0.05) (**Table 3-3**). Similarly, the 1P treatment increased RMD by 26% and 41% in comparison to the 0P and 0.5P treatments, respectively (P>0.05) (**Table 3-1, 3-3**). RMD responded significantly to the interaction of tillage practice, P fertilization and sampling depth (**Table 3-1**), showing that tillage effect on RMD varied among P application rates (**Fig. 3-2**). Under the 0P treatment, tillage had significant effect on RMD only at 0-5 cm depth, with a 74% higher value under MP than NT. Under the 0.5P treatment, tillage had a significant effect on RMD at depths of 0-5 cm and 10-20 cm; RMD under MP was -60% lower than under NT at 0-5 cm, but 75% higher at 10-20 cm. Tillage had no significant effect on RMD under 1P fertilization treatment, even lower RMD was observed under NT for all depths.

3.5.2. Root morphological traits: root surface density, root length density, average root diameter, root diameter distribution and root P content

Considering the soil profile of 40 cm, the average root surface density (RSD) and root length density (RLD) were 0.12 cm^2 cm^{-3} and 1.38 cm cm^{-3}, viz. 4.8×10^4 m^2 ha^{-1} and 5.52×10^4 km ha^{-1}, respectively. Similar to RMD, both RSD and RLD significantly decreased with increasing soil depth along the soil profile. Horizontally, RSD and RLD were significantly greatest at 5-cm distance and significantly smallest at 15-cm distance (**Tables 3-1, 3-2**). Sampling depth and perpendicular distance to the row also interacted significantly, showing that the decrease of RSD and RLD along the soil profile were more obvious at 5-cm distance than at 15-cm and 25-cm distances because of the higher values for each depth (**Fig. 3-1-II, III**).

The RSD was 15% (P>0.05) greater in MP than in NT and was 27% (P>0.05) higher with the 1P than with the 0P and 0.5P applications (**Tables 3-1, 3-3**). Similarly, RLD was 10% (P>0.05) greater in MP than NT and 26% and 32% (P>0.05) higher with the 1P than with the 0P and 0.5P applications, respectively (**Tables 3-1, 3-3**). According to the interaction of tillage practice and sampling depth and distance on RSD (**Table 3-1**), the MP treatment tended to have a higher RSD compared to NT across the sampling zone, with a significant difference at 10□20 cm depth and 25-cm distance (**Table 3-4**). However, the reverse result showed in the surface layer (0-5 cm) at 15-cm and 25-cm distances. The spatial distribution of RLD as affected by tillage showed the same pattern as RSD, even there was no significant interaction effect of tillage, soil depth and distance (**Table 3-4**).

Average root diameter responded significantly to sampling depth and horizontal distance (**Table 3-1**). Vertically, average root diameters were significantly lower at surface layers (0-5, 5-10 cm) than at deep layers (10-20, 20-30, 30-40 cm); horizontally, average root diameter was significantly greater at 5-cm distance than at 15-cm and 25-cm distances (**Table 3-2**). At 5-cm distance, average root diameter, with a higher value at each depth, decreased slightly from the soil surface and maintained relatively constant, whereas, at 15-cm and 25-cm distances, average root diameter increased significantly in the upper layers and then decreased in the deeper layers (**Fig. 3-1-IV**). However, no significant difference in tillage practice or P fertilization was observed for average root diameter (**Table 3-1**).

A detailed study of root morphology was conducted by considering the length of the primary, secondary and tertiary roots, which corresponded to three diameter classes of >0.8, 0.2-0.8, and 0-0.2 mm, respectively. Sampling depth and distance to the row had significant effects on the RLD of primary, secondary and tertiary roots (**Table 3-1**). The RLDs of primary, secondary and tertiary roots (1^{st}RLD, 2^{nd}RLD and 3^{rd}RLD) decreased significantly from the surface along the soil profile. Horizontally, 1^{st}RLD, 2^{nd}RLD and 3^{rd}RLD were significantly higher at 5-cm distance and significantly lower at 15-cm distance than at 25-cm distance (**Table 3-2**).

Tillage practice had no significant effect on the root length density of tertiary, secondary and primary roots (**Table 3-1**). However, the relative change (NT-MP)/MP, was -25%, -21% and -9% for 1^{st}RLD, 2^{nd}RLD and 3^{rd}RLD, respectively (**Table 3-3**). In contrast, the root length density of primary roots was significantly higher in the 1P treatment (**Tables 3-1, 3-3**). The significant interaction of tillage practice and sampling depth was only observed on the 2^{nd}RLD and 1^{st}RLD (**Table 3-1**). Shown in **Fig.s 3-3-I, II**, the 1^{st}RLD and 2^{nd}RLD in MP were higher than in NT along the soil profile, but significant differences were only observed at the depth of 20□30 cm. The interaction of tillage, P fertilization and soil depth significantly affected the 1^{st}RLD and showed the same pattern as RMD (**Table 3-1**); therefore, it was not repeated here.

As the typical compositional data, the root length proportions were transformed into isometric log ratio (*ilr*) forms. Accordingly, a higher RL*ilr*1 indicated a higher proportion of tertiary roots to secondary and primary roots; likewise, a higher RL*ilr*2 stood for a higher proportion of secondary roots to primary roots. Vertically, corn roots tend to have a higher length proportion of tertiary roots and a lower proportion of secondary roots at upper layers, whereas horizontally, higher tertiary root length proportion and lower secondary root length proportion were found in the inter-row than close to the corn row (**Table 3-2**). No significant effect of tillage practice or P fertilization was found for the root length proportions of the three root diameter classes (**Table 3-1**). A significant interaction of sampling distance to corn row, tillage practice and P fertilization was observed for tertiary root length proportion (RL*ilr1*); however, no significant differences were found among the treatments at each corresponding distance to the corn row.

Additionally, tillage and P fertilization showed no significant effects on root P contents (**Table 3-1**). However the effect of tillage was closely deemed as significant with P=0.06. Root P content in root under NT (1313 mg kg^{-1}) was slightly higher than that under MP (1106 mg kg^{-1}) (**Table 3-3**).

3.5.3. Soil properties

Tillage practice primarily influenced the soil properties vertically by interacting with the sampling depth (**Table 3-5**). The NT practice significantly decreased the bulk density in the topsoil (0-5 cm) and increased the soil P status (P-Olsen) along with the contents of soil C, N in the upper soil layers (0-10 cm) (**Table 3-6**). In the deeper layers of the soil profile, the reverse case was observed. Bulk density was higher in NT than in MP with significant difference at the depth of 30-40 cm; soil P-Olsen and the contents of C, N were significantly lower in NT than in MP at 20-30 cm. The pH values in NT and MP were similar. Even indicated in **Table 3-5**, the interaction of tillage and sampling depth significantly influenced pH, but no significant difference was found at corresponding depths between NT and MP (**Table 3-6**); incidentally, it was the same case for the interaction of P fertilization, sampling depth and distance on pH.

P fertilization significantly influenced P-Olsen with the interaction of sampling depth (**Table 3-5**). Higher P fertilization rate led to the higher P-Olsen values, and this trend was consistent along the soil profile (**Table 3-6**).

3.5.4. Corn yield, and cumulative P budget and relation with corn roots

As noted above, the corn yield data were collected from 1992 to 1994 every year and from 1996 to 2014 every two years. The interaction of tillage practice and year significantly affected corn yields (**Table 3-7**). The corn yields in MP were generally higher than those in NT, with significant differences in 2000 (+22%), 2004 (+26%), 2010 (+18%), 2012 (+143%) and 2014 (+105%) (**Fig. 3-4-I)**. After long-term NT practice, the corn-crop productions tend to be more impaired.

P fertilizations showed no significant effect on corn yields, but the highest corn yields were observed in the 1P treatment for 1994 (+8% and +11% over 0P and 0.5P, respectively), 1996 (+3% and +5%), 1998 (+5% and +3%), 2000 (+8% and +6%), 2002 (+7% and +12%), 2010 (+3% and +6%), 2012 (+39% and +65%) and 2014 (+19% and +23%) (**Fig. 3-4-II**). Similarly, after applying various P fertilizations over the long term, the corn-crop productions tend to be more impaired with 0P and 0.5P treatments.

The cumulative P budgets for the 22 years of the experiment (**Fig. 3-4-III**) for the 0P and 0.5P treatments were negative (-357 kg P ha^{-1} and -135 kg P ha^{-1}, respectively after 22 years), whereas it remained slightly positive in 1P treatment (+65 kg P ha^{-1}). The P budgets with a same P fertilization rate were similar under NT and MP; in recent years, however, the cumulative P budgets in NT were slightly higher than in MP because of the reduced corn grain yield in NT in the last years.

Moreover, corn yields and root biomass had different relationships under MP and NT. The results of Pearson correlation between corn yield and average RLD for each field-plot are showed in **Fig. 3-5**. Under MP, corn yield had a significant positive correlation with RLD (Pearson coefficient=0.616, P=0.044); whereas it was a significant negative correlation under NT (Pearson coefficient=-0.706, P=0.023). The results were similar for the correlations between corn yield with RSD (MP: Pearson coefficient=0.640, P=0.034; NT: Pearson coefficient=-0.671, P=0.034) and RMD (MP: Pearson coefficient=0.747, P=0.008; NT: Pearson coefficient=-0.327, P=0.357, not significant).

3.6. Discussion

Corn roots, which have a main nodal root system with branches, are extensive in the soil, reaching as deep as 2 m and spreading throughout the upper 60 cm during growth. Its expansion depends on genotypes (Hajabbasi and Schumacher 1994; Mu et al. 2015), soil types (Chassot et al. 2001) and sampling time (Cai et al. 2014) Sampling design and measurement methods might also explain differences reported in the literature (Buczko et al. 2009). In our field study, the overall root length density (RLD) for the 40-cm soil profile was 1.38 cm cm^{-3}, which is comparable to the RLDs that are widely reported in literature for maize (Qin et al., 2006; Buczko et al. 2009; Guan et al. 2014).

3.6.1. Spatial distribution of corn roots

The distribution of corn roots in the soil varied both vertically and horizontally (Mengel and Barber, 1974; Fernandez and White 2012). More roots are accumulated on the surface of the soil and close to the corn row because of corn root architecture genetically controlled and environmental constraints such as gravity and soil resistance. The greater availability of water and nutrients in surface layers might also explain the higher root biomass in the upper soil layers (Lynch 2011). Regardless of treatment, corn root mass, surface and length densities were higher close to the soil surface and declined along the soil to a depth of 40 cm. Measurements on the 1P treatment showed that at the silking stage the proportion of root length encountered in the 40-60 cm layer represented an average of 9% of the root length

found within the 0-60 cm soil layer at this experimental site. Similar low RLD in deep layers have been reported in other studies (Qin et al. 2006; Cai et al. 2014; Ning et al. 2015). However, Guan et al. (2007) note that corn roots could stretch deeper after silking, e.g., with only 55% of total roots in the 0-40 cm layer just before grain maturity in general. In our study, considering very low soil nutrient status beneath the 40-cm layer, soil humidity and low RLD in deeper layers, the deep root contribution to the water uptake and nutrient acquisition might be low. We also observed greater roots at 25-cm distance to corn row than at 15-cm distance, which is probably attributable to the overlap of roots from every two adjacent corn rows (Qin et al. 2006). Unfortunately, it was impossible to distinguish roots from the both row according our sampling design. The average root diameters also varied with the spatial factors, especially with the sampling distance perpendicular to the corn row. The average root diameter decreased from row to inter-row (corresponding to the observation of Buczko et al. (2009)) as more coarse roots (primary and secondary roots) were close to the corn row.

3.6.2. Corn roots affected by tillage practice

In this study, corn root biomass in NT was -26% smaller than in MP along the soil profile (**Table 3-3**). This was the overall reduction of all root orders, with no significant difference in the root length proportions of primary, secondary and tertiary roots for NT vs. MP (**Table 3-3**). Nevertheless, the tertiary roots in NT were -9% lower in RLD compared to MP, whereas the primary and secondary roots were -25% and -21% lower, respectively, indicating a relatively greater reduction in primary and secondary roots. Corroborated with the reduction of corn roots, we observed a reduction of corn yields in NT in 2014 (**Fig. 3-4-I**). The greater decrease of corn yield in NT was only observed in recent years (2012 and 2014). It is the result of a slow but constant process throughout the 22 years of experimentation. Because root biomass is highly correlated to shoot biomass, we could assume that soil factors modified by NT practice have impaired the C assimilation of shoot and its allocation to the root system. Besides, a negative correlation between corn yields and roots under NT (**Fig. 3-5**) also indicated that corn root confronted pressure in nutrient acquisition, which forced corn crops to allocate more resource and energy to root system. Thus, many soil and climate factors, including climatic conditions, soil structure, weeds, carbon and nutrients contents, could affect root development under no-till (Javeed et al. 2013; Guan et al. 2014; Labreuche et al. 2014).

Firstly, root growth is considered as a function of soil resistance to penetration and water content (Klepper 1990). Numerous studies have shown that higher soil bulk density in NT, which results in higher soil resistance, could impair root proliferation in the soil, especially in deeper layers (Chassot et al. 2001; Qin et al. 2006; Cai et al. 2014; Guan et al. 2014; Nunes et al. 2015). In our study, the NT practice modified the soil bulk density by having higher values at 20-30 and 30-40 cm layers (**Table 3-6**). This is probably the transition layer compaction described in Peigne et al. (2013), which could partly explain the observation of lower primary and secondary RLDs under NT than under MP at 20-30 cm (**Fig. 3-3**). However, similar soil bulk densities of NT and MP in the upper layers were measured and were unable to explain the relative reduction of corn roots in NT in the upper layers. Moreover, NT usually maintains higher soil moisture (Labreuche et al. 2014), but it seems make no difference for corn roots under an annual precipitation of 1000 mm for L'Acadie.

With less soil turbulence, the NT system creates a relatively stable environment in soil, favoring biodiversity and micro- or macro- fauna and flora activities (Helgason et al. 2010; Scopel et al. 2013). The biodiversity in NT has both positive and negative effects on nutrient acquisition, the latter because of the competition for resources. Weed control in NT is often one of the difficulties (Soane et al. 2012; Melander et al. 2013; Nichols et al. 2015; Pakeman et al. 2015) and might limit crop yield because of direct competition for light, water and nutrients (Afifi and Swanton 2011; Silva et al. 2011). Although, weed abundance was not

quantified in our study in 2014, weed colonization was visually more important in NT (**Fig. 3-6**). The previous study of Légère et al. (2008) in 2004 at the same site reported this higher weed proliferation and diversity in NT, whereas nutrient treatments had a much smaller effect. Rizzardi and Wandscheer (2014) noted that root system size usually decreases as a plant grows under competitive conditions. Norris (1992) had observed a reduction of sugarbeet root with the existence of weed as Barnyardgrass. Additionally, Silva et al. (2010; 2011) reported a reduction of corn root biomass without weeding (hoeing). The observed root reduction in NT could be caused by the delayed early growth associated with the competition against weeds for resource acquisition. Even with non-limiting soil resources, weeds delayed the emergence of the corn root radicle and reduced the root relative growth rate until the fourth leaf tip stage (Afifi and Swanton 2011). This delayed early root growth explains that in our study, NT reduced corn roots even with higher soil nutrients (P, N and C). It also explains that the higher degrees of root reduction in NT occurred in primary and secondary roots (which develop in early growth stage) rather than in tertiary roots. The negative correlation between corn yields and roots (**Fig. 3-5**) in NT might also indicate that corn allocated more resource to root system to counteract the weed competition for nutrient, and consequently reduced yields. And weeds were generally considered as better nutrient accumulators than crops (Qasem 1992; Andreasen et al. 2006), nutrient components were supposed to be lower under weed competition. However, our results oppositely showed higher root P content (**Table 3-3**); and it could be due to a lesser dilution effect of P concentration in reduced corn root biomass. Moreover, our results showed that the greater yield gap in 2014 [(NT-MP)/MP=-51%] between NT and MP, compared to the study of Légère et al. (2008) at the same site in 2004 [(NT-MP)/MP=-21%] (**Fig. 3-4-I**), could also indicate that weed competition with corn root in NT tends to become more severe over the long term. Moreover, the seeding was delayed at the beginning of June 2014 because soil moisture conditions did not allow the passage of sowing machine. This can also lead to yield reduction (Muelller 2009) and may contribute to the greater yield difference between NT and MP.

Other studies mention that the NT system tends to have cooler soil temperature in early spring (Chassot et al. 2001; Qin et al. 2006; Fernandez and White 2012) because of higher soil moisture with the greater specific heat capacity of water. Low soil temperature could decelerate the corn germination and growth (Javeed et al., 2013) and have a negative effect on corn root development in the long term (Chassot et al. 2001). The monitoring of soil temperature at L'Acadie in 2014 (Shi et al. 2015) showed that the soil temperature of the 0-10 cm layer in NT was approximately 1-°C lower than its value in MP during April and the beginning of May. This might cause a smaller root system in NT. However, the corn seeding was delayed to early June 2014 and unfortunately, we did not have the data to confirm whether the lower soil temperature in NT would be maintained until that time. Additionally, the higher average air temperature in early June (18.3 °C) than in April (5.6 °C) and May (13.9 °C), which rapidly warmed the soil during daytime, could offset the possibly lower soil temperature effect on corn roots in NT; even 2014's annual average air temperature (6.4 °C) is cooler than the average air temperature over the 22 years (6.8 °C) of the study (Environment Canada 2015).

The higher contents of C, N and P in the soil top layers (0-20 cm) in NT (**Table 3-6**) are caused by the surface placement of residues under NT rather than the mixing of residues within the till-zone under MP (Franzluebbers and Hons 1996). This was not helpful in interpreting the relative corn root reduction in NT in that zone. However, the higher RSD and RLD at the inter-row in NT indicated a more expansive corn root system on the soil surface (0-5 cm) in NT (**Table 3-4**). A plausible explanation could be the corn root's tendency of accumulating to the nutrient-rich patch (Li et al. 2012). A discriminant analysis of corn root partition with compositional data analysis was consistent with this explanation, but because of the high variability of measured root traits, no significant correlation has been shown.

3.6.3. Corn roots affected by P fertilization

P fertilization showed effects on corn roots by having fewer roots, especially primary roots, with 0P and 0.5P applications (**Table 3-3**). However, it was also an overall corn root reduction in 0P and 0.5P with no significant difference in the root length proportion of three root diameter orders within 0P, 0.5P and 1P (**Table 3-3**). Similarly, the fewer corn roots in 0P and 0.5P corroborated to the lower corn yields in 0P and 0.5P in 2014 (**Fig. 3-4-II**). Crop yields decrease with a decrease in soil nutrient levels below a critical value (Ware et al. 1982). Therefore, the fewer corn roots in 0P and 0.5P could result from the continued downward soil P status with negative cumulative P budgets for 22 years of continuous cultivation, which has also increasingly affected the corn grain yields by the time. Meanwhile, the cumulative P budget remained close to zero and was even slightly positive in the 1P rate, maintaining the soil P status. Moreover, the lower soil P status may aggravate weed-crop competition. Thus, the corn root reduction caused by NT seemed to be grosser with 0P than with 1P (**Fig. 3-2**).

On the other hand, Sá et al. (2013) find that corn root growth could have a positive linear relation with soil available P at 0-10 cm, but no response is achieved when the P level is already high. Deng et al. (2014) have provided a critical level of P-Olsen at 8 mg kg^{-1} for the top 20-cm of soil in a calcareous silt loam soil. In this study, the smallest value of P-Olsen of top 20-cm soil measured in MP for 0P (8.5 mg kg^{-1}) was higher than the threshold value of 8 mg kg^{-1} (Deng et al. 2014). This indicates that high soil P status stimulation on corn roots might also work in a higher range of P-Olsen, depending on soil type and corn variety.

3.6.4. Corn roots affected by the interaction of tillage practice and P fertilization

In our study, P fertilization and tillage practice also interacted with depth on root mass density (**Fig. 3-2**). However, the only significant interactive effect of tillage and P fertilization occurred at 0-5 cm with lower RMD in NT with 0P and 1P and higher RMD in NT with 0.5P. The high variability of RMD in the surface layer (Buczko et al. 2009) might explain the unexpectedly higher RMD in NT for 0.5P compared to MP (**Fig. 3-2-II**). To investigate this discrepancy, the sampling effort should be increased in the upper soil layers.

Regardless of the surface layer with 0.5P, more corn roots were observed in MP than in NT for each depth with three P rates, even in the upper layer with a higher soil P status in NT. This might imply that tillage practice has a more critical impact than P fertilization on corn roots.

3.7. Conclusion

In our study, the tillage practices of NT and MP influence the corn root distribution and morphology, by primarily decreasing the corn roots, especially the primary and secondary roots, in the NT system. Lower P fertilization rates (0 and 17.5 kg P ha^{-1} every two years) can decrease the amount of corn roots compared to 35 kg P ha^{-1}, with continued negative cumulative P budgets over 23 years. The combined tillage practices with biennial rates of P fertilization have also changed the soil physical property with higher bulk densities in deeper layers and the distribution of nutrients with higher C, N and P contents on the surface layer in NT. Unlike our hypothesis, the modifications of the soil physical properties and nutrient distributions at L'Acadie after the 23-year experiment were not the determining factors in the variations of corn root distribution and morphology. Other factors, especially weed competition, could be more important drivers to explain both corn root morphology and spatial distribution modifications. This disadvantage of long-term NT practice should be further investigated by simultaneously considering biotic and abiotic factors.

3.8. Acknowledgement

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Table 3-1 Analysis of variance for root mass density (RMD), root surface density (RSD), root length density (RLD), average root diameter, tertiary (diameter<0.2 mm), secondary (0.2 mm<diameter<0.8 mm) and primary (diameter>0.8 mm) root length densities (3^{rd}RLD, 2^{nd}RLD, 1^{st}RLD) and proportion balances (RLilr1 and RLilr2), and P contents in root as affected by tillage and P fertilization and the interactions with sampling depth and perpendicular distance to corn row.

Treatment	RMD	RSD	RLD	Diameter	3^{rd}RLD	2^{nd}RLD	1^{st}RLD	RLilr1	RLilr2	P content
Tillage	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P	NS	NS	NS	NS	NS	NS	**	NS	NS	NS
Tillage × P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Depth	***	***	***	NS	***	***	***	***	***	**
Tillage × Depth	*	*	NS	NS	NS	*	*	NS	NS	NS
P × Depth	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tillage × P × Depth	**	NS	NS	NS	NS	NS	**	NS	NS	NS
Distance	***	***	***	***	***	***	***	***	***	***
Tillage × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P × Distance	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
Tillage × P × Distance	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
Depth × Distance	***	***	NS	**	NS	***	***	NS	NS	*
Tillage × Depth × Distance	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
P × Depth × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tillage × P × Depth × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS not significant ($P \geq 0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 3-2 Root mass density (RMD), root surface density (RSD), root length density (RLD), average root diameter, tertiary (diameter<0.2 mm), secondary (0.2 mm<diameter<0.8 mm) and primary (diameter>0.8 mm) root length densities (3^{rd}RLD, 2^{nd}RLD, 1^{st}RLD), length proportion balances (RL*ilr*1 and RL*ilr*2) and length proportions (3^{rd}RL%, 2^{nd}RL%, 1^{st}RL%), and P contents in root of different sampling depth and perpendicular distance to corn row.

Treatment	RMD	RSD	RLD	Diameter	3^{rd}RLD	2^{nd}RLD	1^{st}RLD	RL*ilr*1	RL*ilr*2	3^{rd}RL%	2^{nd}RL%	1^{st}RL%	P content
	mg cm^{-3}	cm^2 cm^{-3}	cm cm^{-3}	mm	cm cm^{-3}	cm cm^{-3}	cm cm^{-3}						mg kg^{-1}
Depth (cm)													
0–5	0.39a	0.20a	2.29a	0.27b	1.44a	0.79a	0.06a	2.01a	2.53b	64%	33%	3%	1326
5–10	0.30a	0.18a	2.04a	0.28b	1.29a	0.71a	0.05a	1.94a	2.55b	62%	35%	3%	1204
10–20	0.16b	0.11b	1.18b	0.29a	0.69b	0.45b	0.03b	1.62b	2.29b	57%	40%	3%	1216
20–30	0.09c	0.07c	0.82c	0.29ab	0.47c	0.33c	0.02c	1.67b	2.56b	55%	43%	2%	1187
30–40	0.05d	0.05d	0.59d	0.28ab	0.33c	0.24d	0.01d	1.94a	2.99a	56%	43%	1%	1116
Distance (cm)													
5	0.39a	0.18a	1.85a	0.32a	1.07a	0.70a	0.08a	1.30b	1.79b	56%	41%	3%	988
15	0.09b	0.07c	0.91c	0.26b	0.57c	0.32c	0.01b	2.16a	3.09a	60%	39%	1%	1299
25	0.12b	0.11b	1.39b	0.26b	0.88b	0.49b	0.02b	2.03a	2.86a	61%	38%	1%	1343

Different letters in columns indicate significant differences (P<0.05; *t*-test) within depths or distances to corn row.
The means for 3^{rd}RL%, 2^{nd}RL% and 1^{st}RL% are not supposed to be compared statistically.

Table 3-3 Root mass density (RMD), root surface density (RSD), root length density (RLD), average root diameter, tertiary (diameter<0.2 mm), secondary (0.2 mm<diameter<0.8 mm) and primary (diameter>0.8 mm) root length densities (3rdRLD, 2ndRLD, 1stRLD) length proportion balances (RL*ilr*1 and RL*ilr*2) and length proportions (3rdRL%, 2ndRL%, 1stRL%) of different tillage practice and P fertilization.

Treatment	RMD	RSD	RLD	Diameter	3rdRLD	2ndRLD	1stRLD	RL*ilr*1	RL*ilr*2	3rdRL%	2ndRL%	1stRL%	P content
	mg cm^{-3}	cm^2 cm^{-3}	cm cm^{-3}	mm	cm cm^{-3}	cm cm^{-3}	cm cm^{-3}						mg kg^{-1}
Tillage													
MP	0.23	0.13	1.48	0.29	0.88	0.56	0.04	1.77	2.56	60%	38%	2%	1313
NT	0.17	0.11	1.28	0.28	0.80	0.44	0.03	1.90	2.61	63%	35%	2%	1106
P fertilization													
0P	0.19	0.11	1.29	0.28	0.78	0.48	0.03b	1.90	2.76	60%	37%	3%	1202
0.5P	0.17	0.11	1.23	0.28	0.75	0.44	0.03b	1.87	2.61	61%	36%	3%	1173
1P	0.24	0.14	1.62	0.28	0.99	0.58	0.05a	1.74	2.39	61%	36%	3%	1254

Different letters in columns indicate significant differences (P<0.05; t-test)
The means for 3rdRL%, 2ndRL% and 1stRL% are not supposed to be compared statistically.

Table 3-4 Root surface density (RSD) under no-till (NT) and moldboard plough (MP) for the different sampling of soils with depth and perpendicular distance to the corn row.

RSD (cm^2 cm^{-3})	**Perpendicular distance to the corn row**					
	5 cm		**15 cm**		**25 cm**	
	NT	**MP**	**NT**	**MP**	**NT**	**MP**
Depth						
0–5 cm	0.29	0.41	0.13	0.10	0.20	0.11
5–10 cm	0.22	0.28	0.10	0.10	0.14	0.22
10–20 cm	0.14	0.15	0.06	0.07	0.08b	0.14a
20–30cm	0.09	0.12	0.03	0.07	0.05	0.08
30–40 cm	0.07	0.07	0.03	0.04	0.05	0.05

RLD (cm cm^{-3})	**Perpendicular distance to the corn row**					
	5 cm		**15 cm**		**25 cm**	
	NT	**MP**	**NT**	**MP**	**NT**	**MP**
Depth						
0–5 cm	2.85	3.84	1.63	1.36	2.70	1.50
5–10 cm	2.39	2.92	1.19	1.24	1.89	2.65
10–20 cm	1.50	1.46	0.75	0.86	0.93	1.61
20–30cm	1.00	1.22	0.45	0.77	0.53	0.98
30–40 cm	0.73	0.75	0.41	0.44	0.59	0.60

Different letters for each perpendicular distance indicate significant differences between soil tillage for the different depth ($P<0.05$; *t*-test)

Table 3-5 Analysis of variance for soil bulk density (BD), pH, contents of C and N, and soil P status (P-Olsen) as affected by tillage and P fertilization and the interactions with sampling depth and perpendicular distance to corn row.

Treatment	BD	pH	C	N	P-Olsen
Tillage	NS	NS	NS	NS	NS
P	NS	NS	NS	NS	***
Tillage × P	NS	NS	NS	NS	NS
Depth	***	***	***	***	***
Tillage × Depth	*	*	***	***	***
P × Depth	NS	NS	NS	NS	***
Tillage × P × Depth	NS	NS	NS	NS	NS
Distance	-	NS	NS	NS	**
Tillage × Distance	-	NS	NS	NS	NS
P × Distance	-	NS	NS	NS	NS
Tillage × P × Distance	-	NS	NS	NS	NS
Depth × Distance	-	NS	NS	*	*
Tillage × Depth × Distance	-	NS	NS	NS	NS
P × Depth × Distance	-	*	NS	NS	NS
Tillage × P × Depth × Distance	-	***	*	NS	NS

NS not significant ($P \geq 0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 3-6 Soil bulk density (BD), pH, contents of C and N, ratio C/N and soil P status (P-Olsen) for the interactions of tillage practice or P fertilization and sampling depth.

	BD		**pH**		**C**		**N**		**P-Olsen**				
	g cm^{-3}				**%**		**%**		**mg kg^{-1}**				
	NT	**MP**	**NT**	**MP**	**NT**	**MP**	**NT**	**MP**	**NT**	**MP**	**0P**	**0.5P**	**1P**
Depth (cm)													
0–5	1.28b	1.36a	6.43	6.32	2.53a	1.76b	0.24a	0.17b	26.67a	16.66b	14.43b	19.87b	30.7a
5–10	1.43	1.45	6.35	6.2	2.12a	1.81b	0.21a	0.18b	23.89	17.24	10.03b	22.76ab	28.9a
10–20	1.44	1.46	6.26	6.31	1.86	1.82	0.19	0.17	15.91	12.99	9.37b	17.08a	16.91a
20–30	1.55	1.48	6.41	6.45	0.9b	1.52a	0.1b	0.15a	5.41b	12.65a	7.18b	8.55b	11.36a
30–40	1.63a	1.51b	6.82	6.81	0.32	0.4	0.06	0.06	2.6	4.8	3.57	3.54	3.99

Different letters for each variable indicate the significant difference ($P<0.05$; *t*-test) within tillage practices or P fertilization (only for P-Olsen) categories at a certain depth.

Table 3-7 Analysis of variance for corn yield as affected by tillage and P fertilization and the interactions with years.

Treatment	Yield
Tillage	NS
P	NS
Tillage × P	NS
Year	***
Tillage × Year	***
P × Year	NS
Tillage × P × Year	NS

NS not significant ($P \geq 0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Fig. 3-1

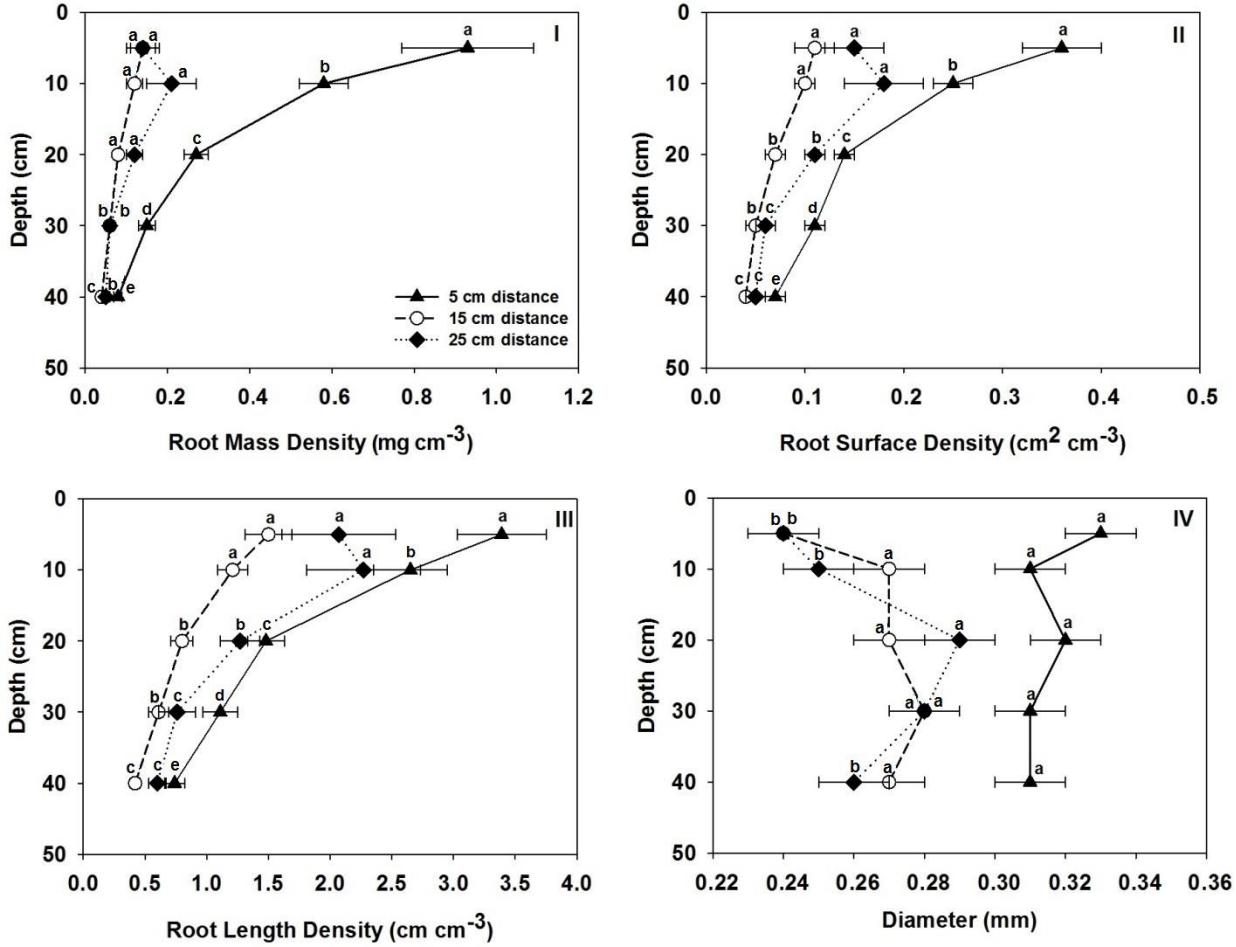
I
II
III
IV
Depth (cm)
Root Mass Density (mg cm-3)
Root Surface Density (cm2 cm-3)
Root Length Density (cm cm-3)
Diameter (mm)
5 cm distance
15 cm distance
25 cm distance

Fig. 3-2

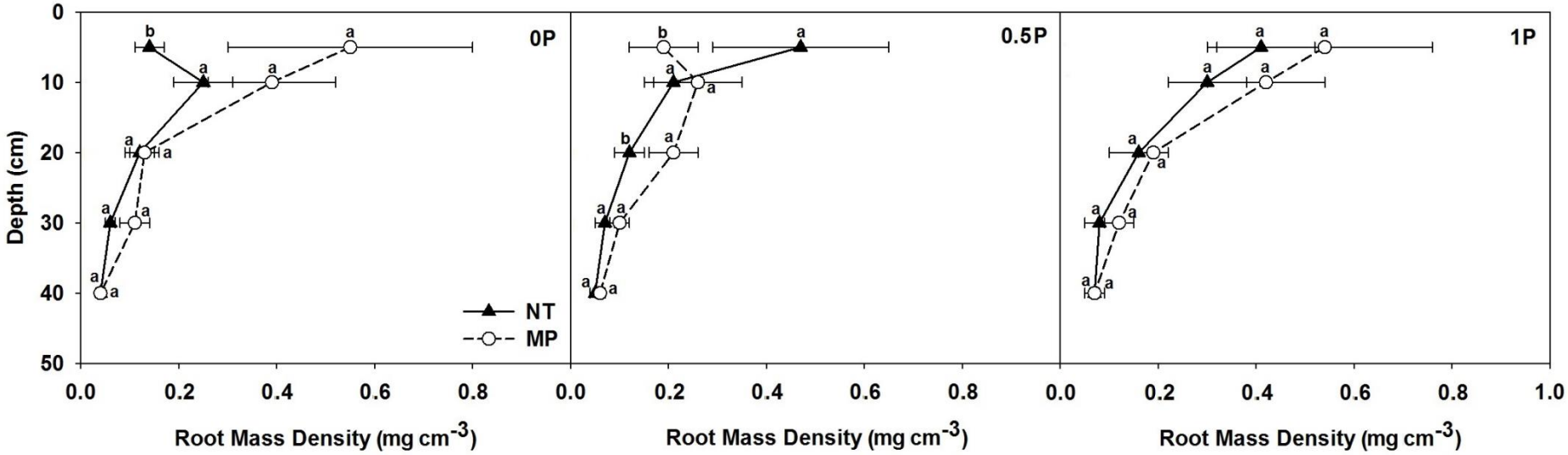
0P
0.5P
1P
NT
MP
Depth (cm)
Root Mass Density (mg cm-3)
Root Mass Density (mg cm-3)
Root Mass Density (mg cm-3)

Fig. 3-3

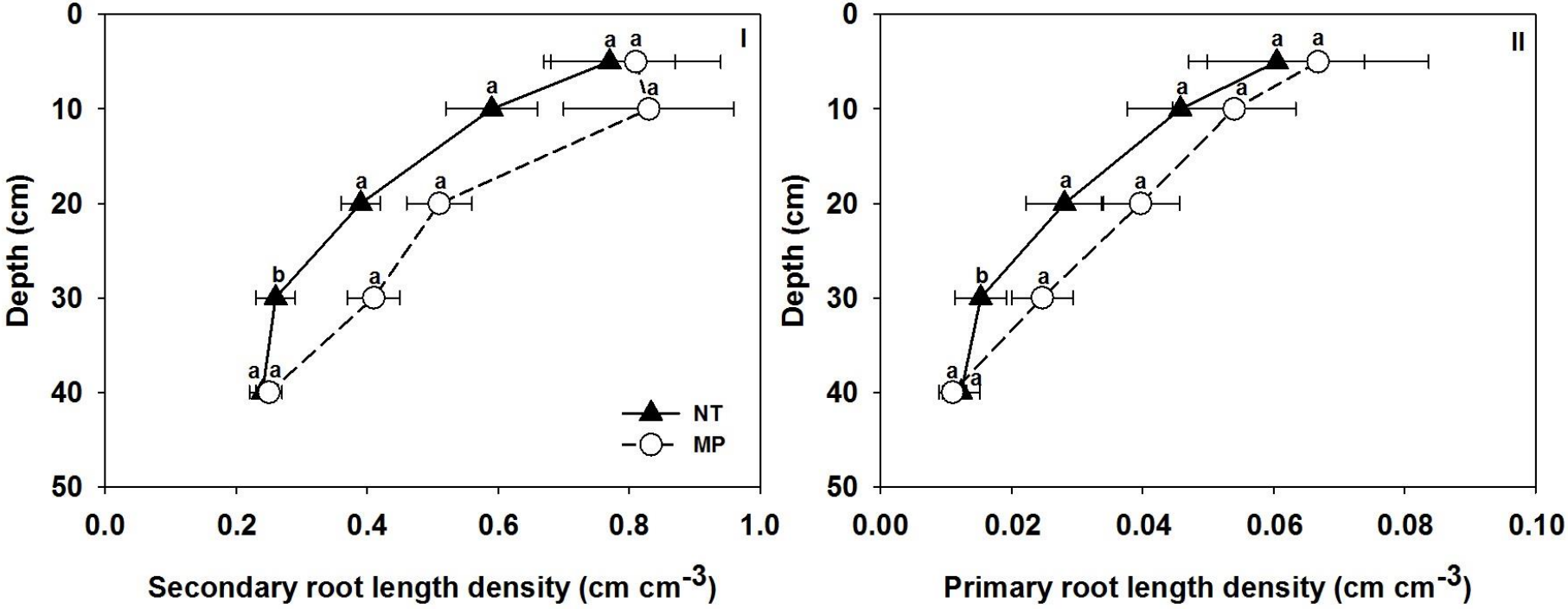
I
II
Depth (cm)
Secondary root length density (cm cm-3)
Primary root length density (cm cm-3)
NT
MP
0
10
20
30
40
50
0.0
0.2
0.4
0.6
0.8
1.0
0.00
0.02
0.04
0.06
0.08
0.10
a
b

Fig. 3-4

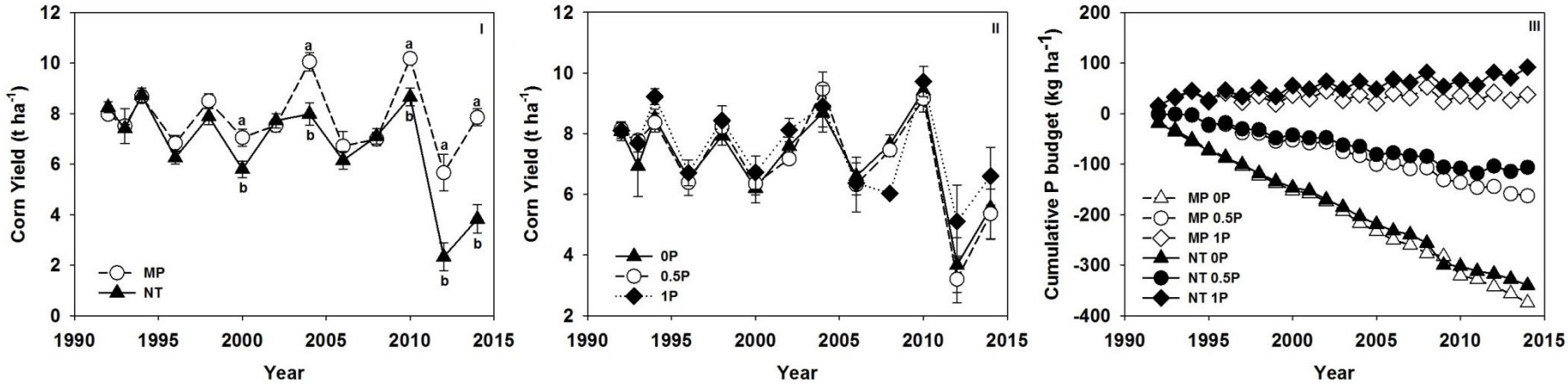
I
Corn Yield (t ha-1)
Year
MP
NT
II
0P
0.5P
1P
III
Cumulative P budget (kg ha-1)
MP 0P
MP 0.5P
MP 1P
NT 0P
NT 0.5P
NT 1P

Fig. 3-5

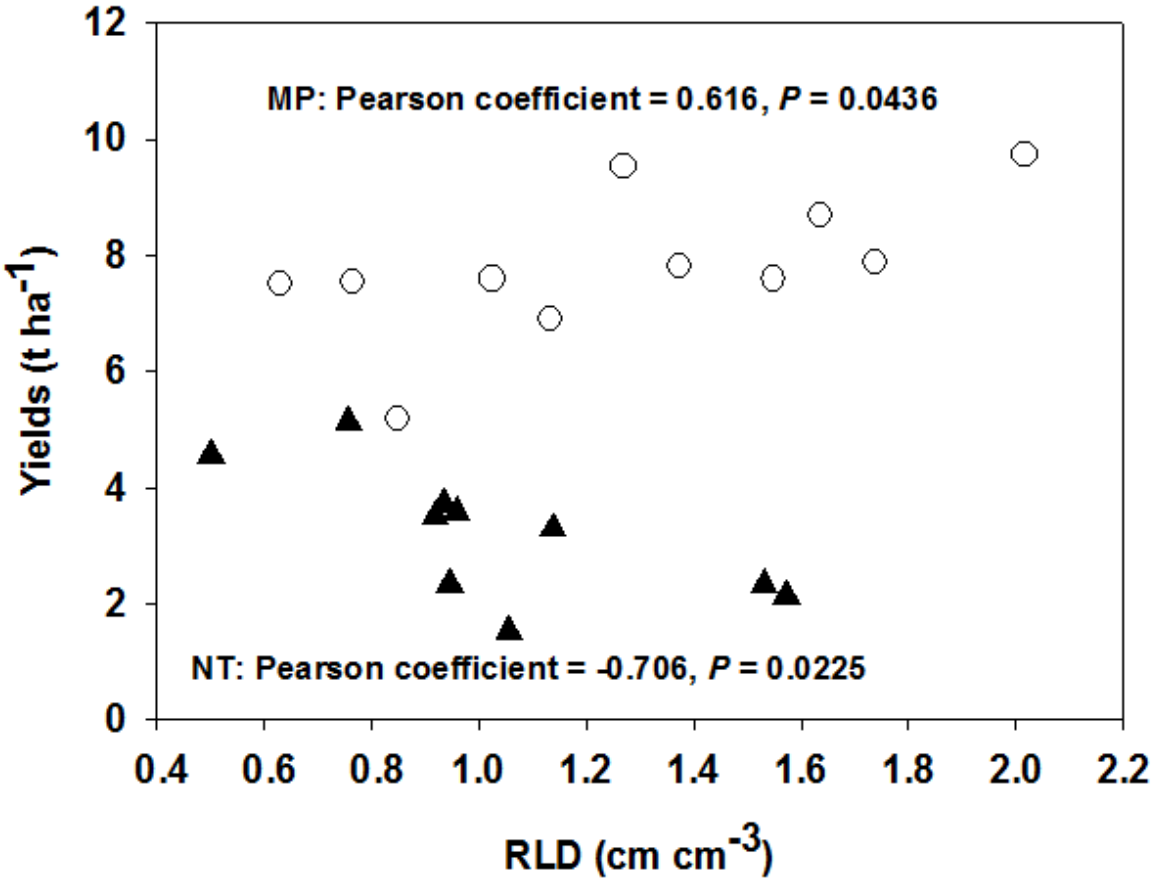
MP: Pearson coefficient = 0.616, P = 0.0436
NT: Pearson coefficient = -0.706, P = 0.0225
Yields (t ha-1)
RLD (cm cm-3)
0
2
4
6
8
10
12
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2

Fig. 3-6

Fig. captions

Fig. 3-1: Root mass density (RMD) (**I**), root surface density (RSD) (**II**), root length density (RLD) (**III**) and average root diameter (**IV**) under different sampling depths and perpendicular distances to the corn row. Bars represent standard errors. Different letters indicate significant differences ($P<0.05$; *t*-test) among depths (0-5, 5-10, 10-20, 20-30 and 30-40 cm) for each perpendicular distance (5-cm, 15-cm and 25-cm).

Fig. 3-2: Root mass density (RMD) as affected by the interaction of tillage (NT and MP), P fertilization (0P, 0.5P and 1P) and sampling depth. Bars represent standard errors. Different letters indicate significant differences ($P<0.05$; *t*-test) between tillage practices (NT and MP).

Fig. 3-3: Secondary root length density (2^{nd}RLD) (**I**) and primary root length density (1^{st}RLD) (**II**) as affected by the interaction of tillage (NT and MP) and sampling depth. Bars represent standard errors. Different letters indicate significant differences ($P<0.05$; *t*-test) between tillage practices (NT and MP).

Fig. 3-4: Corn grain yields as affected by tillage (**I**) or P fertilization (**II**), and cumulative P budget as affected by the interaction of tillage and P fertilization (**III**) in corn phases from 1992□2014. For (**I**) and (**II**): Bars represent standard errors. Different letters in (**I**) indicate significant differences ($P<0.05$; *t*-test) between tillage practices (NT and MP).

Fig. 3-5: Pearson correlation between corn yields at maturity and average root length density over 40-cm profile at corn silking under MP and NT in 2014. Axis X: average root length density (cm cm^{-3}); Axis Y: corn yield (t ha^{-1}). Open circles represent mouldboard plough (MP) and filled triangles represent no-till (NT). Three data were not considered (one for MP and two for NT) because of the five outliers during data screening.

Fig. 3-6: Weed infestation on 4 July 2014 (32 days after sowing) in field-plot number 2 under NT block.

Chapter IV Soybean root traits after 24 years of different soil tillage and mineral phosphorus fertilization management

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***Running title*:** Soybean roots affected by soil tillage and P fertilization

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Soybean root traits after 24 years of different soil tillage and mineral phosphorus fertilization management

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ABSTRACT

Legume crops are widely used in conservation agricultural systems, which are associated with minimum soil tillage, due to their nitrogen-fixing capabilities. However, tillage and fertilization regimes may affect the vertical distribution of legume roots and root traits, hence nutrient and water uptake by altering soil properties in the long term. This study aimed to investigate how tillage and P fertilization affect soybean (*Glycine max*, L.) root distribution and morphology in a long-term experiment.

A 24-year rain-fed corn-soybean rotation was established in 1992 on a clay loam soil in L'Acadie, Quebec, Canada. The split-plot design (four replicates) comprised tillage systems [moldboard plough (MP) and no-till (NT)] as main plot factors and P fertilization [0 (0 P), 17.5 (0.5 P) and 35 (1 P) kg P ha^{-1} every two years during the corn (*Zea mays* L.) phase] as sub-plot factors. Soybean roots and shoots were sampled in 2015, after 24 years, at flowering stage. Root samples were taken by collecting 5.25-cm diameter cores to a depth of 60 cm at 5 cm, 15 cm and 25 cm perpendicular to crop row. Soil cores were cut into 0–5, 5–10, 10–20, 20–30, 30–40 and 40–60 cm layers. After washing and separating the soil and roots, root traits (biomass, length, surface and diameter, and the proportions of primary, secondary and tertiary roots) were quantified using the WinRHIZO software.

Tillage and P fertilization regimes showed no significant effect on soybean root traits. Roots under NT had a relatively higher root length density (RLD) of 1.95 cm cm^{-3} for a 60-cm soil profile compared to roots under MP (1.55 cm cm^{-3}); RLD was relatively smaller at the highest P rate (1.57 cm cm^{-3}) compared to the control and half rate treatment (1.82 and 1.86 cm cm^{-3}, respectively). However, the interaction between tillage and P fertilization significantly influenced the vertical distribution of soybean roots. Roots under NT primarily accumulated at 0–10 cm, containing 44% of the total root length (24% under MP); by contrast, 36% of root length under MP and 21% under NT accumulated at 10–20 cm. However, the difference in vertical root distribution between NT and MP was mitigated as P fertilization increased.

Soybean roots under NT showed higher RLD and greater root accumulation in the upper layers than MP possibly in response to nutrient availability and stratifications with higher nutrient contents in the top layers (0–10 cm) after 24 years of continuous NT practice.

Abbreviations: MP, Moldboard plough; NT, No-till; 0 P, 05 P and 1 P, P fertilization rates of 0 17.5 and 35 kg P ha^{-1} applied every two years on corn phase; RMD, Root mass density (cm cm^{-3}); RSD, Root surface density (cm^2 cm^{-3}); RLD, Root length density (cm cm^{-3}); RND, Root nodule density per root length (cm^{-1}); 3rdRLD, 2ndRLD 1stRLD root length densities of tertiary secondary and primary roots (cm cm^{-3}); 3rdRL%, 2ndRL% 1stRL% root length proportions of tertiary secondary and primary roots; R*Ilr1*, Isometric log ratio transformation of root length proportions (tertiary roots *vs.* secondary and primary roots); R*Ilr2*, Isometric log ratio transformation of root length proportions (secondary roots *vs.* primary roots); V*ilr1*, Isometric log ratio transformation of vertical root length proportions (root lengths at 0–20 cm *vs.* roots at 20–60 cm); V*ilr2*, Isometric log ratio transformation of vertical root length proportions (roots at 0–10 cm *vs.* roots at 10–20 cm); V*ilr3*, Isometric log ratio transformation of vertical root length proportions (roots at 0–5 cm *vs.* roots at 5–10 cm); V*ilr4*, Isometric log ratio transformation of vertical root length proportions (roots at 20–40 cm *vs.* roots at 40–60 cm); V*ilr5*, Isometric log ratio transformation of vertical root length proportions (roots at 20–30 cm *vs.* roots at 30–40 cm).

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4.1. Résumé

Les cultures de légumineuses, en raison de leur capacité de fixer de l'azote, sont largement utilisées dans les systèmes d'agriculture de conservation associés à un travail du sol minimum. Cependant, les modes de travail du sol et le régime de fertilisation peuvent affecter la distribution verticale et les caractéristiques des racines de légumineuses et donc l'absorption des éléments nutritifs et de l'eau en modifiant les propriétés du sol. Cette étude visait à analyser comment le travail du sol et la fertilisation en P affectent la distribution et la morphologie des racines du soja (*Glycine max*, L.) dans un essai de longue durée.

Une expérimentation de 24 ans en rotation maïs-soja a débuté en 1992 sur un sol argilo-limoneux situé à l'Acadie, Québec, Canada. Le dispositif en split-plot avec quatre répétitions comprenait deux régimes de travail du sol (no-till, NT; moldboard plough, MP) et trois doses de fertilisation en P [0 (0P), 17,5 (0,5 P) et 35 (1P) kg P ha^{-1} tous les deux ans au cours du maïs (*Zea mays* L.)] comme facteurs principal et secondaire, respectivement. Les échantillons des racines et des parties aériennes de soja ont été prélevés en 2015 au stade de la floraison. Les racines ont été échantillonnées avec des carottes de sol de 5,25 cm de diamètre à une profondeur de 60 cm sur trois distances (5 cm, 15 cm et 25 cm) perpendiculaires au rang. Les carottes de sol ont été découpées en couches 0-5, 5-10, 10-20, 20-30, 30-40 et 40-60 cm. Après lavage et séparation du sol et des racines, les caractéristiques des racines (biomasse, longueur, surface et diamètre, et les proportions de racines primaires, secondaires et tertiaires) ont été quantifiées en utilisant le logiciel WinRhizo.

Le travail du sol et la fertilisation phosphatée n'ont pas eu d'effet significatif sur les traits des racines de soja. La densité de longueur de racine (RLD) était plus élevée sous NT (1,95 cm cm^{-3}) par rapport à MP (1,55 cm cm^{-3}). Le RLD était plus faible pour la fertilisation en P la plus élevée chez 1P (1,57 cm cm^{-3}) par rapport au témoin 0P et 0,5P (1,82 et 1,86 cm cm^{-3}, respectivement). Cependant, l'interaction entre le labour et la fertilisation du P a influencé significativement la distribution verticale des racines de soja. Les racines sous NT s'accumulaient principalement dans la couche 0-10 cm avec 44% de la longueur totale des racines (24% dans MP). En revanche, 36% (en longueur totale) des racines sous MP et 21% sous NT s'accumulaient à 10-20 cm. La différence de distribution verticale des racines entre NT et MP était moindre avec une fertilisation en P plus élevée.

Au total les densités de longueur de racine de soja étaient plus élevées surtout dans les couches supérieures du traitement sans-labour (NT) par rapport au traitement avec labour. Cette modification de la répartition verticale des racines du soja coïncide avec la stratification verticale des éléments nutritifs après 24 ans de pratique du sans-labour.

Mots-clés: pratique sans labour, distribution verticale des racines, soja

4.2. Abstract

Legume crops are widely used in conservation agricultural systems, which are associated with minimum soil tillage, due to their nitrogen-fixing capabilities. However, tillage and fertilization regimes may affect the vertical distribution of legume roots and root traits, hence nutrient and water uptake by altering soil properties in the long term. This study aimed to investigate how tillage and P fertilization affect soybean (*Glycine max*, L.) root distribution and morphology in a long-term experiment.

A 24-year rain-fed corn-soybean rotation was established in 1992 on a clay loam soil in L'Acadie, Quebec, Canada. The split-plot design (four replicates) comprised tillage systems [moldboard plough (MP) and no-till (NT)] as main plot factors and P fertilization [0 (0P), 17.5 (0.5P) and 35 (1P) kg P ha^{-1} every two years during the corn (*Zea mays* L.) phase] as sub-plot factors. Soybean roots and shoots were sampled in 2015, after 24 years, at flowering stage. Root samples were taken by collecting 5.25-cm diameter cores to a depth of 60 cm at 5 cm, 15 cm and 25 cm perpendicular to crop row. Soil cores were cut into 0-5, 5-10, 10-20, 20-30, 30-40 and 40-60 cm layers. After washing and separating the soil and roots, root traits (biomass, length, surface and diameter, and the proportions of primary, secondary and tertiary roots) were quantified using the WinRHIZO software.

Tillage and P fertilization regimes showed no significant effect on soybean root traits. Roots under NT had a relatively higher root length density (RLD) of 1.95 cm cm^{-3} for a 60-cm soil profile compared to roots under MP (1.55 cm cm^{-3}); RLD was relatively smaller at the highest P rate (1.57 cm cm^{-3}) compared to the control and half rate treatment (1.82 and 1.86 cm cm^{-3}, respectively). However, the interaction between tillage and P fertilization significantly influenced the vertical distribution of soybean roots. Roots under NT primarily accumulated at 0-10 cm, containing 44% of the total root length (24% under MP); by contrast, 36% of root length under MP and 21% under NT accumulated at 10-20 cm. However, the difference in vertical root distribution between NT and MP was mitigated as P fertilization increased.

Soybean roots under NT showed higher RLD and greater root accumulation in the upper layers than MP possibly in response to nutrient availability and stratifications with higher nutrient contents in the top layers (0-10 cm) after 24 years of continuous NT practice.

Highlights:

1. Soybean root biomass tended to be greater under NT rather than under MP.
2. Soybean roots increased under NT but decreased under MP with increasing P rates.
3. Soybean roots accumulated at a 0-10 cm depth under NT and at a 10-20 cm depth under MP.
4. Increasing P rates mitigated the difference between NT and MP in the vertical distribution of roots.

***Key-words*:** no-till, vertical root distribution, legumes

Abbreviations

MP - Moldboard plough
NT - No-till
0P, 0.5P and 1P - P fertilization rates of 0, 17.5 and 35 kg P ha^{-1} applied every two years on corn phase
RMD - Root mass density (mg cm^{-3})
RSD - Root surface density (cm^2 cm^{-3})
RLD - Root length density (cm cm^{-3})

RND - Root nodule density per root length (cm^{-1})
3rdRLD, 2ndRLD, 1stRLD - Root length densities of tertiary, secondary and primary roots ($cm\ cm^{-3}$)
3rdRL%, 2ndRL%, 1stRL% - Root length proportions of tertiary, secondary and primary roots
RL*ilr1* - Isometric log ratio transformation of root length proportions (tertiary roots *vs.* secondary and primary roots)
RL*ilr2* - Isometric log ratio transformation of root length proportions (secondary roots *vs.* primary roots)
V*ilr1* - Isometric log ratio transformation of vertical root length proportions (root lengths at 0-20 cm *vs.* roots at 20-60 cm)
V*ilr2* - Isometric log ratio transformation of vertical root length proportions (roots at 0-10 cm *vs.* roots at 10-20 cm)
V*ilr3* - Isometric log ratio transformation of vertical root length proportions (roots at 0-5 cm *vs.* roots at 5-10 cm)
V*ilr4* - Isometric log ratio transformation of vertical root length proportions (roots at 20-40 cm *vs.* roots at 40-60 cm)
V*ilr5* - Isometric log ratio transformation of vertical root length proportions (roots at 20-30 cm *vs.* roots at 30-40 cm)

4.3. Introduction

Conservation agriculture, as defined by Kassam et al. (2012), has been widely adopted to reduce soil erosion, decrease input costs, and sustain long-term crop productivity (Soane et al. 2012; Pittelkow et al. 2015b). Legumes are typically implemented in conservation agricultural systems as row crops (Vanhie et al. 2015) or cover crops (Sainju et al. 2005) due to their roles in nitrogen fixation and weed control. Long-term conservation systems, especially no-till (NT) without soil inversion, result in the alteration of soil properties (Madejón et al. 2007) and the heterogonous distribution of relatively immobile nutrients (e.g., phosphorus (P)) compared to conventional tillage systems (Messiga et al., 2011). Consequently, root growth, crop yields and plant nutrition can be affected in legume crops (e.g., soybean) under NT.

Greater soil bulk density and resistance usually observed under NT (Gantzer and Blake 1978; Soane et al. 2012; Javeed et al. 2013; Guan et al. 2014); such soil properties can hinder root penetration and reduce root length density (Qin et al. 2005; Nunes et al. 2015). Lal et al. (1989) reported a 45% reduction in soybean root length density (RLD) under NT compared with plow-till in a calcareous soil; this was a result of the greater soil bulk density and penetration resistance caused by wheel traffic. It was also reported that soil compaction could affect soybean root branching and diameter (Cesar Ramos et al. 2010; de Assis Valadao et al. 2015; Colombi and Walter 2016). In contrast, Micucci and Taboada (2006) did not observe any differences in soybean RLD between conventional and NT systems. Therefore, there is a critical soil bulk density or resistance for successful soybean root growth (Keisuke Sato et al. 2015). No-till usually provides a stable soil environment for micro-organisms (Helgason et al. 2010; Scopel et al. 2013) that is favorable for nodulation and biological nitrogen fixation by soybean roots (Muchabi et al. 2014). Modified soil temperature, water availability and crop residues under NT could also influence soybean root growth and nutrient uptake (Farmaha et al. 2012; Vanhie et al. 2015).

The morphological traits of soybean roots are also related to soil P status. Fernandez and Rubio (2015) reported higher specific root length and smaller average root diameter in soybean where P-uptake efficiency increased under P deficit. Higher P concentrations in the topsoil under NT results in P stratification, with a higher P concentration in the topsoil (Lupwayi et al. 2006; Costa et al. 2010; Messiga et al. 2010; Calegari et al. 2013). Such stratification could stimulate the growth of soybean roots (Holanda et al., 1998). It was also reported that soybean roots respond to local P fertility, but only under low soil P test levels (Farmaha et al. 2012).

The effects of tillage and P fertilization on soybean root growth are generally studied separately. In this study, however, we evaluated the combined effects of tillage [moldboard plough (MP) vs. no-till (NT)] and mineral P fertilization (0, 17.5 and 35 kg P ha^{-1} applied every two years as triple-superphosphate) on soybean root distribution and morphology at a long-term (24 years) corn-soybean rotation field experimental site in Eastern Canada. We hypothesized that soybean root distribution and morphology would respond to P fertilization and the subsequent effects of tillage practices on the vertical distribution of nutrients.

4.4. Materials and Methods

4.4.1. Site description

The site was established in 1992 at the L'Acadie Experimental Farm (45°18' N; 73°21' W) of Agriculture and Agri-Food Canada. Details on this rain-fed field experiment are reported in Ziadi et al. (2014). Briefly, the soil is a deep clay loam soil (364 g kg^{-1} of clay and 204 g kg^{-1} of sand in the Ap horizon) and classified as Humic Orthic Gleysol, Typic Haplaquept. From 1992 to 1994, the site was planted with corn (*Zea mays* L.). The corn and soybean (*Glycine max* L.) rotation was initiated in 1995. The experimental set-up was a

split-split-plot replicated four times with two tillage practices [Moldboard Plough (MP) and No-Till (NT)] randomized into main plots and nine combinations of nitrogen (N) and P levels randomized into subplots including three N (0, 80, 160 kg N ha^{-1}) and three P (0, 17.5, and 35 kg P ha^{-1}) regimes, which were applied every two years during the corn phase. The three P rates were referred to as 0P, 0.5P and 1P, which corresponded to approximately 0, 0.5 and 1 time(s) the P exported every two years by harvest, respectively. For the purpose of this study, we only considered the optimal N level of 160 kg N ha^{-1}, the two tillage practices (MP and NT) and the three P fertilization rates. We therefore considered a total of 24 field-plots measuring 25-m in length by 4.5-m in width. The moldboard plough operation to a depth of 20 cm occurred in the fall after crops were harvested. Each spring, the soil was tilled by disking and harrowing to 10 cm before seeding. For the NT treatment, plots were ridge-tilled from 1992 to 1997 and then flat direct-seeded starting in 1998. For all treatments, crop residues were left on the soil surface after harvest. At planting, fertilizers were banded-applied (5 cm from the seeding row with a commercial seeder)). . Whereas, side-dress N was applied using a disk opener (3-4 cm deep; CRAAQ 2003) at approximately the 8-leaf stage of corn growth. The P fertilizers were applied as granules of commercial triple-superphosphate ($Ca(H_2PO_4)_2$, H_2O) during a single operation at seeding. Nitrogen at a rate of 160 kg ha^{-1} was applied first at seeding, as urea at 48 kg N ha^{-1}; this was followed by the addition of 112 kg N ha^{-1} side-dressed as ammonium nitrate. All plots received 50 kg K_2O ha^{-1}, band-applied at planting in 1992 and 2007; this was based on soil analyses and local recommendations (CRAAQ 2003). Herbicides were applied based on provincial recommendations. In soybean years, plots were sprayed with a tank mix of bentazon (0.72 kg ai/ha) and imazethapyr (0.074 kg ai/ha) and 2 L/ha ammonium sulfate (28–0–0) (Légère et al. 2008). Soybean (Pioneer 2510RY) was sown with 75-cm inter-row at a plant density of 45×10^4 plants ha^{-1}. Soybean seeds were inoculated with a commercial formulation of Bradyrhizobium japonicum (Hi Coat N/TS225, Becker Underwood, Saskatoon, SK, Canada). Due to unfavorable climatic conditions, soybeans were sown on June 26, 2015; this was relatively late compared to previous growing seasons (in which sowing occurred in early June).

4.4.2. Root sampling and analysis

Root samples were collected on August 19-20, 2015 (54 days after seeding, at approximately the end of the soybean vegetative stage). Five consecutive soybean plants were randomly selected from the 3rd or 4th row of each field-plot to avoid side effects. The root samples were taken using the soil core method (Bohm 2012) with Giddings soil coring sampler (5.25-cm inner diameter) (Giddings Machine Company, Inc.). In each field-plot, soil cores were sampled perpendicularly from one side of one chosen plant. Cores were taken up to a depth of 60 cm at three horizontal distances (5, 15 and 25 cm) perpendicular to the soybean row. Core samples were sliced as follows: 0-5, 5-10, 10-20, 20-30, 30-40 and 40-60 cm. A total of 432 root samples were collected. Root samples were placed in plastic bags and stored at 4 °C for a few days before soil-root separation was performed. Root samples were first soaked in a 1 M solution of NaCl (1 L per 5-cm soil core) for 16 h to disperse soil aggregates. Samples were then transferred to a hydro-pneumatic elutriation washing machine (Smucker et al., 1982). Cleaned roots were first collected on a 760 micron primary sieve and then on a 400 micron secondary sieve (Bolinder et al. 2002). The roots recovered on the secondary sieve were selected by hand to remove the remaining mineral particles and organic debris. Roots were then rinsed and placed on a transparent tray with distilled water, dispersed with tweezers and scanned in black and white (400 dpi, tagged image file format [TIF], white background) using a Imagery Scan Screen (EPSON Expression 10000XL) (Sheng et al. 2012). Root length, surface area and average diameter were automatically analyzed using the professional image analysis software “WinRhizo” (Regent Instruments Inc., Quebec). Root length was classified into three diameter classes (0-0.2 mm [tertiary roots], 0.2-0.8 mm

[secondary roots] and >0.8 mm [primary roots]). After scanning, the roots were dried with paper towels to determine the fresh weight. Root nodules were separated and counted. Then, roots were oven dried at 55 °C to a constant weight to determine the dry weight. Root length density (RLD), surface area density (RSD) and mass density (RMD) were calculated as root length, surface area and dry mass divided by soil core volume, respectively. Root nodule density (RND) was calculated by dividing the root nodule number by the root length. The root length proportions of tertiary, secondary and primary roots (3^{rd}RL%, 2^{nd}RL% and 1^{st}RL%, respectively) were calculated as the root lengths of each root order divided by total root length in each sample.

4.4.3. Soil sampling and analysis

Soils were sampled on August 19-20, 2014, using a hydraulic power sampler (5.25–cm inner diameter). Samples were air-dried, grinded manually, sieved through a 2-mm sieve and stored before analysis. Phosphorus (P-M3) and potassium (K-M3) were extracted by Mehlich-3 solution (Mehlich, 1984). 2.5 g of soil with 25 mL of Mehlich-3 extractant solution was shaken for 5 min. The P concentrations were determined based on Murphy Riley method (Murphy and Riley 1962) with a spectrophotometer (Jenway 6320D) (at 882 nm) with a 1–cm long optical cell. Potassium concentrations were determined with an Inductively Coupled Plasma Optical Emission Spectrometer (Optima 4300 DV, PerkinElmer Corp., Norwalk, CT). Total carbon (C) and N were quantified by dry combustion with a LECO CNS-1000 analyzer (LECO Corp., St. Joseph, MI).

4.4.4. Crop harvest

Crop-yield data were collected from 1992 to 2015. Crops were harvested at maturity, generally in September for soybean and grain yield was determined by harvesting plants over 10 m in two middle rows of each plot. Grain was threshed and weighed, and yields were adjusted to a moisture content of 14%. Grain P content was measured by mineralizing 0.1 g grain ground (0.2 mm) with a mixture of sulfuric and selenious acids, as described by Isaac and Johnson (1976). The P concentrations were measured with a QuikChem 8000 Lachat autoanalyzer (Zellweger Analytic Inc., Lachat Instruments Division, Milwaukee, WI) using the Lachat method 13-107-06-2-E (Ziadi et al. 2007; Instruments 2008).

4.4.5. Statistical analysis

All root data were first subjected to a screening to identify outliers. No outliers were found; however two samples went missing during transportation. The screened data were tested using the GLIMMIX procedure and transformed (if necessary) using the *transreg* procedure (SAS Institute Inc., 2010) to achieve normality of distribution and homogeneity of variance before conducting ANOVA. Root length proportions were treated as compositional data. To avoid biases, data were transformed into isometric log ratios (*ilr*) according to sequential binary repartitions (RLilr_1: tertiary roots|(secondary and primary roots); RLilr_2: secondary roots|primary roots) and the following equation (Eq. 4-1), which was described previously (Egozcue et al. 2003):

$$\boldsymbol{ilr_i = \sqrt{\frac{rs}{r+s}}\, ln \frac{(\prod_+ x_j)^{\frac{1}{r}}}{(\prod_- x_k)^{\frac{1}{s}}} \quad i = 1, 2, 3, \ldots, D-1; j + k = D} \qquad \text{Eq. 4-1}$$

where $\boldsymbol{D}$ is the number of all data parts in the $\boldsymbol{i}$-th order partition; $\boldsymbol{x_j}$ and $\boldsymbol{x_k}$ is the root lengths of positive and negative parts in the $\boldsymbol{i}$-th order position; the products $\boldsymbol{\Pi}_+$ and $\boldsymbol{\Pi}_-$ only include parts coded with **+** and **-**, and $\boldsymbol{r}$ and $\boldsymbol{s}$ are the numbers of the positive and negative signs (parts) in the $\boldsymbol{i}$-th order partition.

The vertical partitions of soybean root lengths were also analyzed by compositional data analysis with sequential binary partitions (Vilr_1: root lengths at 0-5, 5-10, and 10-20 cm|root lengths at 20-30, 30-40, and 40-60 cm; Vilr_2: root lengths at 0-5 and 5-10 cm|root lengths at 10-20 cm; Vilr_3: root lengths at 0-5 cm|root lengths at 5-10 cm; Vilr_4: root lengths at 20-30 and 30-40 cm|root lengths at 40-60 cm; Vilr_5: root lengths at 20-30 cm|root lengths at 30-40 cm). Canonical discrimination analysis was performed using the CANDISC procedure (SAS Institute Inc., 2010) and V*ilr*s root distribution values for the six depths. Six combined tillage and P fertilization treatments ([NT and 0P], [NT and 0.5P], [NT and 1P], [MP and 0P], [MP and 0.5P] and [MP and 1P]) were tested by discrimination analysis.

ANOVA was performed using the MIXED procedure (SAS Institute Inc., 2010) to evaluate the fixed effects of tillage, P fertilization, sampling depth and perpendicular distance to the soybean row and the interactions between these factors on root distribution and morphology and soil nutrient concentrations. The random effects were set as blocks and block×tillage interactions. In addition, the sampling depth and perpendicular distance to the soybean row were repeated-measures. Crop-yield data were subjected to ANOVA using the MIXED procedure to evaluate the effects of tillage practice, P fertilization, and year along with the interactions between these factors. Random effects were set as the block and its interaction with tillage. Treatment effects were deemed significant when $P<0.05$; differences among least square means for treatment pairs were identified using the LSMEANS (/diff) statement (*t*-test) (SAS Institute Inc., 2010).

4.5. Results and discussion

4.5.1. Soil nutrient stratification

Nutrient stratification refers to a distribution of nutrients that is non-uniform with soil depth and especially to situations with higher concentrations of nutrients near to the soil surface. Phosphorus stratification along the soil profile with high soil P concentrations in the upper layers was observed for the NT system (Fig. 4-1). Soil under NT generally had higher P-M3 values in the upper soil layers (0-20 cm) compared with soil under MP; several of these differences were significant; the differences in P-M3 between NT and MP treatments increased as P rates increased (Table 4-1, Fig. 4-1). In contrast, MP soil had significant higher P-M3 than NT soil at a depth of 20-30 cm. As P fertilization was banded applied, soil P was significantly influenced by sampling distances (Table 4-1) with greater P-M3 (20.43 mg kg^{-1}) at 5-cm distance compared to those at 15- and 25-cm distances (15.70 and 15.84 mg kg^{-1}).

Similar vertical stratifications under NT and MP treatments were also observed for soil C, N and K (Table 4-1, Fig. 4-2). Soil C, N and K contents were distributed homogenously throughout the upper 20 cm, the tilled layer, under MP; the soil was mixed completely during the MP treatment.

This stratification was due to soil perturbation and increases in the crop residues left on the soil surface under NT (Scopel et al. 2013). The low disturbance of upper soil layers in NT limits the dilution of applied fertilizers in deep soil layers. Crops enhance the nutrient stratification by bringing nutrients up from deeper layers and deposing it on the surface and the upper layers through their shoot and root residues, respectively. This plant functioning might explain the accumulation in upper layers and depletion in deeper layers of nutrients observed under NT.

4.5.2. Lateral and vertical root characteristics

Across treatments, soybean roots in the sampled zone had an average root length density of 1.75 cm cm^{-3} and an average root diameter of 0.24 mm. The results were within the reported ranges for RLD (0.7-6.25 cm cm^{-3}) and diameter (0.25-0.48 mm) (Egozcue et al. 2003; Myers et al. 2007; Farmaha et al. 2012; Fenta et al. 2014; da Costa and Crusciol 2016).

Root traits, such as root mass density (RMD), surface density (RSD) and length density (RLD), responded significantly and similarly to the sampling depth, the perpendicular distance from the row and the interaction between these two factors (Table 4-2). The RMD, RSD and RLD increased from the top 5-cm layer to the 5-10 cm layer, where the greatest values were observed; below the 5-10 cm layer, these values decreased as depth increased (Table 4-3, Fig. 4-3). Thus, soybean roots were most abundant in the 0-20 cm layers (66% of the total root biomass) and within a 5-cm distance from the row (58% of the total root biomass). In addition, the average root diameter significantly responded to soil depth and perpendicular distance from the row, however, the interaction between these two factors did not have a significant effect. Root diameters were larger vertically in the 0-20 cm layers than in the deeper layers; diameters were larger horizontally at 5-, 15- than at 25-cm distances (Tables 4-2 and 4-3, Fig. 4-3.4).

These findings suggested that the distribution of soybean roots followed a natural morphology as described by Farmaha et al. (2012), who indicated that soybean roots have a taproot system growing outward into the inter-row position and proliferate in surface layers rather than in subsurface layers.

Larger roots tend to develop near the soil surface and soybean shoots, and the tertiary root proportion increased with increasing depth and distance to the soybean shoot (Table 4-3). The RND responded significantly to only sampling depth (Table 4-2), with higher values in the 0-30 than the 30-60 cm layers. This is primarily because the rhizobium proliferates under aerobic conditions in the upper soil layer (Rupela et al. 1987).

4.5.3. Root quantity (biomass) affected by tillage and P fertilization

After 24 years of treatment, tillage and P fertilization showed no significant effect on average root traits such as RMD, RSD and RLD across the 60-cm profile (Table 4-2). However, NT treatment relatively increased RSD and RLD by +15% and +26%, respectively, compared to MP; 0P and 0.5P fertilization rates also relatively increased RMD (+22% and +22%, respectively), RSD (+25% and +25%, respectively) and RLD (+16% and +18%, respectively) compared to 1P (Table 4-3).

Tillage interacted with sampling depth to have a significant impact on RMD, RSD and RLD (Table 4-2). Compared to MP, RMD was greater at 0-10 cm and lower at 10-20 cm under NT (Fig. 4-4.3). For RSD (data not shown) and RLD, the interaction of tillage practice and sampling depth interacted significantly and similarly with the rates of P fertilization (Table 4-2). No-till increased RLD compared to MP under the three P fertilization treatments in the upper layers (0-10 cm) (Fig. 4-5). At 10-20 cm, RLD was significantly lower under NT than under MP for the 0P treatment but not the 0.5P and 1P treatments. This was primarily caused by an increase in RLD under MP with decreasing P fertilization rates (1P: 1.80 cm cm^{-3}; 0.5P: 2.86 cm cm^{-3}; 0P: 3.48 cm cm^{-3}). These findings were in agreement with those of Fernandez and Rubio (2015) and Ramaekers et al. (2010), who found that the allocation of soybean biomass to the root system increased with decreasing soil P availability. Other studies (Cassman et al. 1980; Drevon and Hartwig 1997) showed conflicting results; soybean roots decreased under P deficit, as root nodulation was inhibited under P deficit. However, we did not observe significant tillage or P fertilization effects on RND (Table 4-2).

Over the entire 60-cm soil profile, soybean roots under MP were similar to the pattern observed at 10-20 cm (1P: 1.14 cm cm^{-3}; 0.5P: 1.72 cm cm^{-3}; 0P: 1.82 cm cm^{-3}). However,

soybean roots under NT tended to increase with increasing P fertilization over the entire soil profile (1P: 2.05 cm cm^{-3}; 0.5P: 2.00 cm cm^{-3}; 0P: 1.81 cm cm^{-3}). This might be caused by soil P stratification, with high soil P status (P-M3) in upper soil layers under NT (Fig. 4-1). Such a soil P stratification could stimulate soybean root growth (Holanda et al. 1998), especially in the early stage of growth (Zia et al. 1988). However, why soybean roots responded to P fertilization differently under NT and MP could not be fully explained in this study.

4.5.4. Vertical root length proportion affected by tillage and P fertilization

According to the RLD results above, tillage and P fertilization affected root length proportions along the soil profile. The results of the canonical discriminant analysis for vertical root length proportions are shown in Fig. 4-6. Along the axis of **Can1**, which explained 65% of the total variance, treatment [NT, 0P] was significantly separated from treatments [MP, 0P] (P=0.0067), [MP, 0.5P] (P=0.0227) and [MP, 1P] (P=0.0191) (Fig. 4-6.1). Treatments [NT, 0.5P] and [NT, 1P] were intermediated. The balance between the root lengths at 0-10 cm and 10-20 cm (**V*ilr*2**) contributed most to the treatment separation (Fig. 4-6.2). Root lengths at 0-10 and 10-20 cm layers under [NT and 0P] accounted for 52% and 18%, respectively, of the total root length over the entire soil profile (Fig. 4-6.3). In contrast, root lengths in the 0-10 and 10-20 cm layers accounted for 27-29% and 33-41%, respectively, of the total length under MP.

One cause of the differential distribution of soybean roots could be nutrient stratifications along the soil profile under NT and MP. As crop roots tend to accumulated in nutrient-rich zones in the soil (Li et al., 2012), increases in soil nutrients (C, N, P, K) in the top layers under NT could lead to a greater proportion of roots in the 0-10 cm layer. Compared with the deep-banded P fertilization observed under NT, Farmaha et al. (2012) reported a greater proportion of soybean roots in the 0-5 cm layer under broad P fertilization treatment. Farmaha et al. (2012) also observed that the response of soybean roots to localized P resources might only occur under low soil P levels; these findings suggest that the disparities in soybean root length proportions along the soil profile between NT and MP tend to be mitigated by increasing rates of P fertilization. In our study, the treatments [NT, 0.5P] and [NT, 1P] could not be significantly distinguished from the MP treatments (Fig. 4-6.1).

Briefly, tillage had significant effects on soybean root proportions along the soil profile. Soybean roots tended to accumulate in the 0-10 cm layer under NT, and in the 10-20 cm layer under MP. Soil bulk density was commonly considered to be a major factor influencing root distribution in the soil. Because of traffic pressure and a lack of tillage, higher soil bulk density and higher soil resistance tend to be observed in NT systems; this could impair root proliferation in the deeper layers (Lal et al. 1989; Chassot et al. 2001; Qin et al. 2006; Cai et al. 2014; Guan et al. 2014; Nunes et al. 2015). However, soil bulk density at the experimental site was found to be lower under NT compared to MP in 0-10 cm soil layer in 2010, as reported by Sheng et al. (2012). Results obtained from measurements performed in 2014 from this experimental site revealed that NT resulted in a greater bulk density compared to MP only in the 20-40 cm layer (Li et al. 2016), where fewer soybean roots accumulated compared to the upper layer. Hence, soil bulk density did not influence the distribution of soybean roots in this study.

In addition, as the absence of soil disturbance under NT, the previous root channels could be preserved, resulting in more biological macro-porosity over the long-term (Peigne et al. 2013). This was reported by Messiga et al. (2011) at the experimental site. Such a net of macro-pores could facilitate root proliferation in soil (Calonego and Rosolem 2013). Our study conducted in 2014 demonstrated a biomass accumulation of corn roots in the upper layers, with 38-49 % of the total root length in the 0-10 cm layer in both NT and MP (Li et al. 2016). However, the high proportions of macro-pores formed by previous corn roots in the

0-10 cm layer was preserved only under NT, possibly contributing to a greater accumulation of soybean roots in the 0-10 cm layer. It was partially confirmed by the previous study of Messiga et al. (2011) on the same site, who observed higher macro aggregation (2000-250 μm) content in NT (60.2% of whole soil weight) than that in MP (48.5% of whole soil weight, which would lead to more macro-pores in NT. Besides, some studies (Francis and Fraser, 1998; Lachnicht et al., 1997) also mentioned that earthworms also contributed to create more macro-pores in soil. Since the activity of earthworms tended to be intensified in relatively stable soil environment in NT (Helgason et al., 2010), it could be another reason why soybean roots in NT accumulated primarily at 0-10 cm depth. In contrast, Chassot et al. (2001) and Costa et al. (2010) mentioned that more vertical macro-pores in NT could also encourage roots to proliferate into deeper layers. Thus, more studies are needed to clarify the relation between macro-pore and crop root distribution in NT system.

4.5.5. Root morphology affected by tillage and P fertilization

In addition to root quantity and distribution, root morphologic traits such as average root diameter and fine root proportion, are important for crop growth. Fine roots are considered more efficient in nutrient uptake (Zobel et al. 2007; Bakker et al. 2009).

Tillage and sampling depth interacted significantly on the average root diameter and the root length proportions of tertiary, secondary and primary roots (1^{st}RL%, 2^{nd}RL% and 3^{rd}RL%) (Table 4-2). Variations in the distribution of tertiary, secondary and primary root lengths (1^{st}RLD, 2^{nd}RLD and 3^{rd}RLD) were similar to those of total RLD (Table 4-3).

Compared to MP, the average root diameter under NT was significantly larger in the 0-5 cm layer and significantly smaller in the 20-30, 30-40 and 40-60 cm layers (Fig. 4-4.4); similar variations were observed for 1^{st}RL%, 2^{nd}RL% and 3^{rd}RL%. The interaction between tillage and sampling depth had a significant effect on root length proportion balances (RL*ilr*1 and RL*ilr*2), with significant differences observed between NT and MP in the 0-5, 10-20 and 20-30 cm layers (Table 4-4). Correspondingly, these results indicated that 3^{rd}RL% was significantly smaller under NT (54.4%) compared with MP (63.3%) in the 0-5 cm layer, resulting in a larger average root diameter. Smaller average root diameters were observed in the 30-40 and 40-60 cm layers under NT, which corresponded with a higher 3^{rd}RL% (Table 4-3).

The proportion of soybean roots along the soil profile might explain the differences in root morphology observed between NT and MP treatments. As soybean roots primarily accumulated in the 0-10 cm layer under NT, more primary and secondary roots were located in the upper soil layers; this results in a smaller proportion of tertiary roots and, consequently, a larger average root diameter. However, tillage and P fertilization had no effect on the root length proportions of tertiary, secondary and primary roots or the average root diameter across the entire soil profile (Table 4-3). Soybeans may prefer to modify the root biomass rather than root morphology in response to variations of soil P status (Fernandez and Rubio 2015).

4.5.6. Soybean yields

Soybean yields obtained in 2015 were much smaller than those in other years over the 24 years of experimentation. This was probably due to delayed seedling, as delayed seeding emergence increases exposure to diseases, the likelihood of soil crusting, and crop injury (Vanhie et al. 2015).

Phosphorus fertilization had no significant effect on soybean yields; however, average grain P contents increased under 0.5P and 1P (Table 4-6). These findings suggest that, even after 24 years of experimentation, the soil P status (P-M3: 9.8-27.9 mg L^{-1} across 40-cm profile) was not the limiting factor for soybean growth. The greater grain P contents observed

under 0.5P and 1P indicated that increased P fertilization could improve soybean quality traits such as protein content (Khaswa et al. 2014).

Only minor differences in soybean yields were observed between NT and MP in 2015 (Table 4-6). This indicates that different patterns of soybean root distribution under these two tillage systems were equally efficient in nutrient uptake. Soybean roots under NT tended to accumulate in upper layers. Indeed, the shallow root system was considered to be efficient for nutrient acquisition in the heterogeneous nutrient stratification observed under NT (Ge et al. 2000). However, this shallow root distribution might be problematic when water availability is low at the surface layer (Farmaha et al. 2012). This could be particularly challenging in arid or semi-arid regions, where the surface soil layers tend to be dry (Richards and Caldwell 1987).

Compared with MP, NT significantly reduced soybean yields by an average of -14% over the long-term (Tables 4-5 and 4-6). Similar observations were reported by Kapusta (1979) and Lal et al. (1989), while Hussain et al. (1999) found NT improved long-term productivity for soybean compared with MP. In addition to root development, weed competition (Bhowmik and Doll 1982; Soane et al. 2012) and increased N immobilization due to lower rates of crop residue mineralization and nitrification (Doran 1980) could reduce soybean yields under NT.

4.6. Conclusion

An increase in soybean root biomass up to 60-cm depth was observed under NT compared with MP. Under MP, root biomass decreased with increasing P fertilization, while the reverse was observed under NT. Tillage significantly affected the distribution of soybean roots along the soil profile. Indeed, roots accumulated in the 0-10 cm layer under NT and in the 10-20 cm layer under MP. These differences in root distribution were probably caused by the adaption of soybean roots to the different stratifications of soil nutrients including P, N, K and C under NT. Different root distribution patterns seemed to be equally efficient in nutrient uptake under both tillage systems, as there were no significant differences in soybean yields between NT and MP in 2015. However, other factors such as weeds and low residue degradation rates could reduce soybean yield under NT over a long-term.

4.7. Acknowledgements

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Table 4-1 Effects of tillage and P fertilization and interactions with depth and distance soil on Mehlich3-P (P-M3), Mehlich3-K (K-M3), C and N contents (%).

Treatment	P-M3	K-M3	C	N
Tillage	NS	NS	NS	NS
P	***	NS	NS	NS
Tillage × P	NS	NS	NS	NS
Depth	***	***	***	***
Tillage × Depth	***	***	***	***
P × Depth	***	NS	NS	NS
Tillage × P × Depth	NS	NS	NS	NS
Distance	***	NS	NS	NS
Tillage × Distance	NS	NS	NS	NS
P × Distance	NS	NS	NS	NS
Tillage × P × Distance	NS	NS	NS	NS
Depth × Distance	**	NS	NS	**
Tillage × Depth × Distance	NS	NS	NS	NS
P × Depth × Distance	NS	NS	NS	NS
Tillage × P × Depth × Distance	NS	NS	NS	NS

NS not significant ($P \geq 0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 4-2 Effects of tillage and P fertilization and interactions with depth and distance on root mass density (RMD), root surface density (RSD), root length density (RLD), average root diameter, tertiary (diameter<0.2 mm), secondary (0.2 mm<diameter<0.8 mm) and primary (diameter>0.8 mm) root length densities (3^{rd}RLD, 2^{nd}RLD, 1^{st}RLD) and proportion balances (RL*ilr*1 and RL*ilr*2) and root nodule density (RND, nodules per root length).

Treatment	RMD	RSD	RLD	Diameter	3^{rd}RLD	2^{nd}RLD	1^{st}RLD	RL*ilr*1	RL*ilr*2	RND
Tillage	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tillage × P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Depth	***	***	***	***	***	***	***	***	**	**
Tillage × Depth	***	***	***	*	***	***	***	*	NS	NS
P × Depth	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tillage × P × Depth	NS	*	*	NS	*	*	*	NS	NS	NS
Distance	***	***	***	**	***	***	***	***	NS	NS
Tillage × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tillage × P × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Depth × Distance	***	***	***	NS	***	***	***	*	*	NS
Tillage × Depth × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P × Depth × Distance	*	NS	NS	*	NS	**	NS	NS	NS	NS
Tillage × P × Depth × Distance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS not significant (P≥0.05); * P<0.05; ** P<0.01; *** P<0.001

Table 4-3 Root mass density (RMD), root surface density (RSD), root length density (RLD), average root diameter, tertiary (diameter<0.2 mm), secondary (0.2 mm<diameter<0.8 mm) and primary (diameter>0.8 mm) root length densities (3^{rd}RLD, 2^{nd}RLD, 1^{st}RLD), length proportion balances (RL*ilr*1 and RL*ilr*2) and length proportions (3^{rd}RL%, 2^{nd}RL%, 1^{st}RL%) and root nodule density (RND, nodules per root length) of different sampling depth, distance to soybean row, tillage practice (MP, moldboard plow; NT, no-till) and P fertilization (0P, 0.5P and 1P).

Treatment	RMD mg cm^{-3}	RSD cm^2 cm^{-3}	RLD cm cm^{-3}	Diameter mm	3^{rd}RLD cm cm^{-3}	2^{nd}RLD cm cm^{-3}	1^{st}RLD*100 cm cm^{-3}	RL*ilr*1	RL*ilr*2	3^{rd}RL%	2^{nd}RL%	1^{st}RL%	RND*100 cm^{-1}
Depth (cm)													
0-5	0.15b	0.19b	2.33b	0.25a	1.32b	0.99b	1.73b	2.81a	4.13a	59%	40%	1%	0.34a
5-10	0.23a	0.29a	3.63a	0.25a	2.14a	1.46a	3.16a	2.48b	3.81a	57%	42%	1%	0.40a
10-20	0.15b	0.19b	2.30b	0.26a	1.34b	0.94b	2.06ab	2.18c	3.32b	56%	42%	1%	0.33a
20-30	0.04c	0.08c	0.99c	0.24b	0.60c	0.39c	0.65c	2.96a	4.39a	61%	38%	1%	0.24ab
30-40	0.03c	0.06d	0.85c	0.22c	0.58c	0.27d	0.51c	3.37a	4.51a	70%	30%	0%	0.09b
40-60	0.01d	0.03e	0.44d	0.23b	0.30d	0.14e	0.39c	2.57b	3.23b	68%	31%	1%	0.02b
Distance (cm)													
5	0.18a	0.23a	2.77a	0.25a	1.64a	1.11a	2.59a	2.41b	3.47b	60%	39%	1%	0.23
15	0.08b	0.12b	1.55b	0.24a	0.89b	0.64b	1.00b	2.64b	3.91a	60%	39%	1%	0.27
25	0.05c	0.07c	0.92c	0.23b	0.59c	0.33c	0.64c	3.14a	4.31a	66%	33%	1%	0.20
Tillage													
MP	0.10	0.13	1.55	0.25	0.91	0.63	1.28	2.63	3.81	61%	39%	1%	0.24
NT	0.10	0.15	1.95	0.24	1.17	0.76	1.54	2.83	3.99	63%	36%	1%	0.24
P fertilization													
0P	0.11	0.15	1.82	0.24	1.09	0.72	1.38	2.62	3.74	62%	38%	1%	0.23
0.5P	0.11	0.15	1.86	0.24	1.08	0.76	1.59	2.74	4.00	61%	38%	1%	0.27
1P	0.09	0.12	1.57	0.24	0.96	0.60	1.26	2.82	3.96	63%	36%	1%	0.20

Different letters in columns indicate significant differences ($P < 0.05$; *t*-test)
The data of root length proportions (3^{rd}RL%, 2^{nd}RL% and 1^{st}RL%) should not be directly subjected to statistical comparison.

Table 4-4 Tertiary (diameter<0.2 mm), secondary (0.2 mm<diameter<0.8 mm) and primary (diameter>0.8 mm) root length proportion balances (RL*ilr*1 and RL*ilr*2) and root length proportions (3^{rd}RL%, 2^{nd}RL% and 1^{st}RL%) for different sampling depth under two tillage practices (MP, moldboard plow; NT, no-till).

Depth	**RL*ilr*1**		**RL*ilr*2**		**3^{rd}RL%**		**2^{nd}RL%**		**1^{st}RL%**	
(cm)	**MP**	**NT**	**MP**	**NT**	**MP**	**NT**	**MP**	**NT**	**MP**	**NT**
0-5	3.26a	2.36b	4.59	3.67	63.6%	54.4%	35.6%	43.8%	0.8%	1.9%
5-10	2.62	2.34	4.11	3.52	56.1%	58.0%	43.4%	41.2%	0.6%	0.7%
10-20	2.00	2.36	2.98b	3.66a	56.6%	56.8%	42.4%	42.5%	1.0%	0.7%
20-30	2.50b	3.42a	3.89	4.89	56.8%	65.7%	42.6%	33.9%	0.7%	0.4%
30-40	3.08	3.67	4.25	4.79	66.7%	73.5%	32.8%	26.1%	0.5%	0.4%
40-60	2.28	2.85	3.05	3.41	64.0%	71.9%	34.7%	27.2%	1.2%	0.8%

Different letters for each variable indicate significant differences ($P < 0.05$; *t*-test) between tillage practices.
The data of root length proportions (3^{rd}RL%, 2^{nd}RL% and 1^{st}RL%) should not be directly subjected to statistical comparison.

Table 4-5 Effects of tillage and P fertilization and interactions with year on soybean yield and grain P content at maturity.

Treatment	yield	grain P content
Tillage	*	NS
P	NS	***
Tillage × P	NS	NS
Year	***	***
Tillage × Year	***	**
P × Year	**	NS
Tillage × P × Year	NS	NS

NS not significant ($P \geq 0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 4-6 Annual soybean yields and soybean grain P contents as affected by tillage (MP, moldboard plow; NT, no-till) and P fertilization (0P, 0.5P, 1P) from 1995 to 2015. The data of soybean grain P were recorded since the year of 2003. Different letters in a row indicate significant differences (P<0.05; t-test) between tillage practices (NT and MP) or P fertilizations (0P, 0.5P and 1P).

	Soybean yields					Soybean grain P contents				
Year	**MP**	**NT**	**0P**	**0.5P**	**1P**	**MP**	**NT**	**0P**	**0.5P**	**1P**
			t ha^{-1}					%		
1995	3.71a	3.32b	3.47	3.54	3.54	-	-	-	-	-
1997	3.24a	2.20b	2.86	2.59	2.70	-	-	-	-	-
1999	2.79	2.91	2.76	2.89	2.91	-	-	-	-	-
2001	1.07	1.11	1.02	1.03	1.22	-	-	-	-	-
2003	3.19a	2.57b	2.89	2.86	2.89	0.59	0.59	0.57	0.59	0.60
2005	2.97	2.78	2.86	2.98	2.79	0.56	0.56	0.55	0.56	0.57
2007	2.00a	1.20b	1.71a	1.73a	1.36b	0.51	0.53	0.50	0.52	0.53
2009	4.76a	4.34b	4.54b	4.04c	5.06a	0.55	0.54	0.54	0.56	0.57
2011	1.76	1.73	1.62	1.80	1.80	0.54b	0.58a	0.55	0.56	0.56
2013	2.67a	1.87b	2.21	2.32	2.28	0.54b	0.57a	0.55	0.54	0.56
2015	1.53	1.57	1.44	1.56	1.64	0.55	0.56	0.54	0.57	0.55
Mean	2.70a	2.33b	2.49	2.49	2.56	0.55	0.56	0.54b	0.56a	0.56a

Different letters for soybean yields indicate significant differences (P <0.05; *t*-test) between tillage practices or P fertilizations in a certain year.

Fig. 4-1

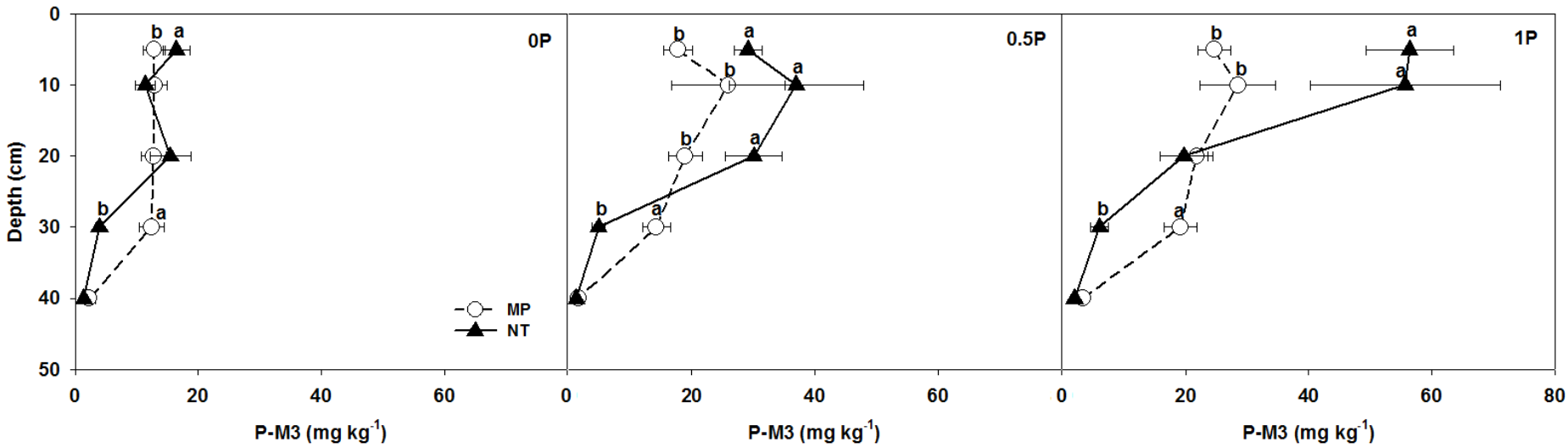

Fig. 4-2

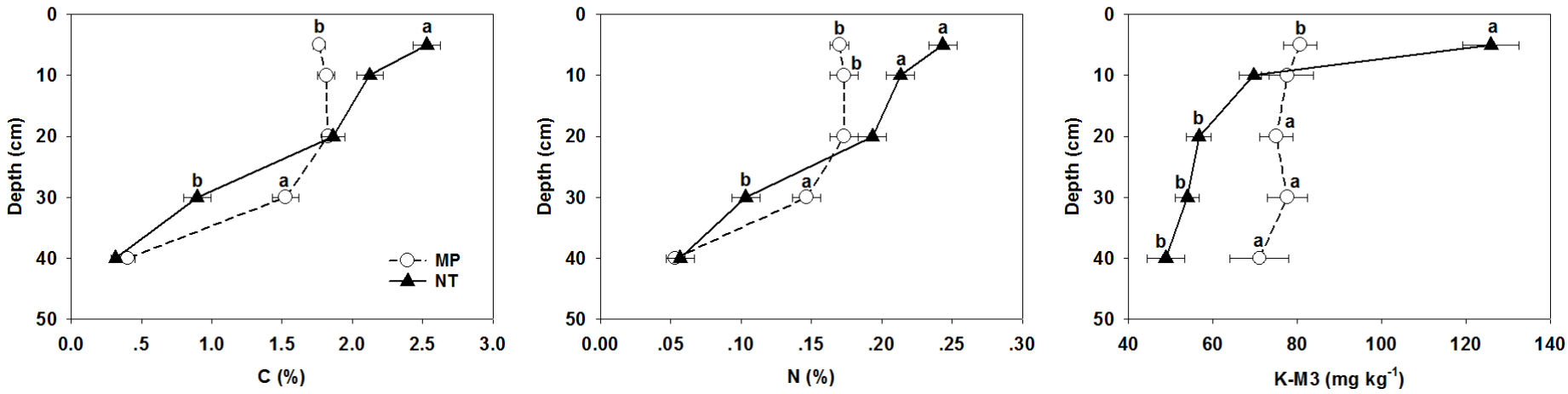

Fig. 4-3

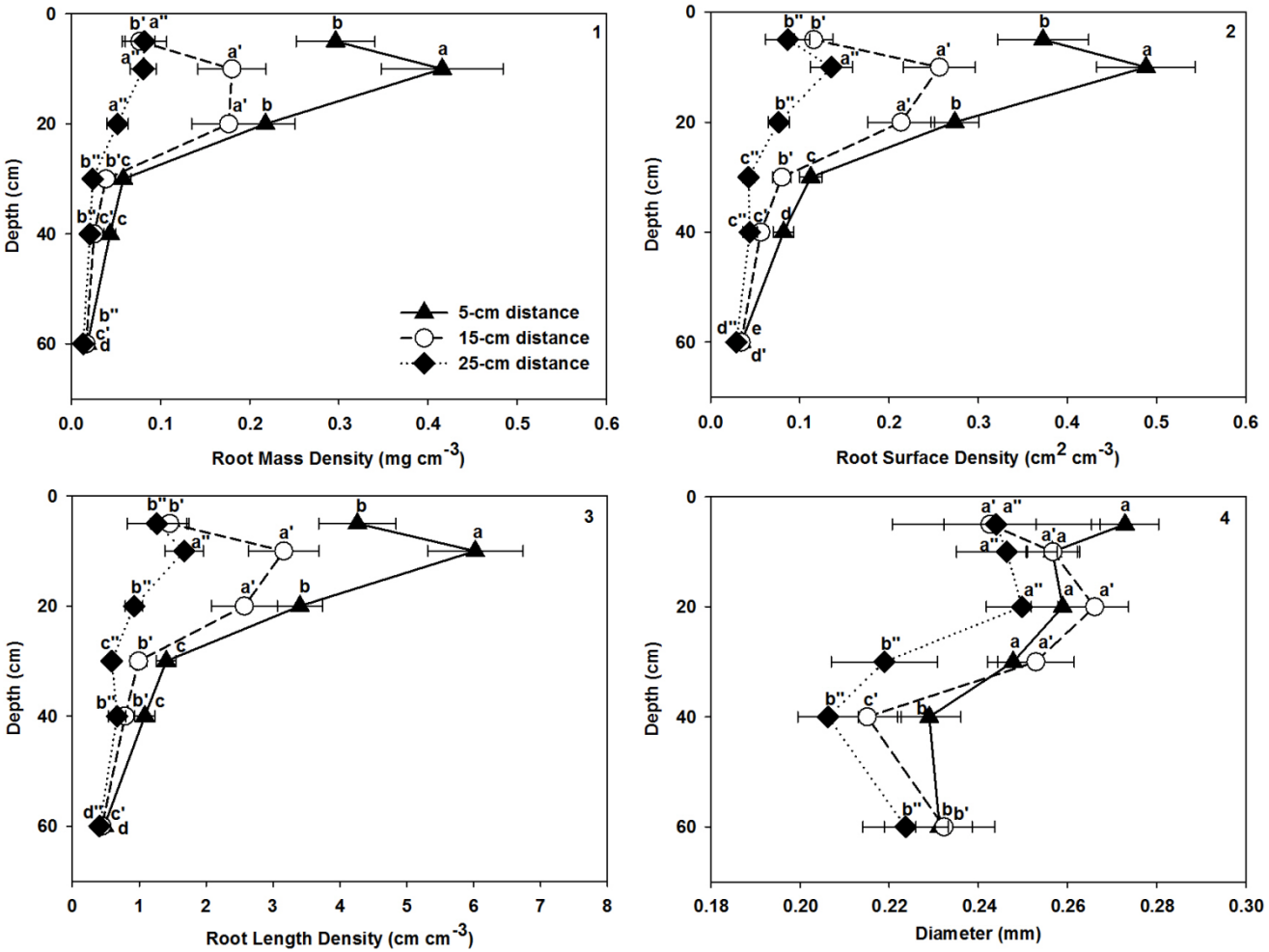
1
2
3
4
Depth (cm)
Root Mass Density (mg cm-3)
Root Surface Density (cm2 cm-3)
Root Length Density (cm cm-3)
Diameter (mm)
5-cm distance
15-cm distance
25-cm distance

Fig. 4-4

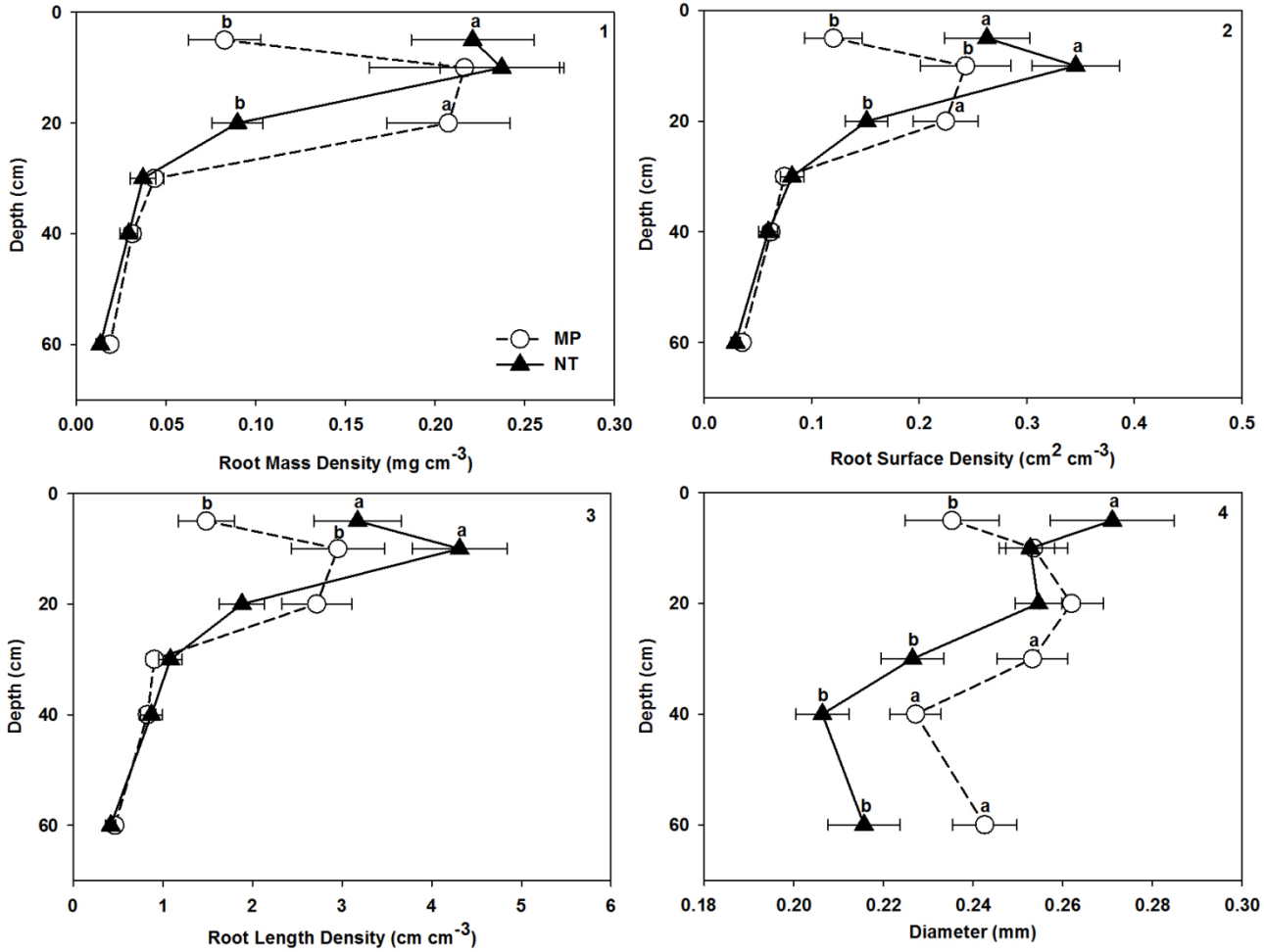
1
2
3
4
MP
NT
Depth (cm)
Root Mass Density (mg cm-3)
Root Surface Density (cm2 cm-3)
Root Length Density (cm cm-3)
Diameter (mm)

Fig. 4-5

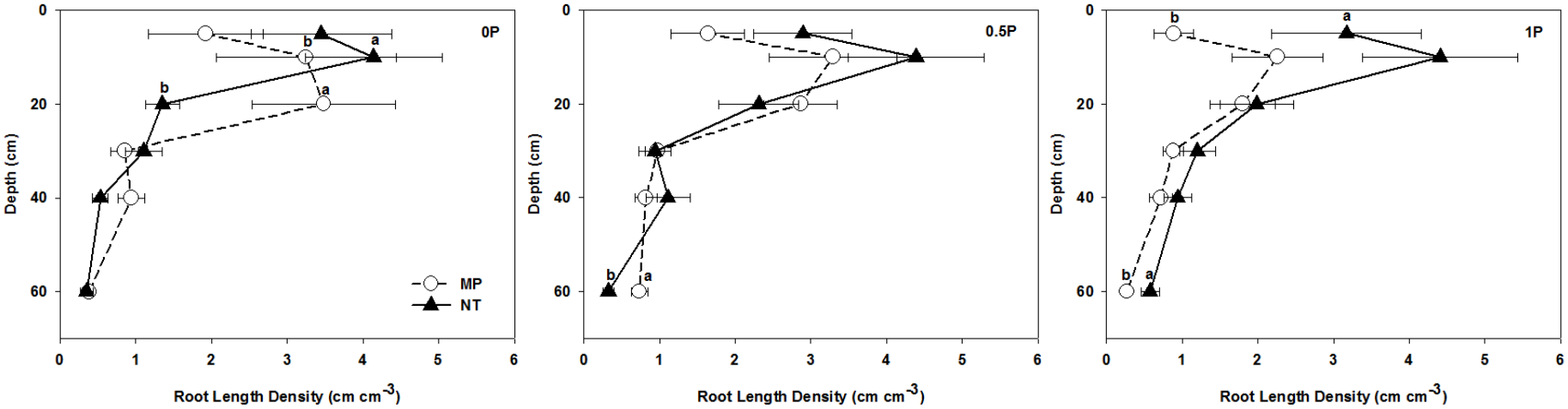

Fig. 4-6

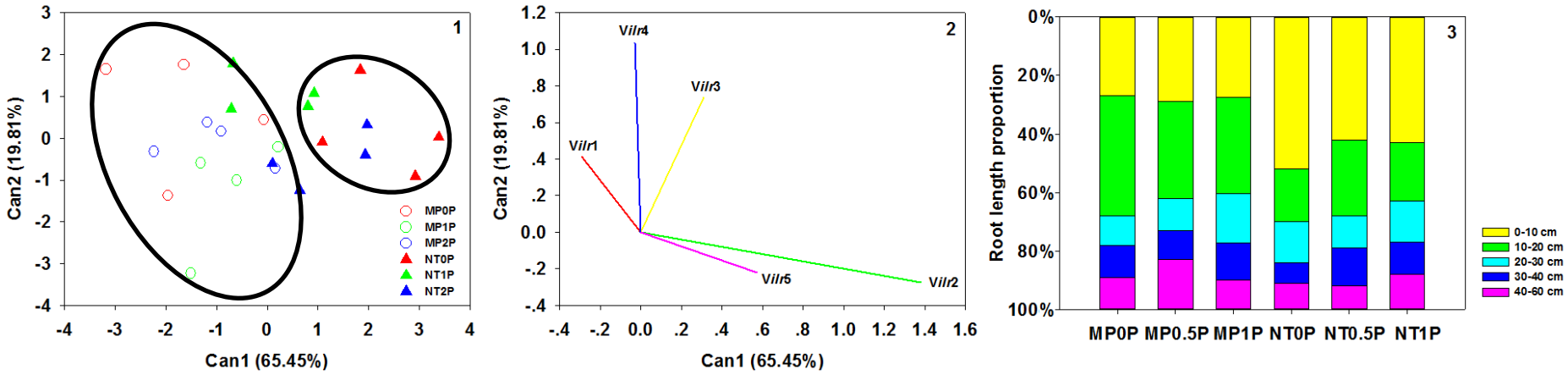
1
Can2 (19.81%)
Can1 (65.45%)
MP0P
MP1P
MP2P
NT0P
NT1P
NT2P
2
Vilr4
Vilr3
Vilr1
Vilr5
Vilr2
Can2 (19.81%)
Can1 (65.45%)
3
Root length proportion
MP0P MP0.5P MP1P NT0P NT0.5P NT1P
0-10 cm
10-20 cm
20-30 cm
30-40 cm
40-60 cm

Fig. captions

Fig. 4-1: Soil P-Mehlich3 (P-M3) values as affected by the interaction of tillage (NT and MP), P fertilization (0P, 0.5P and 1P) and sampling depth. Bars represent standard errors. Different letters indicate significant differences (P<0.05; t-test) between tillage practices (NT and MP).

Fig. 4-2: Soil C contents (**1**), N contents (**2**) and K-Mehlich3 (K-M3) values (**3**) as affected by the interaction of tillage (NT and MP) and sampling depth. Bars represent standard errors. Different letters indicate significant differences (P<0.05; t-test) between tillage practices (NT and MP).

Fig. 4-3: Root mass density (RMD) (**1**), root surface density (RSD) (**2**), root length density (RLD) (**3**) and average root diameter (**4**) under different sampling depths and perpendicular distances to the corn row. Bars represent standard errors. Different letters indicate significant differences (P<0.05; t-test) among depths (0□5, 5□10, 10□20, 20□30 and 30□40 cm) for each perpendicular distance (5-cm, 15-cm and 25-cm).

Fig. 4-4: Root mass density (RMD) (**1**), root surface density (RSD) (**2**), root length density (RLD) (**3**) and average root diameter (**4**) as affected by the interaction of tillage (NT and MP) and sampling depth. Bars represent standard errors. Different letters indicate significant differences (P<0.05; t-test) between tillage practices (NT and MP).

Fig. 4-5: Root surface density (RSD) (**1**) and root length density (RLD) (**2**) as affected by the interaction of tillage (NT and MP), P fertilization (0P, 0.5P and 1P) and sampling depth. Bars represent standard errors. Different letters indicate significant differences (P<0.05; t-test) between tillage practices (NT and MP).

Fig. 4-6: (**1**) Scatterplot for six combined treatments ([MP and 0P], [MP and 0.5P], [MP and 1P], [NT and 0P], [NT and 0.5P] and [NT and 1P]) and (**2**) bi-plot for five vertical root length distribution variables (Vilr1, Vilr2, Vilr3, Vilr4 and Vilr5) of canonical discriminant analysis based on Mahalanobis distance for vertical distribution under combined tillage and P fertilization treatments. The analysis explained 85% of the variance (Axis of Can1 and Can2), suggesting that combined treatments can be discriminated by vertical root length distribution. (**3**) Vertical root length proportions in percentage of each sampled depth for six combined treatments.

Chapter V Validation of an operational phosphorus cycling model CycP for a long-term ploughed and soybean-corn cropped agroecosystem in eastern Canada

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Running title: Validation of a P cycling modeling to a cropped field

Article in preparation

5.1. Résumé

La modélisation du cycle du phosphore (P) est un enjeu crucial pour la gestion de la fertilité du sol et de la durabilité des agroécosystèmes. Récemment, Messiga et al. (2015) ont construit un modèle dynamique, ci-après dénommé CycP, qui décrit le dynamique du P au cours de plusieurs décennies en relation avec les types de sol et la fertilisation phosphatée. La variation annuelle du P disponible dans la couche labourée est influencée par le bilan annuel de P entre les entrées et les sorties de P. CycP suppose que i) des réactions lentes des ions orthophosphates (Pi) telles que la diffusion entre les phases solide et liquide des sols contrôlent la concentration Pi en solution du sol (Cp) et ii) le sous-sol sous la couche labourée ne contribue pas de manière significative au prélèvement du P. L'objectif de cette étude était d'analyser les performances de CycP dans un écosystème canadien. Cette étape de validation requiert une base de données agronomiques (données climatiques, dosage du P, rendements en grains et teneur des grains en P) et une évaluation du P disponible aux plantes dans une expérience de longue durée. Les principales caractéristiques de l'agroécosystème à l'étude sont : un sol argilo-limoneux (Gleysol humique orthique) de l'est du Canada (climat continental humide froid) sous une rotation maïs-soja recevant trois doses de P (0, 17,5 et 35 kg P ha^{-1}) appliquées uniquement sur le maïs depuis 1992. Les couches labourées ont été échantillonnées en 2009 et en 2014. Les sols ont été analysés pour le Cp et le Pi diffusif (Pr) qui réapprovisionne Pi en solution suivant une sorption de 40 h avec dilution isotopique. Les valeurs expérimentales correspondent à l'équation: $Pr = 19.90\ CP^{0.42}\ t^{0.30}$ ($R^2 = 0,96$). La validation a été effectuée pour Cp qui est la variable d'intérêt agronomique pour gérer le dosage du P. Les valeurs simulées de Cp concordaient avec celles mesurées après 19 et 24 ans d'expérimentation. En outre, différents scénarios de fertilisation P ont été réalisés pour évaluer leur impact sur Cp. En conclusion, le modèle CycP pour la couche labourée était approprié pour cet agroécosystème canadien en ignorant la contribution du sous-sol.

Mots-clés: phosphore disponible, sol, agroécosystème, modélisation, fertilisation, dilution isotopique, sorption-désorption, rotation soja-maïs, bilan du phosphore

5.2. Abstract

Modeling of the phosphorus (P) cycle is a crucial issue for managing soil fertility and agroecosystem sustainability. Messiga et al. (2015) built a P model hereafter called CycP that describes soil P dynamics over decades in relation with soil types and P fertilization. The annual change in plant-available P in the ploughed layer was balanced by the annual P budget for P inputs and outputs. The CycP assumed that i) the slow diffusion of orthophosphate ions (Pi) between soil solid and liquid phases controls Pi concentration in the soil solution (*C*p) and ii) subsoil below ploughed layer does not contribute significantly to P nutrition. The objective of this study was to analyze the performance of CycP in a Canadian agroecosystem for a longer period, including P subsoil contribution. This validation step requires an agronomic database (climatic data, P fertilization, grain yields and P contents), an assessment of soil P availability, and a long-term P fertilization experiment. The main agroecosystem features in this study are a clay-loam soil (Orthic Humic Gleysol) in eastern Canada (cold humid continental climate) under a corn-soybean rotation fertilized at three P doses (0, 17.5 and 35 kg P ha^{-1}) applied only to corn since 1992. The ploughed layer was sampled in 2009 and 2014. Soils were analyzed for *C*p and the associated time-dependent diffusive Pi (*Pr*) that replenishes Pi in solution following sorption for 40 h and isotopic dilution. Experimental *Pr* values fitted to the equation: $Pr=19.90\ Cp^{0.42}\ t^{0.30}$ ($R^2=0.96$). The validation focused on C_P, the variable of agronomic interest to manage P fertilization. The simulated C_p agreed with that measured after 19 and 24 years of experiment. The model simulated different P fertilization scenarios that impact on *C*p. In conclusion, the operational CycP for ploughed layer was appropriate for this Canadian agroecosystem ignoring the contribution of sub-soil.

Key-words: available phosphorus, soil, agroecosystems, modeling, fertilization, isotopic dilution, sorption-desorption, soybean-corn rotation, P budget

5.3. Introduction

An agroecosystem is a soil-plant system under agricultural management. It is the basic unit to manage agricultural production within the pre-defined limits of the ecosystem. During the last decades, important land areas have been converted to agriculture to meet the global food demand. Early in 2000s, global cropland and grasslands represented 1.5 and 3.5 10^9 ha, respectively. The disappearing natural ecosystems will continue for the next decades due to population pressure (Tilman et al. 2001). The global phosphorus (P) demand will increase while the economically mineable world reserve of phosphate rocks will decrease (Cordell et al. 2009). Soil fertility under grasslands will drop (Sattari et al. 2016). The modeling of P cycling at field scale will be crucial to manage soil fertility and agroecosystem sustainability over long periods such as one decade and more.

Parton et al. (1988) proposed a mathematical model to describe carbon (C) and nutrient dynamics in grasslands soils. The CENTURY Model embodies an understanding of the biogeochemistry of C, N, P, and S with a P submodel derived from the N submodel except that there are five pools of mineral P (Metherell et al 1993). The Agricultural Nitrogen MOdel (ANIMO) simulates the C, N and P cycles (Kroes and Roelsma 1998). The model was first developed for N leaching from soil surface to groundwater and surface waters (Rijtema and Kroes, 1991), then an optional simulation of the P cycle was added. In the Erosion Productivity Impact Calculator (EPIC) model, the structure of the soil and plant P model was designed to test several components (Jones et al. 1991; Vadas et al. 2006). Such models require many input variables often difficult to quantify. Submodels of the C or N cycling modeling are not well adapted to the soil-plant P transfer mechanisms because long-term soil P changes are mainly controlled by physico-chemical processes associated with mineral constituents and the P budget (Morel et al. 2000; Messiga et al. 2010).

Messiga et al. (2015) developed a prognostic model for biogeochemical P cycling through agroecosystems over decades, hereafter denoted CycP. The conceptual model is composed of information (input data, parameters and equations) that describes fluxes and relevant P stocks in the ploughed layer. It is operational because it only required input variables, the initial status of plant-available soil P, soil-type specific parameters determined in laboratory experiments, and the annual P budget (difference between annual P inputs and outputs). The CycP allows to simulate P changes during decades and different outcomes for P dosage scenarios. The model considers that annual P inputs and outputs only influence the P stocks in the ploughed layer. The CycP model resembles that developed by Uusitalo et al. (2016) except that plant-available soil P is measured as the phosphate ions (Pi) concentration in solution and the time-dependent Pi replenishment by diffusion at the solid-to-solution interface compared to chemical reagents in Uusitalo et al. (2016).

Model predictions must be reliable and discriminate accurately key outcomes when re-tested in agroecosystems with different soils, and climate, crop rotations and follow-up periods. Comparison between simulations and observations allows identifying problem areas for further development. The objectives of this study were to test the ability of CycP model to simulate long term changes in plant-available soil P over decades using a long-term maize-soybean rotation experiment on P fertilization (Ziadi et al. 2014) in a clay-loam soil of eastern Canada (cold humid continental climate).

5.4. Materials and methods

5.4.1. Field experiment

The field trial detailed in Ziadi et al. (2014) was located at L'Acadie Experimental Farm of Agriculture and Agri-Food Canada (http://www.agr.gc.ca/fra/science-et-innovation/centres-de-recherche/quebec/centre-de-recherche-et-de-developpement-de-saint-jean-sur-richelieu/?id=1180632057455). Briefly, the site was established in 1992 on a clay loam soil (Orthic Humic Gleysol; 364 g clay, 432 g silt, 204 g sand kg^{-1} dry soil) and cropped thereafter following an alternate maize-soybean rotation. The statistical design was a split-plot replicated four times. Treatments were conservation tillage and moldboard randomly assigned to the main plots and nine fertilizer combinations (0, 80, and 160 kg N ha^{-1} factorially combined with 0, 17.5, and 35 kg P ha^{-1} applied as triplesuperphosphate (TSP) applied to corn only) randomly assigned to subplots. We focused on ploughed plots receiving three P doses and 160 kg N ha^{-1}. Depth adjustment of the desired plowing was 20 cm. A multi-year agronomic database (recording climatic data, applied P, annual grain yields and P content) was available to allow to compute annual P budgets. The P budgets were computed as the amount of P applied as mineral fertilizer, the amount of P in the seeds at sowing (P_{SEED}), the P removed at harvest in maize and soybean grains (P_{GRAIN}), and the amount of leached Pi ($Pi_{LEACHED}$) from the plow layer (Messiga et al. 2015). Soil erosion, runoff and atmospheric P deposition were negligible. The P_{GRAIN} was obtained by multiplying annual grain yield by grain P concentration measured yearly. The $Pi_{LEACHED}$ was obtained by multiplying leaching rate by soil solution Pi concentration in year *j*. On average, leaching rate beyond the ploughed layer was 100 mm yr^{-1}.

5.4.2. Laboratory determination of soil properties needed to calculate plant-available soil P

The transfer of phosphate ions at the solid-to-solution interface is governed by rapid reactions at particle surfaces followed by subsequent and substantial slow reactions that last for long time periods without reaching equilibrium (Fardeau et al. 1985; Barrow, 2015; Krumina et al. 2016). Several molecular scale mechanisms such as inner sphere complexation and intra- and inter-particle diffusion (Arai and Sparks 2007; Barrow, 2015), monodentate phosphate surface complexes (Krumina et al. 2016) and precipitation have been reported, especially for calcareous soils.

Plant-available P was assessed using a process-based approach considering that roots absorb Pi from solution and that the diffusion process of Pi equilibrated with Pi in solution. Soil solution Pi concentration (Cp, mg P L^{-1} solution)) and the gross amount of diffusive Pi at the solid-to-solution interface (Pr, mg P kg^{-1} soil) were determined in soil suspension experiments (1 g:10 mL) equilibrated for 40 h to reach steady-state, followed by ^{32}Pi isotopic dilution kinetics for a few hours (Morel et al. 2000; Némery et al. 2005; Stroia et al. 2007; Morel et al. 2014; Messiga et al. 2015). The Freudlich kinetics equation (Chardon and Blaauw, 1998) describes *Pr* changing over time (*t*, minutes) and *C*p as follows:

$$Pr = v\, Cp^{w}\, t^{p} \text{ with } Pr < Pr_{LIMIT} \qquad \text{Eq. 5-1}$$

The *v*, *w*, and *p* are soil specific coefficients estimated using non-linear regressions (NLIN, Statistical Analysis Software 9.4, SAS Institute, 2016). The *v* parameter is *Pr* at time t = 1 min and *C*p = 1 mg P L^{-1}; *w* describes the non-linear increase of *Pr* with *C*p; and *p* accounts for the non-linear increase in *Pr* with time (*t*). The P_{rLIMIT} is the soil inorganic P content, *i.e.*, total P minus organic P. The main interest of this equation is to extrapolate *Pr* from a few days to one year (Fardeau, 1993). To parameterize Eq. 5-1 for the ploughed layer, archived soil samples collected in 2009 in the 0-5 cm layer were analyzed for *C*p and *Pr*. We analyzed *C*p in 2014 soils sampled in the ploughed layer (0-20 cm). Each plot was sampled at three depths (0–5, 5–

10, and 10–20 cm) and three distances (5, 15, and 25 cm) perpendicularly to the row. The soil cores were obtained using a hydraulic power sampler (5.25-cm inner diameter). The *C*p was quantified using the green malachite colorimetric method (Messiga et al. 2015). The *C*p values were averaged by block. Similar sampling was carried out to determine soil bulk density. The average bulk density of the ploughed layer was 1.43 g cm^{-3}.

The Messiga et al. (2015) model uses a one-dimensional monolayer soil mass. The upper boundary is soil surface and the lower boundary is the bottom of the ploughed layer. Processes and fluxes included in the model are Pi concentration in soil solution at the solid-to-solution interface by diffusion at equilibration, Pi losses by leaching, crop P offtake, seed P, and P applied as mineral and organic products. The annual amount of seed P was estimated considering the number of seeds and their mass and P content. This P flux is about 0.1 and 0.3 kg ha^{-1} for maize and soybean, respectively, similar to Pi leaching. The annual grain yields of corn and soybean over 24 years (1992-2015) did not differ significantly between P treatments, i.e. 131 (±10), 130 (±11), and 135 (±8) Mg DM ha^{-1}, respectively, when applying 0, 17.5 and 35 kg P ha^{-1}. More effects occurred on P quantities associated to harvested grain yields: 391 (±31), 399 (±36), and 426 (±43) kg P ha^{-1}, respectively, when applying 0, 17.5 and 35 kg P ha^{-1}. The net P budget averaged −16.3, −7.5, and +1.2 kg P ha^{-1} yr^{-1}, respectively. In contrast with many field crop experiments (Boniface and Trocmé, 1988; Shepherd and Withers, 1999; Messiga et al. 2015), there was no P dosage leading to P budget consistently positive over the years.

The model is based on the mass conservation equation between plant-available soil P in the ploughed soil layer and the annual P budget (Eq. 5-1). The process-based evaluation of plant-available P was the amount of Pi in soil solution (*Qw*) plus the amount of Pi that equilibrate with the Pi in soil solution by the diffusion (*Pr*). The difference between annual P inputs minus P outputs provides the P budget for year *j* (B_j) partitioned between Pi in solution and soil Pi for one year (*Pr*(1yr)), as follows:

$$[k_c((Qw\ j + Pr(1\text{yr})j - Qw\ j+1 - Pr(1\text{yr})j+1)] = B_j \qquad \text{Eq. 5-2}$$

where k_c is the coefficient to convert available content in mg P kg^{-1} to kg P ha^{-1}, and *j* denotes the year. The (*Qw* + *Pr*)*j* and $(Qw+ Pr)_{j+1}$ are the amounts of Pi in soil solution (*Qw* = *C*p (V/M) where V is the volume per unit of soil in suspension, i.e. V/M=10) plus diffusive Pi for years *j* and (*j+1*). The model assumes that i) the time-dependent process of Pi diffusion at the solid to solution interface is the dominant process controlling soil solution Pi concentration (other possible rhizospheric processes were neglected), and ii) the P budget influenced only changes in plant-available soil P in the ploughed soil layer (other physico-chemical properties of the soil remain unchanged).

The detailed calculations to simulate *C*p values are presented in Messiga et al. (2015). The initial *C*p value was obtained by linear regression between cumulated P budget (Bcum) for the period 1992 to 2014 and measured *C*p values in 2014 ($Cp = 0.091 + 1.25\times10^{-3}Bcum$, $r^2=0.96$, 3 observations). Soil P available content in 1992 was obtained considering (*v*, *w*, *p*) parameters and one year as te period for Pi equilibration between solid and liquid phases. The Pi concentration of 0.091 mg P L^{-1} was converted to 1090 kg P ha^{-1} for 2860 t soil ha^{-1} in the plough layer.

5.4.3. Validation of the CycP model

The correspondence between model results and observed data was illustrated using graphical plots (Bellocchi et al. 2009). The Cp_{j+1} values were simulated from 1992 to 2015, the last year for available agronomic data. Eq. 5-3 is solved iteratively for Cp_{j+1} using the function "solver" in Microsoft Excel. Simulations are carried on a yearly basis across annual P budgets for the P treatments. Initial *C*p value was assessed at zero P budget (Messiga et al. 2010). Combining Eq. 5-1 and Eq. 5-2 we obtain:

$$[k_c((Cp_j(V/M) + v\, Cp_j^{\,w}\, t^p - Cp_{j+1}(V/M) - v\, Cp_{j+1}^{\,w}\, t^p)] = B_j \qquad \text{Eq. 5-3}$$

We considered several P fertilization scenarios varying in P dosage and P removal as well as different orders of magnitude for initial *C*p values. The Cp_{j+1} values were computed by CycP over 3 decades on a yearly basis for the following scenarios:

- Initial *C*p of 0.2 mg P L^{-1}, P removal of 20 kg P ha^{-1} yr^{-1} across P dosage, i.e. 0, 10, 20, 30, 40 and 50 kg P ha^{-1}, corresponding to -20, -10, 0, +10, +20, +30 kg P ha^{-1} yr^{-1} of net P budget. An additional P fertilization strategy was considered to mimic massive P applications with animal manure (Shepherd and Withers, 1999) at 250 kg P ha yr^{-1} over 2 years, followed by annual applications of 1000 kg P ha-^{1}, 250 kg P ha yr^{-1} over 2 years (total applied P of 2000 kg P ha^{-1} over five years), then no applied P for the subsequent 25 years.
- Initial *C*p values of 0.05, 0.1, 0.5, and 1 mg P L^{-1} at net P budget of +50 kg P ha^{-1}.

5.5. Results and Discussion

5.5.1. Dynamics of diffusive Pi at the solid-to-solution interface of soil

As reported by Morel et al. (2000) and Messiga et al. (2015) for annual crops, by (Stroia et al. 2007) for grasslands soils and by Némery et al. (2005) for river sediments, *Pr* values depend on *C*p and elapsed time (*t*). Our *C*p values ranged from 0.03 to 0.25 mg P L^{-1} and the *Pr* values from 6.4 to 69.1 mg P kg^{-1} (Fig. 5-1). The variation of *Pr* values as a function of *t* and *C*p was taken into account using the Freundlich kinetics equation as follows:

$$Pr = 19.90\, Cp^{0.42}\, t^{030} \quad (90 \text{ observations}, r^2=0.96, P < 0.001) \qquad \text{Eq. 5-4}$$

Simulated and experimental *Pr* values agreed closely (1:1 bisecting line) and the correlation was 0.97, similarly to other studies (Morel et al. 2000; Stroia et al. 2007; Morel et al. 2014). For uniform soil properties, one equation can describe the relationship between *Pr* and *C*p where *C*p increases due to P additions and *C*p decreases due to negative net P budget (Morel et al. 1994). For a given soil, the (*v*, *w*, *p*) parameters hold irrespective of sampling year in the ploughed layer in a French soil (Messiga et al. 2015). Soil types differed significantly in *v*, *w*, and *p* parameters where physico-chemical properties differ (Stroia et al. 2007; Morel et al. 2014; Messiga et al. 2015). For instance, the *v* parameter varied from 1.8 mg P kg^{-1} in a sandy French soil to 35.3 mg P kg^{-1} in a highly sorbing P Malagascan soil of fine texture and high content in iron and aluminum oxi-hydroxides. The *p* parameter also varied from 0.24 in a cropped French soil to 0.43 in a Swiss grassland soil.

5.5.2. Simulations vs. field-observations of Cp

The Cp_{j+1} was simulated from 1993 to 2015, the last year with available data on grain yield and P content. The model was run with different annual P budgets (Fig. 5-2). Simulated *C*p levels were reduced by –56%, and -15 % for P dosage of 0 and 17.5 kg P ha^{-1}. For 35 kg P ha^{-1}, simulated *C*p remained constant while annual P budget oscillated between positive P budget in the corn phase and negative P budget on the soybean phase. The validation was performed after 19 (2009) and 24 (2014) years of experimentation by comparing simulated and observed *C*p values in the plough layer (Fig. 5-2). The mean values of *C*p and intervals ± one time the standard error suggest that CycP predicted *C*p adequately for this Canadian agroecosystem.

Until now, CycP has been tested in one French agroecosystem on a 17-yr irrigated maize (*Zea mays* L.) monoculture cultivated on a neutral sandy loam luvic Arenosol containing 36% of sand (50-2000 µm), 52% of silt (2-50 µm), 12 % of clay (<2 µm), and 0.9% organic carbon (Messiga et al. 2015). Average annual P applications were 0, 27, and 79 kg P ha^{-1}. The model assumed that the time-dependent process of Pi diffusion at the solid to solution interface was the dominant process controlling soil solution Pi, and that the P budget influenced only plant-available soil P (available P) in the plough layer.

Fair agreement between model predictions and field measurements provided further evidence that the assumptions underlying the CycP model held for the Canadian agroecosystem. We included subsoil *C*p (30-40 cm) that proved to be low. 10-cm length cells were required for the colorimetric determinations in order to obtain values above the quantification threshold, which was 0.002 mg P L^{-1} with this procedure. Subsoil *C*p averaged 0.014 mg P L^{-1} across P treatments, more than 6 times smaller than initial *C*p in the plough layer at the beginning of the experiment (0.091 mg P L^{-1}). There was no experimental evidence for P upward or downward movement between subsoil layer and the plough layer due to low P doses applied to a clay soil showing low Pi in solution, high P sorption capacity, and exploration by plant roots essentially limited to the 0-40 cm layers (Li et al. 2015).

5.5.3. Simulations of P fertilization scenarios with CycP model

Fig. 5-3a shows *C*p simulations by CycP for seven scenarios of P budgets for constant initial *C*p of 0.2 mg P L^{-1}. Such simulations reproduced the well-known patterns of the fate of *C*p or any other soil P test for different P budgets and P dosage. *C*p values decreased with time where annual P budget was negative while simulated *C*p increased with time where annual P budget was positive. Simulated *C*p remained constant where the P budget was zero as reported elsewhere for *C*p (Morel et al. 2000; Shepherd and Withers 1999; Messiga et al. 2010) as well as other soil test P methods (Blake et al. 2003) such as Olsen (Boniface and Trocmé, 1988) and Mehlich-1 (McCollum, 1991). The scenario of massive annual P applications of P resulted in *C*p peaking at 1.46 mg P L^{-1} after the five first years followed by a subsequent slow decrease to 1.02 mg P L^{-1} after 30 years. It would take one hundred and five year to come back to the initial *C*p value of 0.2 mg P L^{-1} considering a negative annual budget of -20 kg P ha^{-1}.

Fig. 5-3b shows simulations considering initial *C*p levels of 0.05, 0.2, 0.5, 1 mg P L^{-1} and a positive annual P budget of +30 kg P ha^{-1}. Indeed, simulated *C*p values increased with more positive P budgets. But the interesting point was that the *C*p change for a cumulated P budget of 900 kg P ha^{-1} over 30 years increased with initial *C*p. The higher was initial *C*p, the greater was *C*p variation. Where the initial level of *C*p was 0.05 mg P L^{-1}, the *C*p value reached 0.28 mg P L^{-1} after 30 years hence a variation of +0.23 mg P L^{-1}. Where initial level of *C*p was 1 mg P L^{-1}, the *C*p value reached 1.82 mg P L^{-1} after 30 years hence a variation of +0.82 mg P L^{-1}. The effect of a constant P budget was even more than proportional where soil P was saturated with

possible risk for the environment. This runaway effect has been often reported in Dodd and Sharpley (2015).

5.6. Conclusion

The CycP model and the underlying assumptions proved to be applicable to a Canadian agroecosystem under corn-soybean rotation for over two decades. The CycP simulations allowed to predicting the change in *C*p in the plough layer following different scenarios of P budget and initial conditions. The CycP model could help decision-makers to improve soil P management in this region.

5.7. Acknowledgments

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Fig 5-1

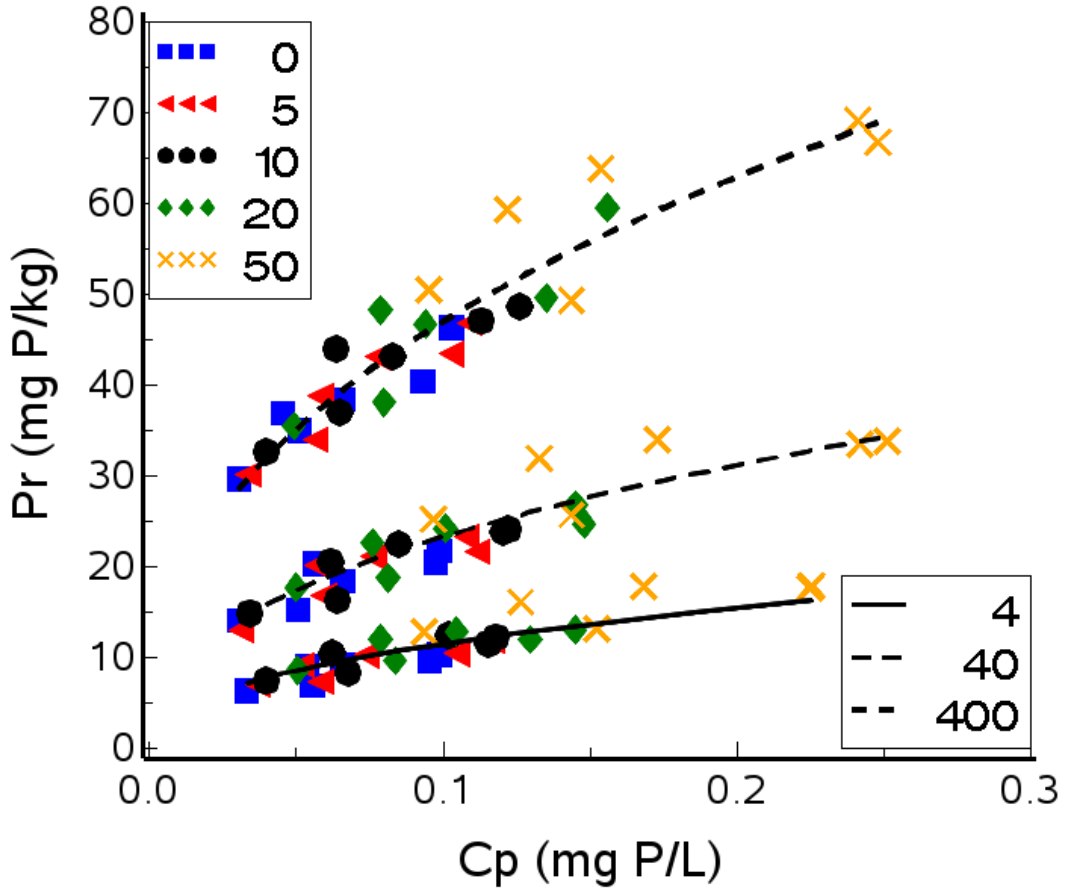
80
70
60
50
40
30
20
10
0
Pr (mg P/kg)
0
5
10
20
50
4
40
400
0.0
0.1
0.2
0.3
Cp (mg P/L)

Fig 5-2

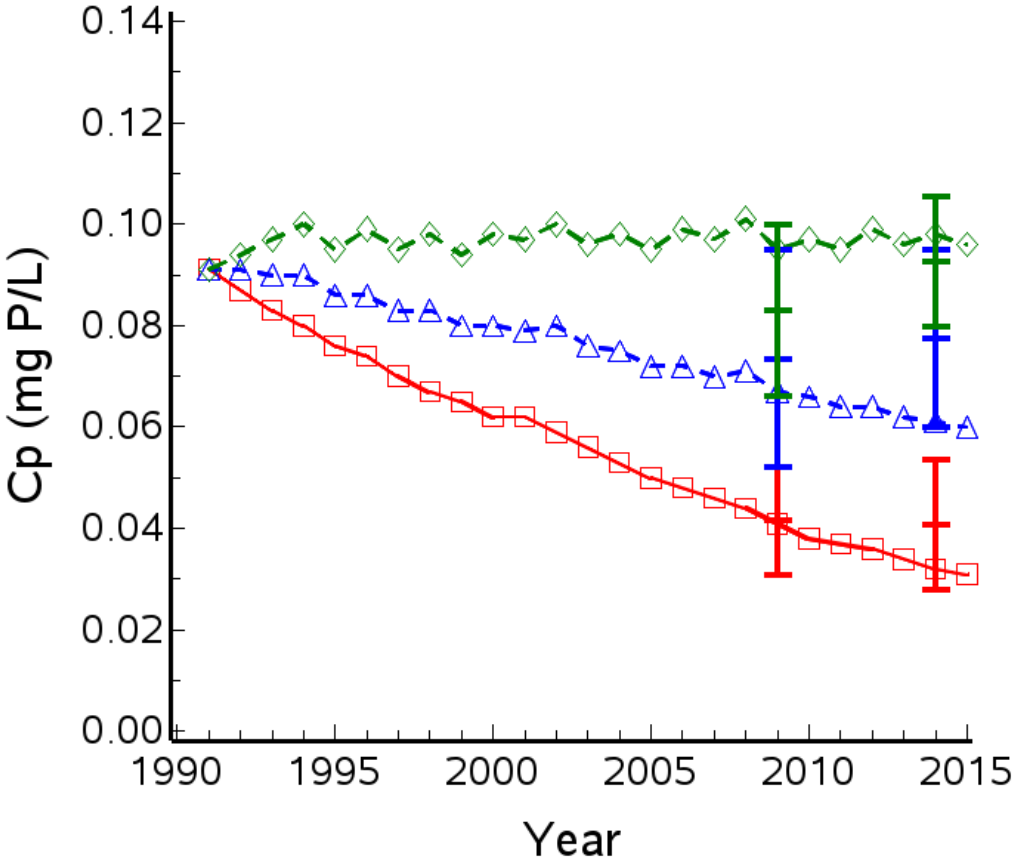
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Cp (mg P/L)
1990
1995
2000
2005
2010
2015
Year

Fig 5-3

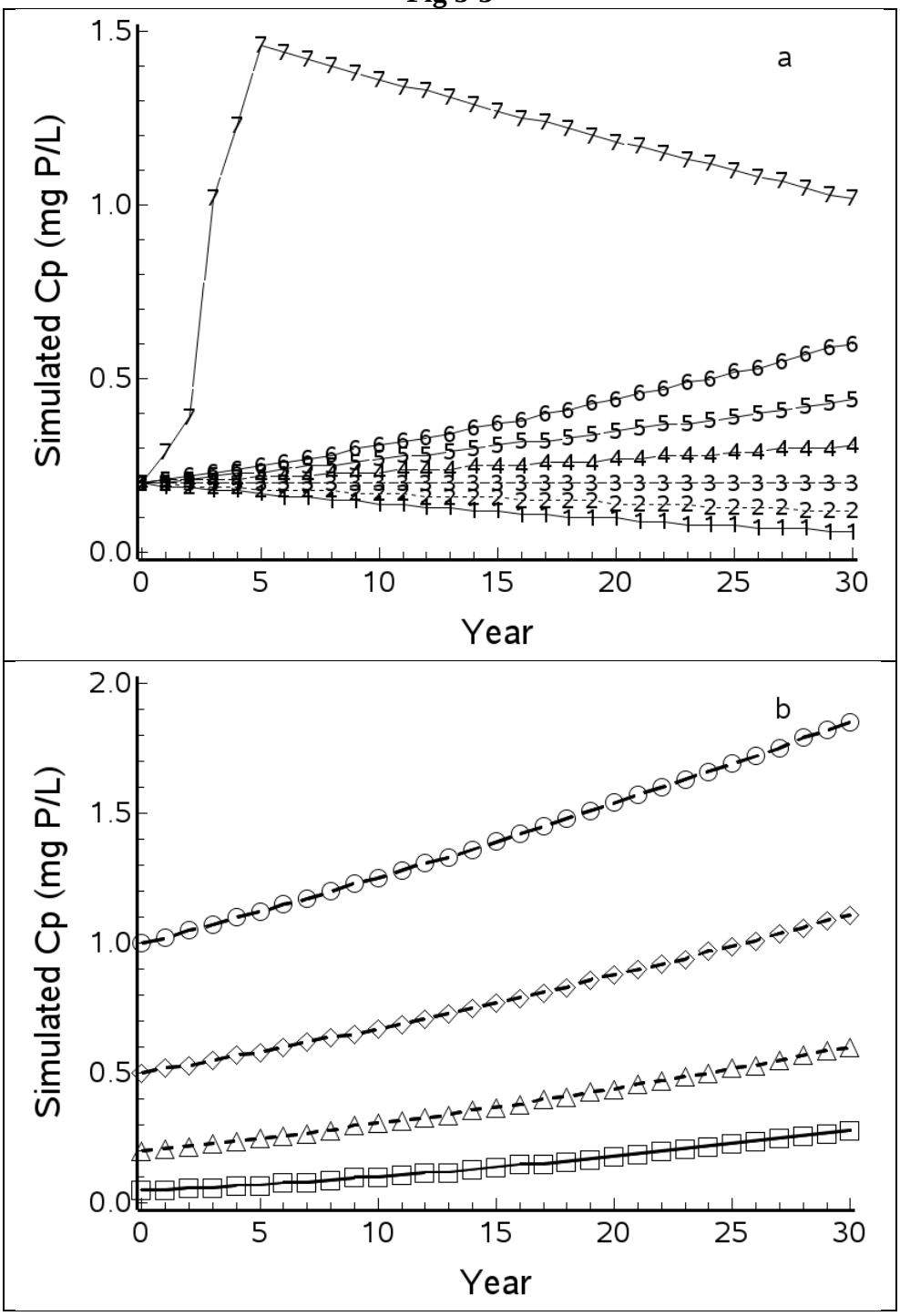

Fig. captions

Fig. 5-1: Experimental (symbols) and computed (lines) values for the gross amount of diffusive Pi (P_r) transferred between solid and liquid phases in soil suspension as a function of Pi concentration in solution (*C*p, in mg P L^{-1}) and elapsed time (4, 40, 400 min). For each of the 6 soils, 5 P applications were added, e. g. 0, 5, 10, 20, and 50 mg P kg^{-1} soil, and sorbed for 40 h before carrying out isotopic dilution kinetics for 4, 40 and 400 minutes

Fig. 5-2: Field-observed (mean ±standard-error) and simulated values of the Pi concentration in soil solution (*C*p) from 1992 to 2015 for the three doses of P fertilization, **0** (□), **17.5** (Δ), and **35** (◊) kg P ha^{-1} applied to corn every two years. The CycP model considered P uptake from cropped plots from the plough layer, and one year to reach equilibrium for Pi reactions between the liquid and solid phases. Solid lines with bars represent the mean of the field-observed *C*p and ±1 standard deviation for 2009 and 2014.

Fig. 5-3: Simulations by CycP model of Pi concentration (*C*p) in soil solution for different scenarios of P dosage and initial *C*p values. a) Seven scenarios at constant annual P budget, -20, -10, 0, +10, +20, and +30 kg P ha^{-1} numbered 1 to 6, respectively, at initial *C*p value of 0.2 mg P L^{-1}. The 7th scenario represents high P dosage during the 5 first years (cumulated P of +2000 kg P ha^{-1}) followed by no P applications. b) Different orders of magnitude for initial Pi concentration, i.e. 0.05 (□), 0.2 (Δ), 0.5 (◊), and 1 (O) mg P L^{-1}, at constant annual P budget of +30 kg P ha^{-1}.

Chapter VI Spatial assessment of the contribution of subsoil P to crop P nutrition

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***Running title*:** Evaluation of phosphorus uptake along soil profile

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6.1. Résumé

L'équation de la conservation de la masse utilisée dans les modèles du cycle du P dans le sol en labour conventionnel est généralement limitée à la couche arable bien que les racines des cultures explorent également les couches plus profondes et que le semis direct change la distribution du P dans le profil du sol. Mais il y a très peu d'information sur la contribution du sous-sol au prélèvement de P. Notre objectif était d'estimer les proportions du prélèvement du P le long profil du sol sous cultures de maïs (*Zea mays* L.) et de soja (*Glycine max* L.). Une rotation de culture maïs-soja a été établie à l'Acadie, Québec, Canada. Le dispositif en split-plot combinait deux approches de travail du sol (moldboard plough, MP; no-till, NT) et trois doses de fertilisation de P (0, 17,5 et 35 kg P ha^{-1}). Les résultats montrent que les proportions du prélèvement de P le long du profil du sol ont été modifiées de façon significative par le travail du sol avec des proportions plus élevées dans les couches 0-10 et 0-20 cm sous NT (46% et 79%, respectivement) par rapport à MP (25% et 68%, respectivement). De plus, 8% du prélèvement de P provenait de la couche de 30-40 cm, ce qui indique que la contribution du sous-sol au prélèvement de P est non négligeable et devrait être prise en considération dans les modèles de cycle du P dans les agrosystèmes.

Mots-clés: ions phosphate diffusifs, labour, sans labour

6.2. Abstract

The mass conservation equation used in soil P cycle models is generally limited to the arable layer under conventional tillage although crop roots also explore sublayers and no-till alter soil P distribution along the soil profile. But very little information has been reported on subsoil P contribution to crop P uptake. Our objective was to estimate the proportions of P uptake along the soil profile under maize (*Zea mays* L.) and soybean (*Glycine max* L.). The maize-soybean cropping sequence was located at l'Acadie, Québec, Canada. The split-plot design combined two tillage practices (moldboard plough, MP; no-till, NT) and three P fertilization rates (0, 17.5 and 35 kg P ha^{-1}). Crop P uptake along soil profile were significantly altered by tillage with higher proportions of P uptake in 0-10 and 0-20 cm layers under NT (46% and 79%, respectively) compared to MP (25% and 68%, respectively). On average, the 30-40 cm layer contributed 8% of total P uptake, indicating that P uptake from subsoil should not be ignored.

Key-words: diffusive phosphate ions, moldboard plough, no-till

6.3. Introduction

Soil P dynamics have been simulated from changes in soil test P in the upper soil layer and cumulative P budgets under conventional tillage (Morel et al. 2014; Messiga et al. 2015). However, Plant P uptake depends on rooting depth and soil P status, and follows Michaelis-Menten kinetics (Barber 1995). Subsoil P as another possible P resource has been largely ignored (Murdock and Engelbert 1958) because of lower soil test P in subsoil and several constraints for P uptake such as higher boron toxicity, salinity, sodicity, water availability (McBeath et al. 2012), higher bulk density (Barber 1995), and compaction affecting rooting depth (Hankansson 2005). Tillage practices and P dosage could influence crop root biomass and morphology by altering soil properties such bulk density, nutrient distribution and biodiversity (Li et al., 2016; 2017).

Beauchemin and Simard (2000) found at 27 sites that while Mehlich-3 extracted P in B (30-50 cm) and C horizons (50-70 cm) averaged 8 and 14 mg P kg^{-1}, respectively, compared to 91 mg P kg^{-1} in the A horizon, total soil P averages were closer, with 932, 703 and 696 mg P kg^{-1} in the A, B and C horizons, respectively, indicating a considerable reservoir of plant-available P in the deeper horizon compared with the upper horizon. Topsoil P could be re-allocated to the subsoil through leaching by preferential flow, earthworm feces, root exudates and root death in deeper layers. Roots can take up subsoil P in hot-spots of high biochemical activity such as the rhizosphere (several mm around roots) and the drilosphere (about 2 mm wide around earthworm burrows) (Kautz et al. 2013). The authors also pointed that the significance of subsoil contribution to plant P nutrition was higher where available P decreased in the topsoil.

Plant-available soil P accumulates primarily in the topsoil due to P fertilization and the P released from crop residues. Conservation tillage could lead to soil P stratification with even much higher P concentrations in the topsoil because crop residues are left on soil surface with little disturbance, compared to conventional tillage system (Messiga et al. 2010; Calegari et al. 2013). Hence, P uptake along soil profile including subsoil should differ between conventional and conservation tillage practices. A long-term trial is required to make such comparison possible because no-till systems take several years to reach their specific soil profile. Literature (Barber 1995; Daroub et al. 2003) have shown that P uptake was primarily controlled by the crop root exploration and soil P status.

The objective of this study was to partition crop P uptake along the soil profile in a 24-years experiment under conventional (moldboard plough) and conservation (no-till) systems, as a function of P dosage. Estimates of P uptake require pairing the distribution of roots and soil P availability that can be quantified as the sum of phosphate ions in soil solution and exchangeable P.

6.4. Materials and Methods

6.4.1 Site description

A permanent site was established in 1992 at the L'Acadie experimental station of Agriculture and Agri-Food Canada in Québec, Canada (45°18' N; 73°21' W). The experiment is described in detail in Ziadi et al. (2014). In brief, the site was planted with maize from 1992 to 1994. A maize (*Zea mays* L.) and soybean (*Glycine max* L.) rotation was initiated in 1995.
The experimental set-up is a split-split-plot replicated four times with two tillage practices [Moldboard Plough (MP) and No-Till (NT)] randomized in main plots, and nine combinations of nitrogen (N) and phosphorous (P) levels (0, 80, 160 kg N ha^{-1} and 0, 17.5, and 35 kg P ha^{-1} applied every two years during the maize phase) randomized in subplots. Hereinafter, the three P treatments are referred to as 0P, 0.5P and 1P, each receiving 160 kg N ha^{-1}. The plots are 25-m in length and 4.5-m in width and row space is 75 cm. Conventional tillage consisted

of moldboard-ploughing to 20 cm in the fall after harvest, and disking and harrowing to 10 cm before seeding next spring. The NT treatment consists of ridge-tillage from 1992 to 1997 and flat direct-seeding from 1998 on. Crop residues are left on soil surface after harvest. Fertilizers are band-applied (5-cm from seeding row) as commercial granulated triple superphosphate ($Ca(H_2PO_4)_2$, H_2O). The N dose is split-applied as urea at seeding (48 kg N ha^{-1}) and side-dressed as ammonium nitrate (112 kg N ha^{-1}) at the 8-leaf maize stage. All plots received 50 kg K_2O ha^{-1}, band-applied at planting in 1992 and 2007, based on soil analyses and local recommendations (CRAAQ 2003).

Pioneer P9623AM maize (2850 CTU) was sown 2 June 2014 at a rate of 83 000 plants ha^{-1}. Soybean was sown 26 June 2015 at a rate of 450 000 plants ha^{-1}. Climatic conditions (precipitation and temperature) for the cultural seasons (in general from June to October) are presented in **Fig. 5-1**. Soil analyses (P-Olsen and P-Mehlich3) have been reported in previous studies (Li et al., 2016a; Li et al., 2016b).

6.4.2 Soil sampling for phosphate ion transfer kinetics

The soil sampling for phosphate ion transfer kinetics was conducted in the spring of 2009 before fertilizer application and sowing. Soil cores (5.25-cm inner diameter) were collected in the arable layer (0–5 cm) between rows in MP plots of two blocks (12 sub-plots). Four soil cores were composited per plot, air dried, 2-mm sieved and stored at room temperature.

The transfer kinetics of phosphate ion at the solid-to-liquid interface was determined as a function of time using an isotopic labeling and dilution procedure (Stroia et al. 2007; Achat et al. 2009). It consisted of two steps. The first step was to prepare soil suspension by mixing 2.0 g of oven-dry (105 °C) soil with 18.0 ml of ultra-pure water (18 MΩ) and 0.2 ml of toluene. For each soil sample, five different P concentration (0, 5, 10, 20 and 50 mg KH_2PO_4-P kg^{-1}) were added to 1 g of soil suspended in distilled water to produce a range of phosphate ion concentrations (***Cp***) in soil suspensions having a final volume to mass ratio of 10. Suspensions were shaken for 40 h to reach steady state for subsequent isotopic dilution. Then, 100 µL of carrier-free ^{32}P was added to soil suspensions at time zero. Soil suspensions were sampled after 4, 40 and 400 min of equilibration and immediately filtered through 0.2 µm membrane filters. Molybdate-reactive P (***Cp***) in filtered soil solution was determined using the malachite green colorimetric method (Van Veldhoven and Mannaerts, 1987). The radioactivity remaining in solution at time ***t*** (***r***) and the amount of initially added carrier-free ^{32}P ion radioactivity (***R***) values were counted by liquid scintillation counter (Packard TR 1100).

At equilibrium, the gross rate of P desorption from the soil solid phase to solution is equal to the gross rate of P sorption onto the solid phase. It is thus possible to measure the amount of unlabeled phosphate ions newly transferred to solution, by determining the amount of unlabeled phosphate ions present in the solution (***Pw***) and the ratio of radioactivity to the total amount of P ions in the solution (***r***/***Pw***), called the isotopic composition ratio (***IC***). Since all the fractions of phosphate ions in solid and liquid phases have the same ***IC***, giving:

$$IC = R / (Pw + Pr) = r / Pw = (R - r) / Pr \qquad \textbf{Eq 6-1}$$

where ***R*** and ***r*** are the radioactivity in the added labelled phosphate ions and that remaining in solution after several time ***t***; ***Pw*** and ***Pr*** are the amounts of diffusive phosphate ions in liquid and solid phases after several time ***t***.

The ***Pr*** is computed as:

$$Pr = Pw \times (R / r - 1) = Cp \times V \times (R / r - 1) \qquad \textbf{Eq 6-2}$$

where ***Cp*** is the phosphate ion concentration in present soil solution; ***V*** is the volume of soil solution, and ***Pw*** = ***Cp*** × ***V***. Total amount of diffusive P ions is computed as (***Pr*** + ***Pw***).

Those data allow the parameterization of the mathematical function that relates the amount of diffusive phosphate ions in solid phase (***Pr***, mg P kg^{-1} soil) with time (***t***, min) and phosphate ion concentration in soil solution (***Cp***, mg P L^{-1}). Such kinetic is in form of Freundlich equation as described in (Morel et al. 2014):

$$Pr = v \times Cp^{w}\, t^{p} \qquad Pr < Pr_{limit} \qquad \textbf{Eq 6-3}$$

where ***Pr*** is the amount of diffusive phosphate ions in solid phase (mg P kg^{-1} soil) at time ***t***; Pr_{limit} is the upper limit of diffusive phosphate ions that could be adsorbed on the solid phase (mg P kg^{-1} soil); ***Cp*** is the concentration of phosphate ions in soil solution (mg P L^{-1}); ***v***, ***w*** and ***p*** are fitted parameters. The time (***t***) for equilibration of phosphate ions between solid and liquid phases was deemed as one day (1440 min) (Morel et al. 2014).

6.4.3 Soil sampling for Cp along soil profile

The soil sampling for soil P measurement was conducted on 19-20 August 2014. Samples were collected about five consecutive plants randomly selected in the 3rd or 4th row to avoid side effects, using the core method for soils (Bohm 2012) with a hydraulic power sampler (5.25–cm inner diameter) to a depth of 40 cm at 5-, 15- and 25-cm distances perpendicularly to the crop row. The 40-cm samples were cut as follows: 0–5, 5–10, 10–20, 20–30 and 30–40 cm, for a total of 360 soil samples. Samples were air-dried, ground manually and sieved through a 2-mm sieve, then stored. The phosphate ion concentration in soil solution (***Cp***) was determined by agitating 2.0 g of oven-dry (105 °C) soil with 18.0 ml of ultra-pure water (18 MΩ) and 0.2 ml of toluene on a roller mixer (40 cycles min^{-1}) for 16 h, then < 0.2-µm filtered (Stroia et al. 2007). The P concentrations were determined using the malachite green method (Van Veldhoven and Mannaerts 1987) and quantified at 610 nm using a 1-cm long optical cell spectrophotometer (Jenway 6320D).

The amount of phosphate ions in both soil solution and solid phase was combined with soil bulk density (***BD***) data. Soil sampling for bulk density was conducted on 3 July 2014 just before split N application at five depths (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm and 30-40 cm) in 18 subplots (3 blocks) using the core method (Carter 1993) with a hand-held power sampler (5.25-cm inner diameter). Soil samples were air-dried and soil bulk density analysis determined. The ***BD*** data were reported in previous study (Li et al., 2016a). Similar soil bulk densities of MP and NT in the upper layers (0-20 cm) were measured (1.43 and 1.40 g cm^{-3} on average for MP and NT, respectively). But in the deeper layers (20-40 cm), higher soil bulk density was observed in NT (1.59 g cm^{-3} on average) compared to that in MP (1.50 g cm^{-3} on average). The data of ***BD*** in 2014 are presented in **Addendum 6-1**.

6.4.4 Root sampling and analysis

Maize root samples were collected on 19–20 August 2014 (80 days after seeding, approximately at silk stage) about five consecutive plants randomly selected in the 3rd or 4th row to avoid side effects. Samples were taken using the core method for soils (Bohm 2012) and a hand-held power sampler (8–cm inner diameter) for roots, to a depth of 40 cm at 5-, 15- and 25-cm distances perpendicularly to the crop row. The 40-cm samples were cut as follows: 0–5, 5–10, 10–20, 20–30 and 30–40 cm, for a total of 360 root samples. Soybean root samples were collected on 19-20 August 2015 (54 days after seeding, at approximately the end of soybean vegetative stage) following the same pattern above but using a hydraulic power

sampler (5.25-cm inner diameter). Root samples were placed in plastic bags and stored at 4 °C for a few days before performing soil-root separation.

Root samples were soaked into a 1 M solution of NaCl (1 L per 5-cm soil core) for 16 h to disperse soil aggregates. Samples were transferred to a hydro-pneumatic elutriation washing machine (Smucker et al., 1982). Cleaned roots were collected on a 760 µm sieve then on a 400 µm sieve (Bolinder et al. 2002). The roots recovered on the 400 µm sieve were cleaned by hand from remaining mineral particles and organic debris. Roots were rinsed and spread on a transparent tray using distilled water, dispersed with tweezers and scanned in black and white (400 dpi, tagged image file format [TIF], white background) using a Imagery Scan Screen (EPSON Expression 10000XL) (Sheng et al. 2012). Root surface area was determined using the professional image analysis software "WinRhizo" (Regent Instruments Inc., Québec). Root surface density (***RSD***) was calculated as root surface area divided by soil core volume.

6.4.5 Phosphorus uptake weighting factor and proportion

As Daroub et al. (2003) suggested, the weighting factor of P uptake along the soil profile requires measuring root distribution, soil water availability, labile P in soil solution and labile P in solid phase to replenish soil solution. We assumed that water availability did not affect P uptake due to annual precipitation of 900 mm at the experimental site. The weighting factor accounted for the distribution of roots and labile P to 40 cm vertically and 60 cm horizontally was computed as follows:

$$Weight_{P\ uptake,\ i\text{-}j} = (RS_{i\text{-}j} \times P_{total,\ i\text{-}j}) / (\sum(RS_{i\text{-}j} \times P_{total,\ i\text{-}j})) \qquad \textbf{Eq 6-4}$$

where ***i*** is layer number; ***j*** is distance numbering from the row; $Weight_{P\ uptake,\ i\text{-}j}$ is the weighting factor of P uptake for ***i-j*** grid unit; $RS_{i\text{-}j}$ is root surface (cm^2) for ***i-j*** grid unit; $P_{total,\ i\text{-}j}$ is exchangeable phosphate ions in soil solution and solid phase (kg P ha^{-1}) for ***i-j*** grid unit.

Expanding **Eq 6-4** we obtain:

$$Weight_{P\ uptake,\ i\text{-}j} = [RSD_{i\text{-}j} \times V_{i\text{-}j} \times (Pr_{i\text{-}j} \times BD_{i\text{-}j} \times V_{i\text{-}j} + 10 \times Cp_{i\text{-}j} \times BD_{i\text{-}j} \times V_{i\text{-}j})] / [\sum[RSD_{i\text{-}j} \times V_{i\text{-}j} \times (Pr_{i\text{-}j} \times BD_{i\text{-}j} \times V_{i\text{-}j} + 10 \times Cp_{i\text{-}j} \times BD_{i\text{-}j} \times V_{i\text{-}j})]] \qquad \textbf{Eq 6-5}$$

where $Weight_{P\ uptake,\ i\text{-}j}$ is the weighting factor for the ***i-j*** grid unit; $RSD_{i\text{-}j}$ is root surface density (cm^2 cm^{-3}) for ***i-j*** grid unit; $V_{i\text{-}j}$ is the volume (cm^3) for ***i-j*** grid unit; $BD_{i\text{-}j}$ is soil bulk density (g cm^{-3}) for ***i-j*** grid unit; $Pr_{i\text{-}j}$ is the diffusive phosphate ion concentration in the solid phase (mg P kg^{-1} soil) for ***i-j*** grid unit; $Cp_{i\text{-}j}$ is the effective phosphate ion concentration in soil solution (mg P L^{-1}) for ***i-j*** grid unit; and **10** is the ratio of solution volume to soil mass in the suspension (cm^{-3} g^{-1}).

The critical ***Cp*** value (Cp_{min}) for P uptake from soil solution is 0.003 mg P L^{-1} for maize and soybean (Edwards and Barber 1976; Schenk and Barber 1980). Hence, the effective ***Cp*** values for root uptake in **Eq 6-5** are computed as the difference between measured ***Cp*** and Cp_{min}.

The P uptake proportions are estimated for 30 soil grids (**Fig. 6-2**). Because soil properties including P status were measured in 2014 only on the fertilized side of the row, we assumed that:

1. ***Cp*** distribution was asymmetric for the fertilized and unfertilized sides for NT and MP. Indeed, there was no tillage for NT and tillage operation occurred before P fertilization for MP, while applied P was constrained to a very limited zone around the fertilizer granules (Stecker et al. 2001). The ***Cp*** values for the unfertilized side

were assumed to be the ***Cp*** means of 10-20 and 20-30 cm distances on the fertilized side.

2. The Freundlich kinetics parameters for the 0-5 cm layer in MP plots in 2009 did not change across the soil profile (0-40 cm depth) in MP and NT plots of 2014 and 2015.
3. Because of the small root interception (in contact with only 1-2% of soil) (Baligar 1985; Johnston et al. 2014) and the slow diffusion coefficient of phosphate ions (1 x 10^{-8} to 10^{-10} cm^2 s^{-1}) in the soil (Barber 1995), P uptake during one season made a small fraction of soil P stock. Total soil P stock (***P_{total}*** in **Eq 6-4**) estimated from ***Cp*** in 2015 was assumed to be the same as in 2014. The ploughing practice would homogenize soil P stocks across grids in the 0-20 cm layer.
4. Root distribution was symmetric about rows because P fertilization had no significant effect on lateral root distribution in this trial (Li et al. 2016a and 2016b).

The proportion of total P uptake in each soil grid was computed as follows:

$$Pup\%_{i\text{-}j} = Weight_{P\ uptake,\ i\text{-}j} / \sum Weight_{P\ uptake,\ i\text{-}j} \qquad \textbf{Eq 6-6}$$

where ***$Pup\%_{i\text{-}j}$*** is the proportion of P uptake in the ***i-j*** grid unit.

6.4.6 Statistical analysis

For phosphate ion transfer kinetics, the ***v***, ***w*** and ***p*** parameters are estimated by non-linear regressions using Proc NLIN (SAS Institute, Inc. 2010).

For ***Cp*** data, ANOVA was performed using the MIXED procedure (SAS Institute Inc., 2010) to evaluate the fixed effects of tillage, P fertilization, sampling depth and perpendicular distance to the crop row and the interactions between these factors on ***Cp*** values. The random effects were assigned to blocks and block × tillage interactions. In addition, the sampling depth and perpendicular distance to the soybean row were set as repeated-measures. The similar ANOVA was conducted for root data (***RSD***) and the results in details were reported in previous studies (Li et al. 2016; 2017). So in this study, only synthetic results were presented.

Additive log ratio (*alr*) transformation was conducted to run statistical analyses on P uptake proportions then back-transformed to proportion to obtain unbiased means (Aitchison 1982). There are three additive log ratios based on adhoc binary contrasts (*alr*1: [P uptake from the 0-10 cm layer | P uptake from the 10-40 cm layer]; *alr*2: [P uptake from the 0-20 cm layer | P uptake from the 20-40 cm layer]; *alr*3: [P uptake from the 0-30 cm layer | P uptake from the 30-40 cm layer]). The alrs were computed as follows:

$$alr1 = \ln(Pup\%_{0\text{-}10cm} / Pup\%_{10\text{-}40cm});$$
$$alr2 = \ln(Pup\%_{0\text{-}20cm} / Pup\%_{20\text{-}40cm});$$
$$alr3 = \ln(Pup\%_{0\text{-}30cm} / Pup\%_{30\text{-}40cm}) \qquad \textbf{Eq 6-7}$$

In terms of P uptake proportions, ANOVA was performed using the MIXED procedure (SAS Institute Inc., 2010) to evaluate the fixed effects of culture, tillage, P fertilization, and the interactions between these factors on the *alr* data. The random effects were set as blocks (within culture) and block (within culture) × tillage interactions. For all the ANOVA, treatment effects were deemed significant when $P<0.05$; differences among least square means for treatment pairs were identified using the LSMEANS (/diff) statement (*t*-test) (SAS Institute Inc., 2010).

6.5. Results

6.5.1 Soil P status (Cp) data

The P fertilization increased soil P status along the soil profile. Compared to MP, NT tended to show higher soil P status in the upper soil layer and lower soil P status in the deeper soil layer. The ***Cp*** values shared the similar patterns as P-Olsen and P-Mehlich3 (**Table 6-1**). The ***Cp*** value decreased significantly from 0.17 and 0.14 mg P L^{-1} in the 0-5 and 5-10 cm layers, respectively, to 0.07, 0.04 and 0.01 mg P L^{-1} in the 10-20, 20-30 and 30-40 cm layers, respectively. Such variation was slightly but significantly affected by sampling distance to crop row. At 5-cm distance, ***Cp*** was higher in the 5-10 cm layer (0.23 mg P L^{-1}) than that in the 0-5 cm layer (0.17 mg P L^{-1}), mainly because P fertilizers were applied at 5-cm depth (data in details were not shown). The P fertilization could significantly increase ***Cp*** values from 0.05 mg P L^{-1} with 0P to 0.09 and 0.13 mg P L^{-1} with 0.5P and 1P, respectively; and this tendency was almost consistent along the soil profile with no interaction between P fertilization and sampling depth (**Fig. 6-3a**). Tillage practice also significantly influenced ***Cp*** value in interaction with sampling depth. The NT had significantly higher ***Cp*** in the 0-5 and 5-10 cm layers (0.28 and 0.19 mg P L^{-1}, respectively) compared to MP (0.07 and 0.09 mg P L^{-1}, respectively). In contrast, NT showed significantly smaller Cp in the 20-30 cm layer (0.02 mg P L^{-1}) compared to MP (0.05 mg P L^{-1}) (**Fig. 6-3b**).

The ***Cp*** data in 2014 used to estimate for P uptake proportions in the 2D model in **Fig. 6-2** are presented in **Addendum 6-2**.

6.5.2 Crop root distributions

The most important factor for P upake is roots distribution in the soil to provide access to immobile soil P. Tillage practices and P dosage could influence crop root biomass and morphology by altering bulk density, nutrient distribution and biodiversity as shown by Li et al. (2016) and Li et al. (2017).

In terms of lateral distribution, regardless of treatment, maize roots accumulated laterally in greatest amounts 5-cm (49% of total root surface on average) and 25-cm (30% of total root surface) from row center compared to 15-cm (21% of total root surface). Maize roots expanded laterally following an S-shape distribution (Guan et al., 2006). Soybean roots had a different pattern of lateral distribution. Soybean roots accumulated mostly 5-cm (52% of total root surface on average) from row center and decreased toward inter-row with 32% and 16% of root surface 15- and 25-cm from row center, respectively.

For vertical distribution, a previous study (Li et al. 2016) showed that tillage and P fertilization had no significant effects on maize roots. However, the vertical root distribution of soybean was significantly affected by tillage practice ($P<0.05$). Soybean roots under NT primarily accumulated in the 0-10 cm layer with 46% of total root surface on average, with only 34% of total root surface under MP at 0-10 cm. Soybean roots under MP mostly accumulated in the 10-20 cm layer (41% of total root surface) with only 28% of total root surface in 10-20 cm layer under NT.

Data for root surface density and root surface proportion in 2014 and 2015 used for P uptake proportion estimation are reported **Fig. 6-2** and estimates are given in **Addenda 6-3** and **6-4**.

6.5.3 Phosphate ion transfer kinetic and soil P availability estimation

Parameters (***v***, ***w*** and ***p***) of **Eq 6-3** for phosphate ion transfer kinetics at the solid-to-liquid interface were determined by non-linear regression. The results are plotted in **Fig. 6-3**. The Freundlich equation for the 0-5 cm soil layers under MP was:

$$\boldsymbol{Pr = 19.9 \times Cp^{0.42}\, t^{0.30}}\ (\mathbf{r^2=0.99;\ n = 90}) \qquad \textbf{Eq 6-8}$$

The amount of diffusive phosphate ions in soil was computed based on measurements of ***BD*** and ***Cp*** (**Addenda 6-1** and **6-2**) in 2014 and estimated parameters (***v***, ***w*** and ***p***). Considering P uptake by crop roots, the time (***t***) for equilibration of phosphate ions between solid and liquid phases was fixed at one day (1440 min) (Morel et al. 2014). The results are presented in **Adendum 6-5**. Total diffusive phosphate ion in the sampling zone (0-40 cm) was found to be less than 400 kg P ha^{-1}, less than average total P of 500 mg P kg^{-1} in soils (Bray and Kurtz 1945; Bowman 1988), viz. 2800 kg P ha^{-1} for the 0-40 cm layer.

The seasonal P uptake makes a very small part of soil P stock and total soil P status in 2015 was assumed to be similar compared to 2014. The MP would homogenize soil P status in the 0-20 cm layer. Hence the amounts of diffusive phosphate ions computed for one-day equilibration period in 2015 are presented in **Adendum 6-6**.

6.5.4 Phosphate uptake proportions

With data for soil bulk density (***BD***), soil P status (***Cp***) and root surface density (***RSD***) (**Addenda 6-1**, **6-2**, **6-3** and **6-4**) and amounts of diffusive phosphate ions (**Addenda 6-5** and **6-6**), weighting factors for P uptake and proportions of P uptake for individual grid unit along soil profile (**Fig. 6-2**) could be estimated for 2014 and 2015 using **Eqs 6-5** and **6-6**. Results are reported in **Addenda 6-7** and **6-8**.

In this study, we focused on the vertical distribution of P uptake, especially that from the subsoil. Effects of the crop, tillage and P fertilization on the proportions of P uptake from the 0-40 cm layers (transformed into *alr*1, *alr*2 and *alr*3) were tested as shown in **Table 6-3**. The proportions of P uptake from the 0-10, 0-20 and 0-30 cm layers averaged 36%, 74% and 92%, respectively, across treatments (**Fig. 6-3**). Tillage practice significantly influenced proportions from the 0-10 and 0-20 cm layers. The proportions of P uptake were 46% and 79% for the 0-10 and 0-20 cm layers under NT, respectively, and were higher compared to MP (25% and 68% for the 0-10 and 0-20 cm layers, respectively) (**Table 6-3**). In the 0-10 cm layer, tillage significantly interacted with crop. For soybean, the difference between the P uptake proportions at the 0-10 cm for NT (48%) and MP (21%) was much higher than that for maize (NT, 44%; MP, 29%). For th 0-30 cm layer, there was no significant effect of crop, tillage and P fertilization. In addition, there was no P fertilization effect on the selected vertical P uptake proportions

6.6. Discussion

6.6.1 P uptake effected by tillage practice

Tillage practice influenced soil properties (i.e. bulk density), soil P distribution and crop root system, hence root P uptake along the soil profile. We found that NT showed significantly higher estimated proportions of total P uptake (Pup%) in the upper soil layer (0-20 cm) compared to MP due undoubtedly to soil P stratification, as found elsewhere (Lupwayi et al. 2006; Messiga et al. 2010; Calegari et al. 2013).

In the 0-10 cm layer, the difference in Pup% between NT and MP was greater for soybean than for maize as related to crop root distribution in the soil. Maize has a tap root system with several radical roots that would root penetration into deeper soil layers while the soybean root system is fibrous and tends to proliferate in upper layers (Farmaha et al. 2012). Li et al. (2016b) observed that soybean roots was more sensitive than maize roots to soil P variations and tended to proliferate primarily in the 0-10 cm layer under NT because of the nutrient stratifications, including P. Ho et al. (2004) pointed out shallow root systems such as soybean could be more effective in P uptake where P distribution is heterogeneous under NT.

6.6.2 P uptake proportion from subsoil P stock

Most of P uptake (92%) was from the 0-30 cm layer. Indeed, most soil P accumulated in the 0-30 cm layer (MP, 85% and NT, 87%) due to P immobilization in the soil (Hinsinger et al. 2011). Most maize and soybean roots also accumulated in the 0-30 cm layer (maize, 87%; soybean, 89%) because of their architecture and possibly to higher soil bulk density in deeper layers that hinders root proliferation (Qin et al. 2005).

We found that, in average, 8% of total P uptake must orignate from the 30-40 cm layer, indicating that P uptake from subsoil should not be ignored as stressed by Kautz et al. (2013). Considering subsoil as the untilled soil layer (the 20-40 cm layer), subsoil contribution to P uptake increased to 26%. Hence subsoil P could become even more crucial for crop growth where topsoil P decreased on the long run without fertilizer input. It was partially supported by our results with smaller P uptake proportion from subsoil under NT with higher soil P status in the upper layers compared to MP. However, we found no P fertilization effect found on P uptake proportions. One reason may be that P fertilization could increase soil P status across the soil profile, as shown by ***Cp*** value. The P flow to subsoil could be driven by P leaching by preferential flow, earthworm faeces, root exudates and P released from dead roots in deeper layers (Kautz et al. 2013).

Very little information is available to confirm our estimates. Many other factors should also be taken into account such as water availability (Daroub et al. 2003), that controls the P transport from bulk soil to crop roots, fine roots, that are efficient to absorb P (Fernandez and Rubio 2015), and soil air pores that influence root respiration (Grable and Siemer 1968) and energy flow for root P uptake..

6.7. Summary and conclusion

In this study, we propose an approach to estimate the proportions of P uptake from layers along the soil profile from the distribution of roots and soil available P. Our estimates were in line with other studies. The P uptake from subsoil was high enough (8% in average) to be included in P cycle models, still based on the tilled soil layer under conventional tillage. However, to validate and improve this approach, more factors should be considered.

6.8. Acknowledgements

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Table 6-1 Effects of tillage and P fertilization and their interactions with depth and distance soil on phosphate ion concentration in soil solution (***Cp***)

Treatment	***Cp***
Tillage	NS
P	***
Tillage × P	NS
Depth	***
Tillage × Depth	***
P × Depth	NS
Tillage × P × Depth	NS
Distance	NS
Tillage × Distance	NS
P × Distance	NS
Tillage × P × Distance	NS
Depth × Distance	*
Tillage × Depth × Distance	NS
P × Depth × Distance	NS
Tillage × P × Depth × Distance	NS

NS not significant ($P \geq 0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 6-2 Effects of crop, tillage, P fertilization and their interactions on proportions of total P uptake from the 0-10, 0-20 and 0-30 cm layers after transformation into *alr*1, *alr*2 and *alr*3.

Treatment	*alr*1	*alr*2	*alr*3
Crop	NS	NS	NS
Tillage	***	**	NS
Crop × Tillage	*	NS	NS
P	NS	NS	NS
Crop × Tillage	NS	NS	NS
Tillage × P	NS	NS	NS
Crop × Tillage × P	NS	NS	NS

NS not significant (P≥0.05); * P<0.05; ** P<0.01; *** P<0.001

Table 6-3 Average values of *alr*1, *alr*2, *alr*3 and proportions of total P uptake (Pup%) from the 0-10, 0-20 and 0-30 cm layers under different crops, tillage practices, interaction of tillage × crop and P treatments

Treatment	*alr*1	*alr*2	*alr*3	Pup% (0-10 cm)	Pup% (0-20 cm)	Pup% (0-30%)
Tillage						
NT	-0.17a	1.40a	2.61	46%	79%	92%
MP	-1.10b	0.86b	2.47	25%	68%	91%
Crop						
Maize	-0.52	1.00	2.51	37%	71%	91%
Soybean	-0.74	1.26	2.57	34%	77%	92%
Tillage × Crop						
Maize NT	-0.24ab	1.29a	2.44	44%	77%	91%
Maize MP	-0.81b	0.70b	2.59	29%	64%	91%
Soybean NT	-0.10a	1.51a	2.78	48%	80%	93%
Soybean MP	-1.39b	1.01b	2.36	21%	73%	91%
P fertilization						
0P	-3.87	2.56	3.47	34%	71%	91%
0.5P	-4.49	4.53	4.19	34%	76%	92%
1P	-3.21	4.14	4.12	39%	75%	92%

Different letters after ***alr*** data indicated significant ($P<0.05$) difference between mean values within the treatments; **Pup%** data, as compositional data, were not supposed to be compared directly.

Fig. 6-1

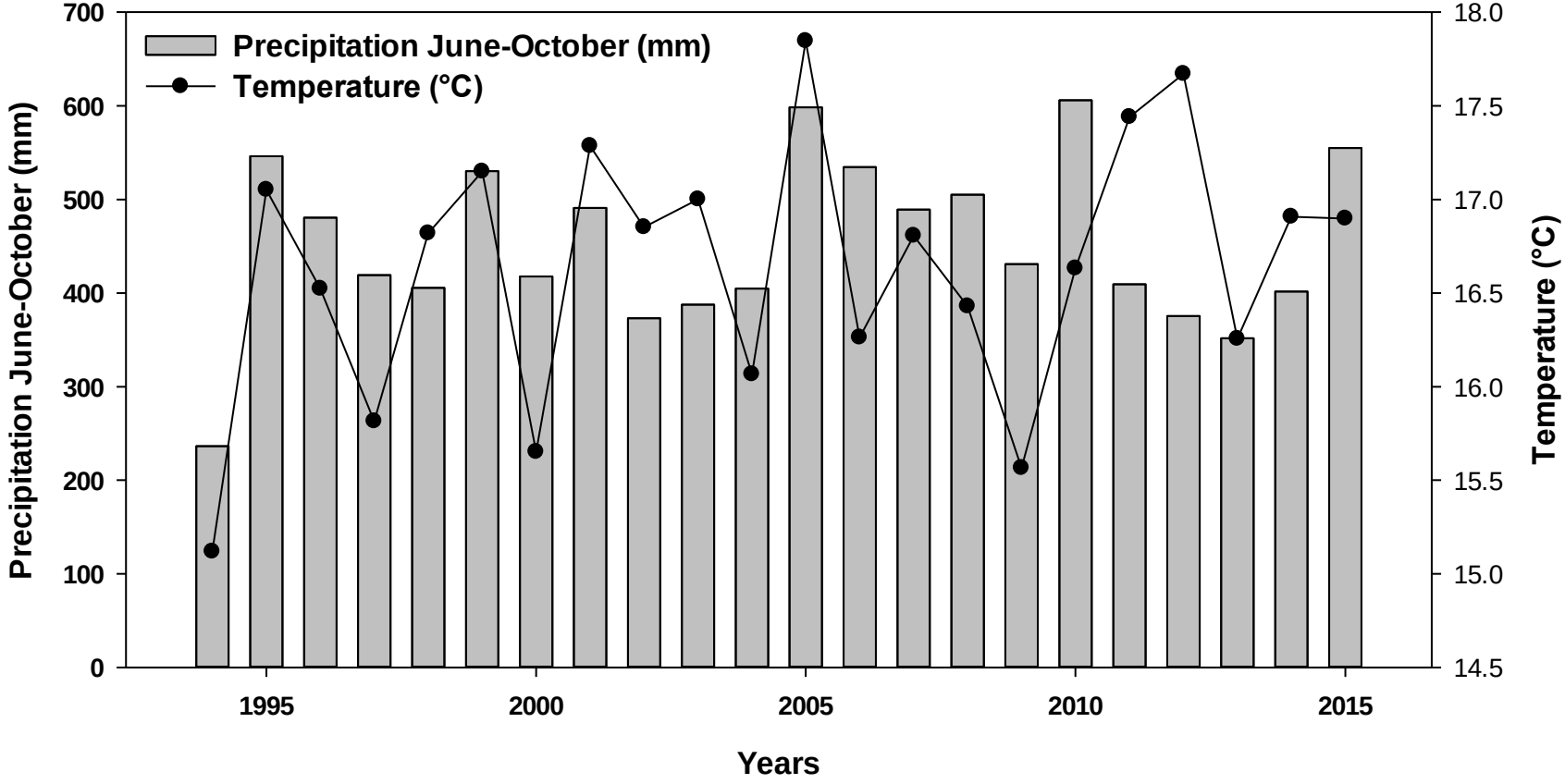
Precipitation June-October (mm)
Temperature (°C)
Precipitation June-October (mm)
Temperature (°C)
Years
700
600
500
400
300
200
100
0
18.0
17.5
17.0
16.5
16.0
15.5
15.0
14.5
1995
2000
2005
2010
2015

Fig. 6-2

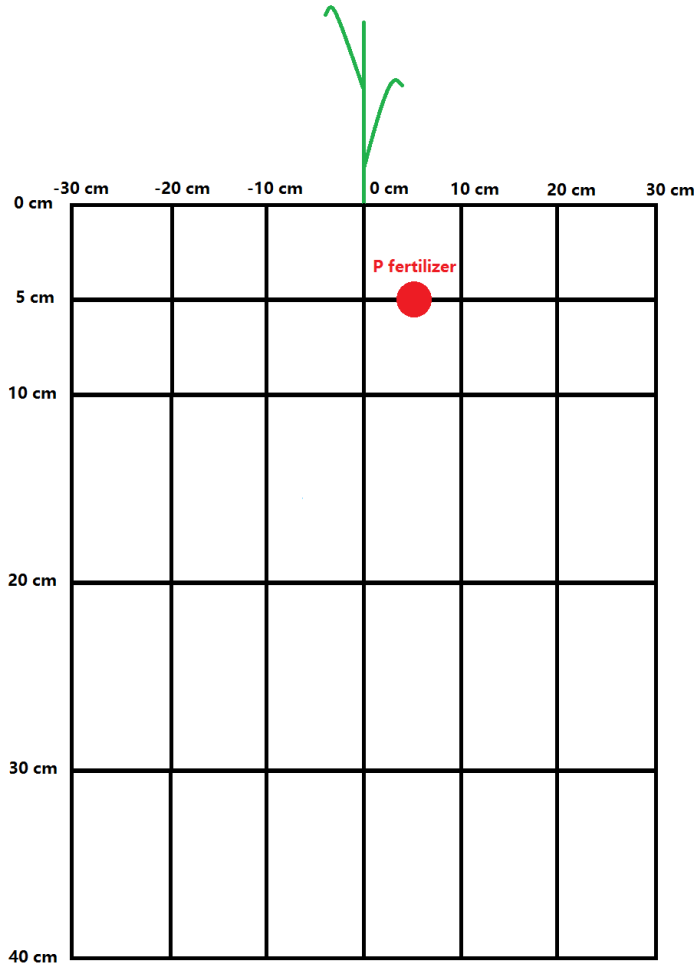
-30 cm
-20 cm
-10 cm
0 cm
10 cm
20 cm
30 cm
0 cm
5 cm
10 cm
20 cm
30 cm
40 cm
P fertilizer

Fig. 6-3

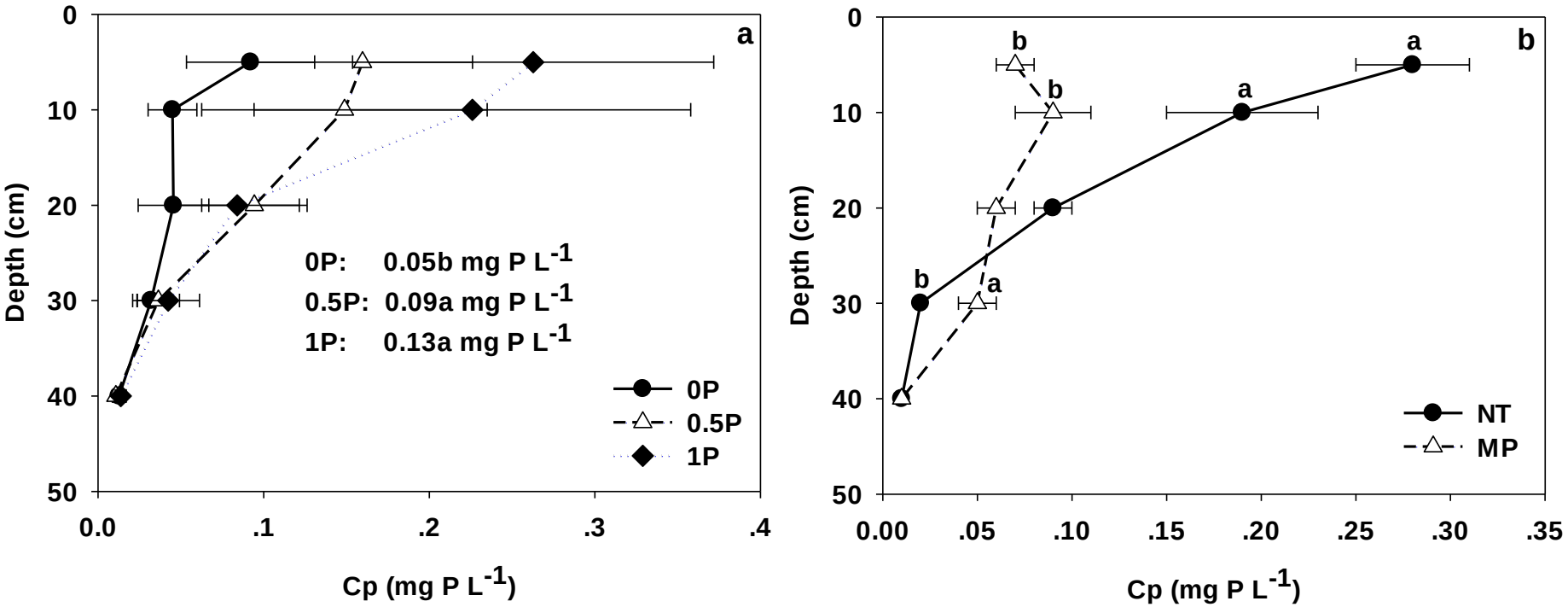
a
0P: 0.05b mg P L-1
0.5P: 0.09a mg P L-1
1P: 0.13a mg P L-1
0P
0.5P
1P
Depth (cm)
Cp (mg P L-1)
b
NT
MP
Depth (cm)
Cp (mg P L-1)

Fig. 6-4

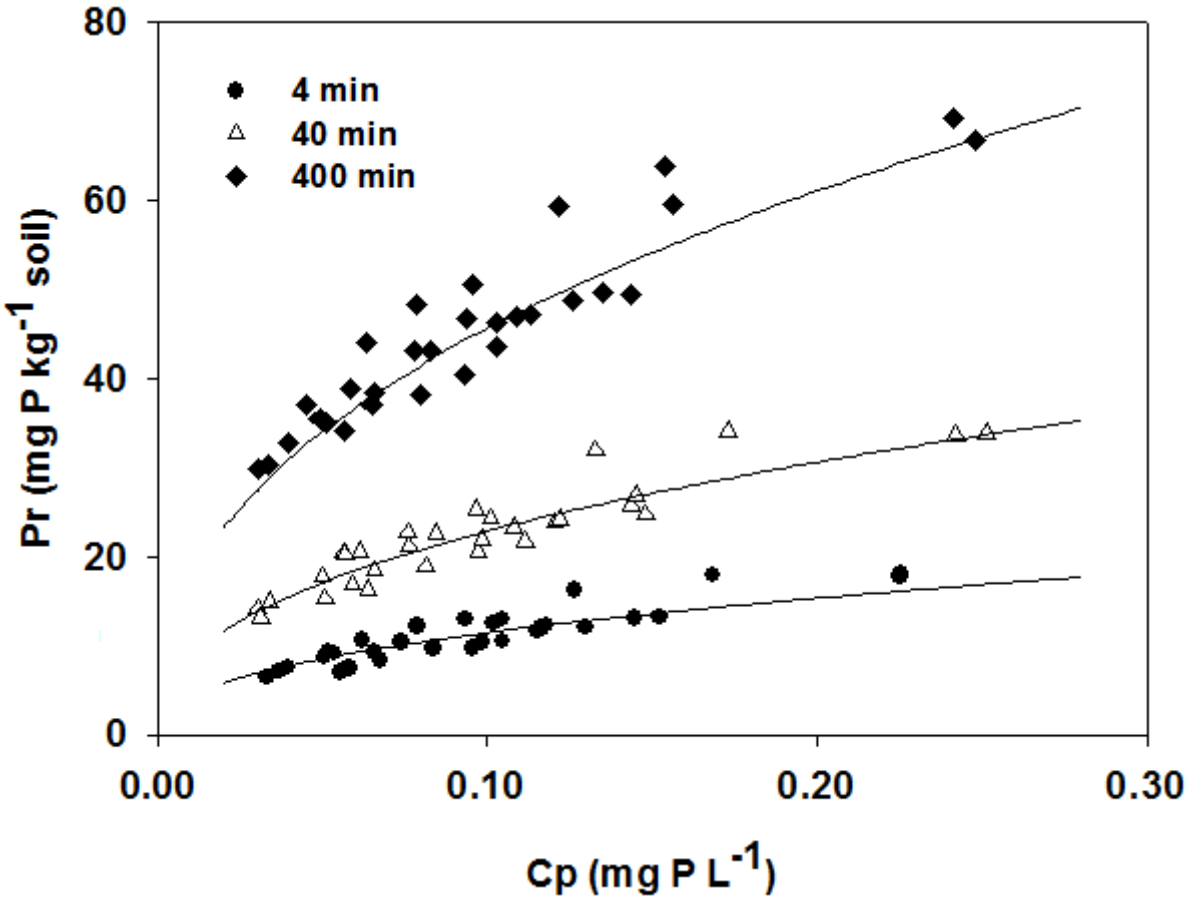
80
60
40
20
0
0.00
0.10
0.20
0.30
4 min
40 min
400 min
Pr (mg P kg-1 soil)
Cp (mg P L-1)

Fig. 6-5

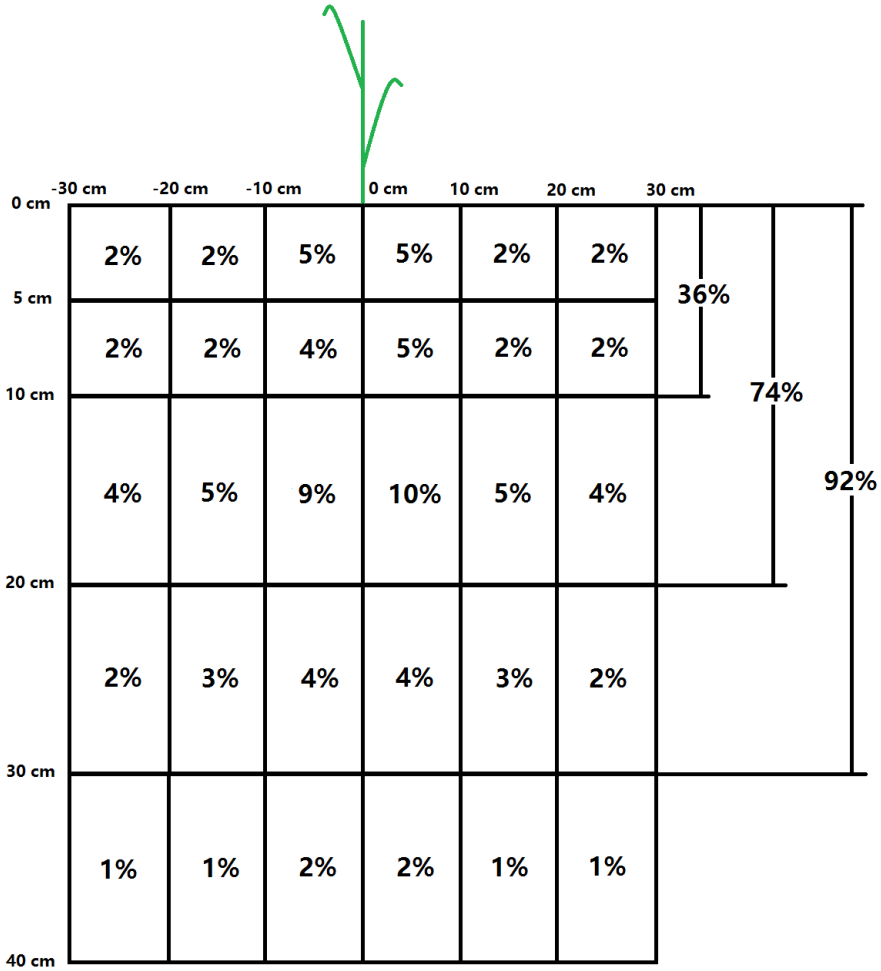
-30 cm
-20 cm
-10 cm
0 cm
10 cm
20 cm
30 cm
0 cm
5 cm
10 cm
20 cm
30 cm
40 cm
2% 2% 5% 5% 2% 2%
2% 2% 4% 5% 2% 2%
4% 5% 9% 10% 5% 4%
2% 3% 4% 4% 3% 2%
1% 1% 2% 2% 1% 1%
36%
74%
92%

Fig. captions

Fig. 6-1: Average temperature (°C) and precipitation (mm) in the growth season from June to October at the experimental site of l'Acadie from 1994 to 2015 (no data for 1992 and 1993).

Fig. 6-2: Schematic structure of 2D soil profile to estimate proportions of total P uptake.

Fig. 6-3: Average values of phosphate ion concentrations (***Cp***, mg P L^{-1}) in the soil profile for three P doses (0P, 0.5P and 1P) (a) and two tillage (MP and NT) (b). Different letters at a depth indicated significant (P<0.05) differences in ***Cp*** values between treatments (P dosage or tillage practice).

Fig. 6-4: Experimental (symbols) and calculated (lines) values for diffusive phosphate ions (***Pr***) in the solid phase of soil suspensions as a function of phosphate ion concentration in solution (***Cp***, mg P L^{-1}) and elapsed time of isotopic dilution (4, 40, 400 min) in the MP system.

Fig. 6-5: 2D distribution of estimated proportions of total P uptake.

Addendum 6-1. Average 2D distribution of soil bulk density (***BD***, g cm^{-3}) of three blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2014

P fertilization	**Distance from row (cm)**					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	1.35	1.35	1.35	1.35	1.35	1.35
5-10	1.46	1.46	1.46	1.46	1.46	1.46
10-20	1.46	1.46	1.46	1.46	1.46	1.46
20-30	1.51	1.51	1.51	1.51	1.51	1.51
30-40	1.55	1.55	1.55	1.55	1.55	1.55
	Soil tillage : NT					
0-5	1.25	1.25	1.25	1.25	1.25	1.25
5-10	1.40	1.40	1.40	1.40	1.40	1.40
10-20	1.45	1.45	1.45	1.45	1.45	1.45
20-30	1.52	1.52	1.52	1.52	1.52	1.52
30-40	1.65	1.65	1.65	1.65	1.65	1.65

P fertilization	**Distance from row (cm)**					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	1.25	1.25	1.25	1.25	1.25	1.25
5-10	1.44	1.44	1.44	1.44	1.44	1.44
10-20	1.42	1.42	1.42	1.42	1.42	1.42
20-30	1.50	1.50	1.50	1.50	1.50	1.50
30-40	1.53	1.53	1.53	1.53	1.53	1.53
	Soil tillage : NT					
0-5	1.41	1.41	1.41	1.41	1.41	1.41
5-10	1.45	1.45	1.45	1.45	1.45	1.45
10-20	1.46	1.46	1.46	1.46	1.46	1.46
20-30	1.46	1.46	1.46	1.46	1.46	1.46
30-40	1.51	1.51	1.51	1.51	1.51	1.51

P fertilization	**Distance from row (cm)**					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					

0-5	1.32	1.32	1.32	1.32	1.32	1.32
5-10	1.44	1.44	1.44	1.44	1.44	1.44
10-20	1.45	1.45	1.45	1.45	1.45	1.45
20-30	1.48	1.48	1.48	1.48	1.48	1.48
30-40	1.47	1.47	1.47	1.47	1.47	1.47
	Soil tillage : NT					
0-5	1.34	1.34	1.34	1.34	1.34	1.34
5-10	1.46	1.46	1.46	1.46	1.46	1.46
10-20	1.46	1.46	1.46	1.46	1.46	1.46
20-30	1.62	1.62	1.62	1.62	1.62	1.62
30-40	1.70	1.70	1.70	1.70	1.70	1.70

Addendum 6-2. Average 2D distribution of effective phosphate ion concentrations in soil solution (***Cp***, mg P L^{-1}) of four blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2014

P fertilization	**Distance from row (cm)**					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.04	0.04	0.05	0.05	0.04	0.04
5-10	0.03	0.04	0.04	0.04	0.04	0.03
10-20	0.03	0.04	0.03	0.03	0.04	0.03
20-30	0.04	0.05	0.04	0.04	0.05	0.04
30-40	0.02	0.01	0.01	0.01	0.01	0.02
	Soil tillage : NT					
0-5	0.17	0.13	0.11	0.11	0.13	0.17
5-10	0.06	0.04	0.04	0.04	0.04	0.06
10-20	0.06	0.05	0.04	0.04	0.05	0.06
20-30	0.02	0.01	0.01	0.01	0.01	0.02
30-40	0.01	0.01	0.01	0.01	0.01	0.01

P fertilization	**Distance from row (cm)**					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.06	0.06	0.06	0.06	0.06	0.06
5-10	0.06	0.06	0.06	0.20	0.08	0.04

10-20	0.07	0.07	0.07	0.07	0.09	0.05
20-30	0.05	0.05	0.05	0.03	0.04	0.06
30-40	0.01	0.01	0.01	0.01	0.01	0.01
	Soil tillage : NT					
0-5	0.23	0.23	0.23	0.31	0.22	0.24
5-10	0.13	0.13	0.13	0.29	0.08	0.19
10-20	0.09	0.09	0.09	0.18	0.08	0.09
20-30	0.02	0.02	0.02	0.02	0.03	0.02
30-40	0.01	0.01	0.01	0.01	0.01	0.01

P fertilization	**Distance from row (cm)**					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.10	0.10	0.10	0.08	0.11	0.10
5-10	0.07	0.07	0.07	0.22	0.08	0.06
10-20	0.07	0.07	0.07	0.09	0.08	0.06
20-30	0.06	0.06	0.06	0.05	0.07	0.06
30-40	0.01	0.01	0.01	0.01	0.01	0.02
	Soil tillage : NT					
0-5	0.43	0.43	0.43	0.40	0.42	0.45
5-10	0.19	0.19	0.19	0.60	0.25	0.14
10-20	0.08	0.08	0.08	0.10	0.10	0.06
20-30	0.03	0.03	0.03	0.02	0.03	0.02
30-40	0.01	0.01	0.01	0.01	0.01	0.01

Addendum 6-3. Average 2D distribution of maize root surface density (***RSD***, $cm^2\ cm^{-3}$) of four blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2014. Numbers in parentheses are the proportion of root surface for a given horizontal and lateral localization in the soil semi-profile.

P fertilization	**Distance from row (cm)**					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.12 (2%)	0.08 (2%)	0.48 (10%)	0.48 (10%)	0.08 (2%)	0.12 (2%)
5-10	0.31 (4%)	0.10 (2%)	0.25 (5%)	0.25 (5%)	0.10 (2%)	0.31 (4%)
10-20	0.14 (5%)	0.05 (2%)	0.11 (5%)	0.11 (5%)	0.05 (2%)	0.14 (5%)
20-30	0.11 (3%)	0.06 (2%)	0.11 (4%)	0.11 (4%)	0.06 (2%)	0.11 (3%)

30-40	0.04 (1%)	0.04 (1%)	0.06 (2%)	0.06 (2%)	0.04 (1%)	0.04 (1%)
	Soil tillage : NT					
0-5	0.06 (2%)	0.09 (2%)	0.19 (5%)	0.19 (5%)	0.09 (2%)	0.06 (2%)
5-10	0.18 (4%)	0.09 (2%)	0.23 (6%)	0.23 (6%)	0.09 (2%)	0.18 (4%)
10-20	0.09 (4%)	0.05 (3%)	0.12 (7%)	0.12 (7%)	0.05 (3%)	0.09 (4%)
20-30	0.05 (3%)	0.04 (2%)	0.08 (4%)	0.08 (4%)	0.04 (2%)	0.05 (3%)
30-40	0.04 (2%)	0.03 (1%)	0.06 (3%)	0.06 (3%)	0.03 (1%)	0.04 (2%)

P fertilization	**Distance from row (cm)**					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.05 (1%)	0.13 (3%)	0.20 (4%)	0.20 (4%)	0.13 (3%)	0.05 (1%)
5-10	0.13 (3%)	0.09 (2%)	0.25 (5%)	0.25 (5%)	0.09 (2%)	0.13 (3%)
10-20	0.14 (6%)	0.07 (3%)	0.17 (7%)	0.17 (7%)	0.07 (3%)	0.14 (6%)
20-30	0.07 (3%)	0.06 (3%)	0.10 (4%)	0.10 (4%)	0.06 (3%)	0.07 (3%)
30-40	0.05 (2%)	0.04 (2%)	0.08 (3%)	0.08 (3%)	0.04 (2%)	0.05 (2%)
	Soil tillage : NT					
0-5	0.20 (4%)	0.14 (3%)	0.30 (7%)	0.30 (7%)	0.14 (3%)	0.20 (4%)
5-10	0.07 (1%)	0.11 (2%)	0.21 (5%)	0.21 (5%)	0.11 (2%)	0.07 (1%)
10-20	0.06 (3%)	0.07 (3%)	0.14 (6%)	0.14 (6%)	0.07 (3%)	0.06 (3%)
20-30	0.05 (2%)	0.04 (2%)	0.09 (4%)	0.09 (4%)	0.04 (2%)	0.05 (2%)
30-40	0.05 (2%)	0.04 (2%)	0.07 (3%)	0.07 (3%)	0.04 (2%)	0.05 (2%)

P fertilization	**Distance from row (cm)**					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.17 (2%)	0.10 (2%)	0.55 (9%)	0.55 (9%)	0.10 (2%)	0.17 (2%)
5-10	0.22 (3%)	0.12 (2%)	0.33 (5%)	0.33 (5%)	0.12 (2%)	0.22 (3%)
10-20	0.13 (4%)	0.10 (3%)	0.16 (5%)	0.16 (5%)	0.10 (3%)	0.13 (4%)
20-30	0.06 (2%)	0.08 (3%)	0.15 (5%)	0.15 (5%)	0.08 (3%)	0.06 (2%)
30-40	0.06 (2%)	0.05 (2%)	0.08 (3%)	0.08 (3%)	0.05 (2%)	0.06 (2%)
	Soil tillage : NT					
0-5	0.30 (5%)	0.15 (3%)	0.34 (7%)	0.34 (7%)	0.15 (3%)	0.30 (5%)
5-10	0.18 (3%)	0.10 (2%)	0.22 (4%)	0.22 (4%)	0.10 (2%)	0.18 (3%)
10-20	0.10 (4%)	0.06 (3%)	0.16 (6%)	0.16 (6%)	0.06 (3%)	0.10 (4%)

20-30	0.04 (2%)	0.03 (1%)	0.12 (4%)	0.12 (4%)	0.03 (1%)	0.04 (2%)
30-40	0.05 (2%)	0.03 (1%)	0.09 (4%)	0.09 (4%)	0.03 (1%)	0.05 (2%)

Addendum 6-4. Average 2D distribution of soybean root surface density (***RSD***, cm^2 cm^{-3}) of four blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2015. Numbers in parentheses are the proportion of root surface for a given horizontal and lateral localization in the soil semi-profile.

P fertilization	**Distance from row (cm)**					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.04 (1%)	0.07 (1%)	0.36 (4%)	0.36 (4%)	0.07 (1%)	0.04 (1%)
5-10	0.06 (1%)	0.30 (3%)	0.43 (5%)	0.43 (5%)	0.30 (3%)	0.06 (1%)
10-20	0.07 (2%)	0.48 (13%)	0.32 (9%)	0.32 (9%)	0.48 (13%)	0.07 (2%)
20-30	0.04 (1%)	0.07 (2%)	0.10 (3%)	0.10 (3%)	0.07 (2%)	0.04 (1%)
30-40	0.06 (1%)	0.07 (2%)	0.08 (2%)	0.08 (2%)	0.07 (2%)	0.06 (1%)
	Soil tillage : NT					
0-5	0.09 (1%)	0.17 (3%)	0.64 (10%)	0.64 (10%)	0.17 (3%)	0.09 (1%)
5-10	0.24 (4%)	0.26 (4%)	0.48 (7%)	0.48 (7%)	0.26 (4%)	0.24 (4%)
10-20	0.06 (2%)	0.15 (5%)	0.12 (4%)	0.12 (4%)	0.15 (5%)	0.06 (2%)
20-30	0.03 (1%)	0.09 (3%)	0.12 (4%)	0.12 (4%)	0.09 (3%)	0.03 (1%)
30-40	0.03 (1%)	0.03 (1%)	0.05 (1%)	0.05 (1%)	0.03 (1%)	0.03 (1%)

P fertilization	**Distance from row (cm)**					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.04 (1%)	0.08 (1%)	0.30 (5%)	0.30 (5%)	0.08 (1%)	0.04 (1%)
5-10	0.10 (1%)	0.18 (3%)	0.54 (8%)	0.54 (8%)	0.18 (3%)	0.10 (1%)
10-20	0.13 (3%)	0.21 (6%)	0.38 (11%)	0.38 (11%)	0.21 (6%)	0.13 (3%)
20-30	0.05 (2%)	0.09 (3%)	0.09 (3%)	0.09 (3%)	0.09 (3%)	0.05 (2%)
30-40	0.04 (1%)	0.08 (2%)	0.08 (2%)	0.08 (2%)	0.08 (2%)	0.04 (1%)
	Soil tillage : NT					
0-5	0.13 (2%)	0.20 (3%)	0.38 (5%)	0.38 (5%)	0.20 (3%)	0.13 (2%)
5-10	0.21 (3%)	0.28 (4%)	0.61 (8%)	0.61 (8%)	0.28 (4%)	0.21 (3%)
10-20	0.07 (2%)	0.15 (4%)	0.34 (9%)	0.34 (9%)	0.15 (4%)	0.07 (2%)
20-30	0.03 (1%)	0.07 (2%)	0.13 (3%)	0.13 (3%)	0.07 (2%)	0.03 (1%)

30-40	0.06 (2%)	0.06 (2%)	0.11 (3%)	0.11 (3%)	0.06 (2%)	0.06 (2%)

P fertilization	**Distance from row (cm)**					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0.04 (1%)	0.04 (1%)	0.11 (2%)	0.11 (2%)	0.04 (1%)	0.04 (1%)
5-10	0.07 (1%)	0.17 (4%)	0.32 (7%)	0.32 (7%)	0.17 (4%)	0.07 (1%)
10-20	0.08 (3%)	0.12 (4%)	0.25 (11%)	0.25 (11%)	0.12 (4%)	0.08 (3%)
20-30	0.06 (3%)	0.07 (3%)	0.08 (4%)	0.08 (4%)	0.07 (3%)	0.06 (3%)
30-40	0.02 (1%)	0.07 (2%)	0.07 (4%)	0.07 (4%)	0.07 (2%)	0.02 (1%)
	Soil tillage : NT					
0-5	0.18 (3%)	0.13 (2%)	0.44 (6%)	0.44 (6%)	0.13 (2%)	0.18 (3%)
5-10	0.14 (2%)	0.35 (5%)	0.54 (7%)	0.54 (7%)	0.35 (5%)	0.14 (2%)
10-20	0.06 (2%)	0.18 (4%)	0.24 (6%)	0.24 (6%)	0.18 (4%)	0.06 (2%)
20-30	0.03 (1%)	0.09 (2%)	0.14 (4%)	0.14 (4%)	0.09 (2%)	0.03 (1%)
30-40	0.04 (1%)	0.05 (1%)	0.11 (3%)	0.11 (3%)	0.05 (1%)	0.04 (1%)

Addendum 6-5. Average 2D distribution of effective phosphate ion amounts in solid and liquid phases (kg P ha^{-1}) of four blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2014.

P fertilization	**Distance from row (cm)**					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	4.80	4.69	5.29	5.29	4.69	4.80
5-10	4.77	5.48	5.16	5.16	5.48	4.77
10-20	9.33	11.06	9.44	9.44	11.06	9.33
20-30	11.30	12.87	11.89	11.89	12.87	11.30
30-40	7.62	6.68	6.01	6.01	6.68	7.62
	Soil tillage : NT					
0-5	8.67	7.69	7.01	7.01	7.69	8.67
5-10	5.87	5.24	4.87	4.87	5.24	5.87
10-20	11.10	10.95	10.05	10.05	10.95	11.10
20-30	7.76	6.88	6.04	6.04	6.88	7.76
30-40	5.54	5.70	6.27	6.27	5.70	5.54

P fertilization	Distance from row (cm)					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	6.07	6.07	6.07	6.22	6.05	6.09
5-10	6.34	6.34	6.34	8.99	6.98	5.52
10-20	13.20	13.20	13.20	13.52	14.46	11.48
20-30	11.76	11.76	11.76	9.97	10.44	12.73
30-40	5.61	5.61	5.61	5.61	5.97	5.14
	Soil tillage : NT					
0-5	10.00	10.00	10.00	11.32	9.76	10.14
5-10	8.61	8.61	8.61	12.64	7.15	9.27
10-20	14.23	14.23	14.23	20.28	13.66	14.66
20-30	9.16	9.16	9.16	8.66	9.40	8.89
30-40	5.47	5.47	5.47	6.01	5.55	5.37

P fertilization	Distance from row (cm)					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	7.29	7.29	7.29	6.66	7.32	7.23
5-10	6.67	6.67	6.67	10.23	6.98	6.28
10-20	13.45	13.45	13.45	14.19	14.16	12.61
20-30	13.18	13.18	13.18	11.54	13.68	12.55
30-40	7.37	7.37	7.37	6.97	7.35	7.35
	Soil tillage : NT					
0-5	14.10	14.10	14.10	13.48	13.87	14.27
5-10	10.35	10.35	10.35	16.78	10.95	9.25
10-20	14.61	14.61	14.61	14.86	14.87	13.02
20-30	9.75	9.75	9.75	9.05	10.17	9.14
30-40	6.31	6.31	6.31	6.11	5.71	6.81

Addendum 6-6. Average 2D distribution of effective phosphate ion amounts in solid and liquid phases (kg P ha^{-1}) of four blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2015.

P fertilization	Distance from row (cm)					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**

Layers (cm)	Soil tillage : MP					
0-5	5.00	5.00	5.00	5.00	5.00	5.00
5-10	5.00	5.00	5.00	5.00	5.00	5.00
10-20	10.00	10.00	10.00	10.00	10.00	10.00
20-30	11.30	12.87	11.89	11.89	12.87	11.30
30-40	7.62	6.68	6.01	6.01	6.68	7.62
	Soil tillage : NT					
0-5	8.67	7.69	7.01	7.01	7.69	8.67
5-10	5.87	5.24	4.87	4.87	5.24	5.87
10-20	11.10	10.95	10.05	10.05	10.95	11.10
20-30	7.76	6.88	6.04	6.04	6.88	7.76
30-40	5.54	5.70	6.27	6.27	5.70	5.54

P fertilization	Distance from row (cm)					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	6.51	6.51	6.51	6.51	6.51	6.51
5-10	6.51	6.51	6.51	6.51	6.51	6.51
10-20	13.01	13.01	13.01	13.01	13.01	13.01
20-30	11.76	11.76	11.76	9.97	10.44	12.73
30-40	5.61	5.61	5.61	5.61	5.97	5.14
	Soil tillage : NT					
0-5	10.00	10.00	10.00	11.32	9.76	10.14
5-10	8.61	8.61	8.61	12.64	7.15	9.27
10-20	14.23	14.23	14.23	20.28	13.66	14.66
20-30	9.16	9.16	9.16	8.66	9.40	8.89
30-40	5.47	5.47	5.47	6.01	5.55	5.37

P fertilization	Distance from row (cm)					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	7.00	7.00	7.00	7.00	7.00	7.00
5-10	7.00	7.00	7.00	7.00	7.00	7.00
10-20	13.99	13.99	13.99	13.99	13.99	13.99
20-30	13.18	13.18	13.18	11.54	13.68	12.55

30-40	7.37	7.37	7.37	6.97	7.35	7.35
	Soil tillage : NT					
0-5	14.10	14.10	14.10	13.48	13.87	14.27
5-10	10.35	10.35	10.35	16.78	10.95	9.25
10-20	14.61	14.61	14.61	14.86	14.87	13.02
20-30	9.75	9.75	9.75	9.05	10.17	9.14
30-40	6.31	6.31	6.31	6.11	5.71	6.81

Addendum 6-7. Average P uptake proportions (***Pup%***) by maize roots along soil profile in four blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2014.

P fertilization	**Distance from row (cm)**					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	1%	1%	8%	8%	1%	1%
5-10	3%	2%	4%	4%	2%	3%
10-20	5%	2%	6%	6%	2%	5%
20-30	5%	4%	6%	6%	4%	5%
30-40	1%	1%	2%	2%	1%	1%
	Soil tillage : NT					
0-5	2%	3%	5%	5%	3%	2%
5-10	3%	2%	4%	4%	2%	3%
10-20	6%	3%	9%	9%	3%	6%
20-30	3%	2%	3%	3%	2%	3%
30-40	2%	1%	3%	3%	1%	2%

P fertilization	**Distance from row (cm)**					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	1%	2%	3%	3%	2%	1%
5-10	2%	1%	4%	5%	2%	2%
10-20	7%	4%	10%	10%	4%	7%
20-30	4%	3%	6%	5%	3%	4%
30-40	1%	1%	2%	2%	1%	1%
	Soil tillage : NT					
0-5	4%	3%	7%	8%	3%	4%

5-10	1%	2%	4%	5%	1%	1%
10-20	3%	4%	8%	12%	4%	3%
20-30	2%	1%	3%	3%	1%	2%
30-40	1%	1%	2%	2%	1%	1%

P fertilization	**Distance from row (cm)**					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	2%	1%	6%	6%	1%	2%
5-10	2%	1%	4%	5%	1%	2%
10-20	6%	4%	7%	7%	4%	5%
20-30	3%	3%	6%	6%	4%	2%
30-40	2%	1%	2%	2%	1%	1%
	Soil tillage : NT					
0-5	6%	3%	8%	7%	3%	6%
5-10	3%	1%	3%	5%	2%	3%
10-20	5%	3%	7%	7%	3%	5%
20-30	1%	1%	4%	3%	1%	1%
30-40	1%	1%	2%	2%	1%	1%

Addendum 6-8. Average P up take proportions (***Pup%***) along soil profile by soybean roots of four blocks under MP and NT receiving 0P, 0.5P and 1P treatments in 2015.

P fertilization	**Distance from row (cm)**					
0P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0%	1%	3%	3%	1%	0%
5-10	0%	2%	3%	3%	2%	0%
10-20	3%	15%	10%	10%	15%	3%
20-30	1%	3%	4%	4%	3%	1%
30-40	1%	1%	2%	2%	1%	1%
	Soil tillage : NT					
0-5	2%	3%	10%	10%	3%	2%
5-10	3%	3%	5%	5%	3%	3%
10-20	2%	7%	5%	5%	7%	2%
20-30	1%	3%	3%	3%	3%	1%

30-40	1%	1%	1%	1%	1%	1%

P fertilization	**Distance from row (cm)**					
0.5P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	0%	1%	3%	3%	1%	0%
5-10	1%	2%	5%	5%	2%	1%
10-20	5%	8%	14%	14%	8%	5%
20-30	2%	4%	3%	3%	3%	2%
30-40	1%	1%	1%	1%	1%	1%
	Soil tillage : NT					
0-5	2%	3%	5%	5%	2%	2%
5-10	2%	2%	6%	10%	2%	2%
10-20	2%	4%	12%	16%	4%	2%
20-30	1%	1%	3%	2%	2%	1%
30-40	1%	1%	2%	2%	1%	1%

P fertilization	**Distance from row (cm)**					
1P	**(-30)-(-20)**	**(-20)-(-10)**	**(-10)-0**	**0-10**	**10-20**	**20-30**
Layers (cm)	**Soil tillage : MP**					
0-5	1%	1%	1%	1%	1%	1%
5-10	1%	3%	4%	4%	3%	1%
10-20	4%	6%	14%	14%	6%	4%
20-30	3%	4%	5%	4%	4%	3%
30-40	1%	2%	2%	2%	2%	1%
	Soil tillage : NT					
0-5	4%	2%	7%	7%	2%	4%
5-10	2%	4%	6%	10%	5%	2%
10-20	2%	5%	8%	7%	5%	2%
20-30	1%	2%	4%	4%	2%	1%
30-40	1%	1%	1%	1%	1%	1%

Chapter VII Simplified model for phosphorus cycle in long-term no-till agroecosystems

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***Running title*:** Simplified simulation P model in different tillage systems

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7.1. Résumé

Un modèle simplifié a été élaboré pour la couche labourée où la distribution de P du sol est considérée comme homogène. Cependant, l'hétérogénéité de la distribution de P sous semis direct (no-till ou NT) doit considérer la stratification du P dans le sol, et donc subdivision du sol en zones de distribution relativement homogène du P. Dans cette étude, un modèle simplifié permet d'estime le P disponible à long-terme dans chaque maille de la zone du sol sous NT à partir du bilan du P. Le modèle simule l'évolution des concentrations en ions phosphates dans la solution du sol en NT recevant trois doses de fertilisation de P (0, 17,5 et 35 kg P ha^{-1}) sur une prériode de 24 ans.

Mots-clés: racines, ions phosphates, bilan du phosphore

7.2. Abstract

Models have been developed to simulate available P in the plough layer where soil P distribution is considered homogeneous. However, soil P stratification under no-till (NT) require to divide soil zone into smaller grids assumed to be homogeneous. We elaborated a P model to estimate soil P availability under NT by grid unit from P budget for each grid. The model simulates changes in phosphate ion concentrations in soil solution along soil profile under NT receiving three P dosages (0, 17.5 and 35 kg P ha^{-1}) for a period of 24 years.

Key-words: roots, phosphorus budget, phosphate ions

7.3. Introduction

Phosphorus (P) from fertilization and crop residue restitution can be retained in arable layer while soil P can be lost to watercourses and groundwater by erosion, runoff or leaching. Simulation models have been developed to describe the P cycle in agroecosystem. Most models are process-based (Kautz et al., 2013) with mathematical descriptions of fundamental physio-chemical mechanisms (Jones et al., 1984). Silberbush and Barber (1983) used a Cushman model to simulate P uptake of plants by considering root size change with time, P flux into the root as related to P concentration in the soil solution at the root surface, and P supply to the root by mass-flow and diffusion. Groenendijk et al. (2005) developed the Agricultural Nutrient Model (ANIMO) that simulates hydrological processes, water and nutrient transfers, crop production and soil P cycle to predict P leaching. For more complex models, it is costly and time consuming to acquire more data for initial state estimation and boundary conditions (Tague and Band, 2004). Simulation models usually run on a small time-step e.g. sub-daily, daily or multi-daily (Daroub et al., 2003; Groenendijk et al., 2005; Jones et al., 1984). Every time-step can cause biases and ensuing corrections (Ines and Hansen, 2006). Simpler empirical approach could suffice (Jones et al., 1984; Lewis and McGechan, 2002).

Simplified model at large time-step might be useful for long-term simulation of soil-plant P cycles in agroecosystem. Soil P status at a field-scale is closely related over a long run to the cumulative annual P budget computed as the sum of P inputs and outputs (Messiga et al., 2010; Oehl et al., 2002; Simpson et al., 2015). Messiga et al. (2015) have developed a mass-balance model to estimate soil P availability using P budget and phosphate ion transfer kinetic between solid and liquid soil phases. However, this P cycle model was elaborated for the plough layer under conventional tillage where soil P distribution is homogeneous. Conservation agriculture including no-till (NT) has been implemented over the world (Derpsch and Friedrich, 2009; Kassam et al., 2012). The NT leads to heterogeneous soil P distribution, viz. P stratification, along soil profile (Calegari et al., 2013; Lupwayi et al., 2006). The (Messiga et al., 2015) model should be stratified into smaller soil units where P distribution can be assumed to be relatively homogeneous.

In this study, we intended to elaborate a model structure based on the combination of P budget estimation and phosphate ion transfer kinetics for soil grid units. The model was tested with data collected at a long-term experimental site under NT at l'Acadie, Quebec, Canada, with three P dosages.

7.4. Materials and methods

7.4.1. Model structure

A soil profile 40 cm deep and 60 cm wide represented the rooted zone from crop-row to inter-row in a long-term corn-soybean rotation experiment established in 1992 at the l'Acadie experimental station of Agriculture and Agri-Food Canada (45°18' N; 73°21' W). The design was a split-plot design with two tillage (moldboard plough, MP; no-till, NT) and three P fertilization (0, 17.5 and 35 kg P ha-1 every two years on corn phase, referring to 0P, 0.5P and 1P, respectively) treatments. Details are reported in Ziadi et al. (2014) and Li et al. (2016).

The structure of model is presented in **Fig. 7-1**. The soil profile is divided into 30 soil grid units with five depths (0-5, 5-10, 10-20, 20-30 and 30-40 cm) and three lateral distances on both sides of the row [(-30)-(-20), (-20)-(-10), (-10)-0, 10-20, 20-30 cm]. For each unit, P stock is defined according to Messiga et al. (2012) as the sum of diffusive phosphate ions in solid phase and liquid phase (soil solution). The P inputs (P fertilizer, P in shoot and root residues, P leaching from upper unit) and outputs (P offtake by grain, P leaching to deeper units and P runoff) over one year participate in phosphate ion equilibrium between the solid

and liquid phases. The inputs and outputs are accounted for in the P budget for each unit at every time-step, influencing P stocks (phosphate ion concentration in soil solution). The P stocks and fluxes are expressed in kg P ha^{-1}.

To compute P flows, the following data were collected: 1. Soil bulk density in 2014 considered constant during the 1992-2015 period; 2. Corn and soybean root data analyzed in 2014 and 2015, respectively, and considered constant during the 1992-2015 period; 3. Crop grain yields and grain P contents measured every year during the 1992-2015 period; 4. Annual precipitation and annual temperature collected from the web-site of Climate-Environment Canada during the 1992-2015 period. The data for soil bulk density and crop roots were measured on one side of the row. We assumed that root distribution was symmetric about the row. Data collected in MP plots allowed computing the initial variables to launch the simulation, while the data collected from NT plots simulated soil P status over the long run.

7.4.2. P flux estimation

P stock

There are several definitions of soil P stocks (Barber, 1995). Plants extract phosphate ions from soil solution, and this quantity is mainly controlled by sorption and desorption mechanism between the solid and liquid phases (Balemi and Negisho, 2012). The P stock is thus defined as the amount of diffusive phosphate ions in soil including phosphate ions sorbed on solid phase (***Pr***) and phosphate ions in soil solution. The ***Cp*** was the index of soil P status (Achat et al., 2011). The long-term relationship between ***Cp*** and ***Pr*** is described by Freundlich kinetics as follows (**Eq 7-1**) (Messiga et al., 2012) :

$$Pr = v \times Cp^{w}\, t^{p} \qquad Pr < Pr_{limit} \qquad \textbf{Eq 7-1}$$

where ***Pr*** is the amount of diffusive phosphate ions in solid phase (mg P kg^{-1} soil); Pr_{limit} is the limited amount of diffusive phosphate ions that could be adsorbed onto the solid phase (mg P kg^{-1} soil); ***Cp*** is the concentration of phosphate ions in soil solution (mg P L^{-1}); ***t*** is the time (min); ***v***, ***w*** and ***p*** are fitted parameters.

The ***Cp*** values were measured in 1:10 (1g soil in 10ml water) soil suspensions according to Stroia et al. (2007). The Freundlich kinetics equation parameters were found to be ***v***=19.9, ***w***=0.42 and ***p***=0.30 for the 0-5 cm layer in the experimental site, mentioned in Chapter VI. We assumed that such parameters can be used across the 40-cm soil profile under NT. The P stock for each soil grid unit was computed as folows (**Eq 7-2**):

$$P_{stock,\, i\text{-}j} = (Dep_{i\text{-}j} / 10 \times BD_{i\text{-}j} \times Pr_{i\text{-}j} + Dep_{i\text{-}j} \times Cp_{i\text{-}j} \times BD_{i\text{-}j})/6$$
$$i = 1, 2, 3, 4, 5;\ j = -3, -2, -1, 1, 2, 3 \qquad \textbf{Eq 7-2}$$

where ***i*** and ***j*** refer to vertical and lateral distances $P_{stock,\, i\text{-}j}$ is P stock for soil grid unit ***i-j*** (kg P ha^{-1}); $Dep_{i\text{-}j}$ and $BD_{i\text{-}j}$ are the depth (cm) and soil bulk density (g cm^{-3}) for ***i-j*** unit.

The ***BD*** value measured in 2014 were considered constant. The time (***t***) to reach sorption-desorption equilibrium was fixed at one year (525 600 min). Initial ***Cp*** was assumed to be uniform values in the 0-20 cm layer and to decrease at 20-30 and 30-40 cm. Initial ***Cp*** values were estimated from the ***Cp*** measured in 2014 in MP plots. According to Messiga et al. (2010), soil P status, including ***Cp***, are linearly related with cumulative P budget for the plough layer as shown for MP 0-20 cm layer in 2014 (**Fig. 7-2**). Initial ***Cp*** for the 20 cm layer was fixed at 0.0916 mg P L^{-1}. Although the contribution of P uptake from below the plough layer was ignored (Messiga et al., 2010), initial ***Cp*** for the 20-30 and 30-40 cm layers was computed as average values of measured ***Cp*** in MP plots in 2014 (**Table 7-1**). Initial P stock

for each grid unit was estimated from initial ***Cp*** computed according to **Eq 7-2**. The output of a new P stock after one time-step is used as initial P stock at next time-step.

P fertilizer

The P dosage can increase soil P content (Sims et al., 1998). Most of added mineral P is retained by soil constituents by adsorption (Balemi and Negisho, 2012). The soil volume perturbed by P fertilizer is constrained to a very limited zone around the fertilizer band. Stecker et al. (2001) observed that the zone affected by fertilizer in one year did not exceed 5-cm radially. At l'Acadie the P fertilizer was banded 5-cm to the rows at a depth of 5 cm. The P fertilizer could thus influence the soil grid units 1-1 and 2-1 (**Fig. 7-1**). We assumed that the P fertilizer was uniformly distributed in the 1-1 and 2-1 grid units (**Eq 7-3**). The inputs of P fertilizer for the other grid units were zero at every time-step.

$$P_{fertilizer,\, i\text{-}1} = P_{fertilizer} / 2 \qquad i = 1, 2 \qquad \textbf{Eq 7-3}$$

where $P_{fertilizer,\, i\text{-}1}$ is P fertilizer input (kg P ha^{-1}) for soil grid unit ***i-1***; $P_{fertilizer}$ is the total P fertilizer input (kg P ha^{-1}).

P uptake

The P uptake depends on root growth and soil P status. The dynamic process for absorption of dissolved inorganic phosphate ions through the root surface follows Michaelis-Menten kinetics (Barber, 1995). Dynamic modules were developed to compute the amount of P uptake (Barber and Mackay, 1986; Silberbush and Barber, 1983), requiring P uptake at root surface and soil P movement. Another approach is to calculate the weighted P uptake of each grid unit by multiplying total P uptake and weighting factor for each unit. Daroub et al. (2003) estimated the weighting factor from measurements of roots, water availability, labile P in soil solution and labile P in solid phase that replenishes soil solution.

We assumed that soil water availability does not affect P uptake annual precipitation is about 900 mm. Over a relative short time-step such as daily or monthly time-step in the model of Daroub et al. (2003), phosphate ions in solution (***Cp***) and in solid phase (***Pr***) could be assessed separately and assumed tro be constant at each time-step. With a yearly time-step, ***Cp*** and ***Pr*** values are not stationary. It is reasonable to use P stock to estimate the P uptake weighting factor at each time-step. Although P from fertilizer and crop residues can be taken up, only a small part is used by plants during the season (Linères and Morel, 2004).

Unlike specific models (Barber and Mackay, 1986; Ge et al., 2000), root growth was not considered in the present model. Root distribution was the indicator of nutrient acquisition (Ho et al., 2004). Corn root distribution at stage R1 and soybean root distribution at the beginning of flowering analyzed in 2014 and 2015, respectively, are assumed to be constant and representative of yearly distribution (Guan et al., 2007; Kaspar et al., 1978; Mengel and Barber, 1974; Torrion et al., 2012). The symbiosis between roots and mycorrhiza, that also contributes to crop P acquisition (Atwell et al., 1999; Richardson et al., 2011), was not taken into account in this model. The weighting factors for P uptake were computed as follows:

$$Weight_{P\ uptake,\, i\text{-}j} = RS_{i\text{-}j} \times P_{stock,\, i\text{-}j} / \sum(RS_{i\text{-}j} \times P_{stock,\, i\text{-}j})$$
$$i = 1, 2, 3, 4, 5;\ j = -3, -2, -1, 1, 2, 3 \qquad \textbf{Eq 7-4}$$

where $Weight_{P\ uptake,\, i\text{-}j}$ is the weighting factor of P uptake for grid unit ***i-j***; $RS_{i\text{-}j}$ is root surface (cm^2) for grid unit ***i-j*** ; $P\ stock_{i\text{-}j}$ is the P stock (kg P ha^{-1}), including phosphate ions in soil solution and solid phase, for grid unit ***i-j***.

Total amount of P uptake consisted of three parts: 1. P in grains, 2. P in shoot, 3. P in root. Only P in grain was measured by multiplying grain yields by P concentration. Crop residue P could be estimated from P in grain.

The ratio of P amount in corn grain to shoot averages 2.5 according to Parentoni and Souza Júnior (2008), but could be lower at 30%. Brunel-Muguet et al. (2014) reported that at the end of corn reproductive stage, the amount of P in corn roots was 5-10% of the P in corn shoot. Similar results were reported by Yu et al. (2015) at corn maturity. Assuming constant ratio until maturity, corn root P should be 2-4% of corn grain P. This value was 6.1% computed from our data of average corn grain P exportation (15.85 kg P ha^{-1}), average corn root mass density (0.08 mg m^{-2}) and average P content in corn roots (0.12 mg P kg^{-1}) in 2014. Araújo and Teixeira (2008) found, for common bean cultivars, an average phosphorus harvest index (PHI) of 0.89, viz. P in shoot was about 12.5% of P in grain. We assumed that the value for common bean could be used for soybean. For soybean grain P exportation in 2015 (8.62 kg P ha^{-1}), soybean root mass density (0.06 mg m^{-2}) and P content in soybean roots (0.12 mg P kg^{-1}) (we assumed to be the same as corn roots), P in soybean roots was about 8% of P in grain. Total P uptake for one time-step in the model was estimated as follows:

$$\boldsymbol{P_{uptake} = P_{grain} \times (1 + a + b)} \qquad \textbf{Eq 7-5}$$

where $\boldsymbol{P_{uptake}}$ is total amount of P uptake (kg P ha^{-1}); $\boldsymbol{P_{grain}}$ is amount of P in grain (kg P ha^{-1}); $\boldsymbol{a}$ and $\boldsymbol{b}$ are ratios of P in shoot residues and root residues, respectively, divided by P in grain. The $\boldsymbol{a}$ and $\boldsymbol{b}$ are kept constants (0.3 and 0.05 for corn; 0.125 and 0.08 for soybean).

The amount of P uptake for each soil grid unit for one time-step is computed as follows:

$$\boldsymbol{P_{uptake,\, i\text{-}j} = P_{uptake} \times Weight_{P\ uptake,\, i\text{-}j}}$$
$$\boldsymbol{i = 1, 2, 3, 4, 5;\ j = -3, -2, -1, 1, 2, 3} \qquad \textbf{Eq 7-6}$$

where $\boldsymbol{P_{uptake,\, i\text{-}j}}$ is the amount of P uptake (kg P ha^{-1}) in the $\boldsymbol{i\text{-}j}$ soil grid unit.

Combining **Eqs 7-4**, **7-5** and **7-6**, we obtain:

$$\boldsymbol{P_{uptake,\, i\text{-}j} = P_{grain} \times (1 + a + b) \times RS_{i\text{-}j} \times P_{stock,\, i\text{-}j} \,/\, \sum RS_{i\text{-}j} \times P_{stock,\, i\text{-}j}} \qquad \textbf{Eq 7-7}$$

P in residues

Because crop residues were left on soil surface, P restitution from crop residue replenishes P stock at every time-step. Gilmour et al. (1998) measured 60% decomposition of soybean shoot residues within 60 days. The decomposition rate of corn residues could be even faster (Ajwa and Tabatabai, 1994). Shoot residues decompose completely in a year (one time-step). Moreover, it was reasonable to think that root residues had a similar or even higher decomposition rate as shoot residues because they are in direct contact with soil to stimulate decomposition (Henriksen and Breland, 2002). Because shoot residues were left on soil surface uniformly, P restitution from shoot residues occurred only on soil grid units 1-(-3), 1-(-2), 1-(-1), 1-1, 1-2 and 1-3. For the other grids, the P input from shoot residues were set at zero. Restitution was computed as follows:

$$\boldsymbol{P_{shoot\ residue,\, 1\text{-}j} = P_{shoot\ residue} \,/\, 6 = P_{grain} \times a \,/\, 6}$$
$$\boldsymbol{j = -3, -2, -1, 1, 2, 3} \qquad \textbf{Eq 7-8}$$

where $\boldsymbol{P_{shoot\ residue,\, 1\text{-}j}}$ is the amount of P restitution from shoot residues (kg P ha^{-1}) for the $\boldsymbol{1\text{-}j}$ soil grid unit; $\boldsymbol{P_{shoot\ residue}}$ is total amount of P restitution from shoot residues (kg P ha^{-1}).

On the other hand, P restitution from root residues that depends on the distribution of root biomass was estimated as follow:

$$P_{root\ residue,\ i\text{-}j} = P_{root\ residue} \times RM_{i\text{-}j} / \sum RM_{i\text{-}j} = P_{grain} \times b \times RM_{i\text{-}j} / \sum RM_{i\text{-}j}$$
$$i = 1, 2, 3, 4, 5;\ j = -3, -2, -1, 1, 2, 3 \qquad \textbf{Eq 7-9}$$

where $P_{root\ residue,\ i\text{-}j}$ is the amount of P restitution from root residues (kg P ha^{-1}) for the ***i-j*** soil grid unit; $P_{root\ residu}$ is total amount of P restitution from root residues (kg P ha^{-1}); $RM_{i\text{-}j}$ is root biomass (mg) for the ***i-j*** soil grid unit.

P runoff and P leaching

Surface runoff and leaching are two pathways for P loss from agricultural soil to receiving waters (Zhang et al., 2003). The P losses are quantified by the concentrations of dissolved P and particle P (Tan and Zhang, 2011). Loss of particle P is related to soil erosion (Carroll et al., 2000). At l'Acadie, the slope was 0.2%, thus P in runoff and leaching was set to be in the dissolved form, viz. phosphate ions in soil solution.

The estimation of runoff and leaching fluxes allows computing the amount of P loss by runoff and leaching estimated according to Ghiglieri et al. (2014) based on annual precipitation and temperature. Evaporation, runoff flux and infiltration flux werecomputed as follows:

$$Eva = Pre/\{[0.9 + Pre^2 / (300 + 25T + 0.05T^3)]^2\}^{0.5} \qquad \textbf{Eq 7-10}$$
$$Runoff = (Pre - Eva) \times C_{runoff} \qquad \textbf{Eq 7-11}$$
$$Leaching = Pre - Eva - Runoff \qquad \textbf{Eq 7-12}$$

where ***Pre*** is annual precipitation (mm); ***T*** is annual temperature (°C); C_{runoff} is runoff coefficient that depends on slope, land cover and soil permeability. The C_{runoff} value was 0.19 in the model for soil with slope of < 3.5% and medium permeability. ***Eva***, ***Runoff*** and ***Leaching*** are amount of evaporation, runoff and leaching (mm).

The P runoff was assumed to occur on soil grid units 1-(-3), 1-(-2), 1-(-1), 1-1, 1-2 and 1-3. Assuming uniform distribution of runoff flux, the P runoff could be estimated as follows:

$$P_{runoff,\ 1\text{-}j} = Runoff / 6 \times Cp_{1\text{-}j} / 100$$
$$j = -3, -2, -1, 1, 2, 3 \qquad \textbf{Eq 7-13}$$

where $P_{runoff,\ 1\text{-}j}$ is the P output in runoff (kg P ha^{-1}) for the ***i-j*** soil grid unit ; $Cp_{1\text{-}j}$ is the phosphate ion concentration (mg P L^{-1}) for the ***i-j*** soil grid unit . For one grid unit, P leaching could be an input or an output of P computed as follows:

$$P_{leaching,\ i\text{-}j} = Leaching / 6 \times (Cp_{(i\text{-}1)\text{-}j} - Cp_{i\text{-}j})/100$$
$$i = 1, 2, 3, 4, 5;\ j = -3, -2, -1, 1, 2, 3 \qquad \textbf{Eq 7-14}$$

where $P_{leaching,\ i\text{-}j}$ is the P leaching amount (kg P ha^{-1}) for ***i-j*** soil grid; $Cp_{(i\text{-}1)\text{-}j}$ and $Cp_{i\text{-}j}$ are the phosphate ion concentrations (mg P L^{-1}) for ***(i-1)-j*** and ***i-j*** grid unit. When ***i*** =1, $Cp_{(i\text{-}1)\text{-}j}$ = 0.

Lateral mass flux of water and P diffusion could also be considered between every two neighboring soil grid units (Cameron and Klute, 1977). Flatness at l'Acadie site precluded lateral mass flux of water. The average P diffusion coefficient in soil is 1 x 10^{-8} to 10^{-10} cm^2 s^{-1} (Barber, 1995). Fick's first law showed that even under optimum conditions (maximum ***Cp*** and zero at two boundaries), the diffusive phosphate ion could not exceed 5-cm over one year. Hence lateral transport between soil grid units were not taken into account. At every time-step

the new P stock in each grid is computed as the sum of P stock, P inputs and P outputs (**Eq 7-15**).

$$\textit{New } P_{stock,i\text{-}j} = P_{stock,\ i\text{-}j} + P_{fertilizer,\ i\text{-}j} + P_{shoot\ residue,\ i\text{-}j} + P_{root\ residue,\ i\text{-}j} - P_{uptake,\ i\text{-}j} - P_{runoff,\ i\text{-}j} + P_{leaching,\ i\text{-}j}$$

$$i = 1, 2, 3, 4, 5;\ j = -3, -2, -1, 1, 2, 3 \qquad \textbf{Eq 7-15}$$

where ***New*** $P_{stock,i\text{-}j}$ is output of new P stock (kg P ha^{-1}) in ***i-j*** soil grid unit. Once the new P stock was calculated, the new ***Cp*** values is reversely computed according to **Eq 7-2** using the new P stock for use for the next time-step simulation.

7.5. Software

The model was elaborated with SAS (SAS Institute Inc., 2010).

7.6. Results and discussion

7.6.1. P stock and flux

The P stock and flux in **Fig. 7-1** were computed for each soil grid unit at each time-step. Results for NT1P plots (highest soil P status) in 2014 and 2015 are presented in **Tables 7-2** and **7-3**. The simulated P stocks in the 0-40 cm layer were 1916 and 1939 kg P ha^{-1} in 2014 and 2015, respectively. Considering average total P of about 500 mg P kg^{-1} (Bowman, 1988; Bray and Kurtz, 1945), viz. 2800 kg P ha^{-1}, the simulated P stock was within Pr_{limit}. Kautz et al. (2013) reported that the proportion of P stock for crop uptake in the subsoil (beneath 20 cm depth) ranged from 25 to 70%. The presented results of the model were within the range with about 34% of P stock under 20-cm depth along soil profile. The simulated results also showed that 29% and 25% of the P uptake for corn and soybean came from the subsoil in 2014 and 2015, respectively. Soybean had smaller P uptake proportion from subsoil than corn, it was because soybean roots explored more the high-P zone in the upper layers under NT compared to corn (Li et al., 2016). The simulated results for P uptake indicated that the P uptake from subsoil should not be ignored as in previous studies (Kautz et al., 2013; Messiga et al., 2010).

The simulated runoff P in **Tables 7-2** and **7-3** were 1.08 and 1.24 kg P ha^{-1} compared to 0.04 to 1.96 kg P ha^{-1} in New Zealand agricultural soils (Hart et al. 2004). The simulated P leaching was 0.06 kg P ha^{-1} across a 40-cm soil profile, much smaller than the estimated annual P of 2 kg P ha^{-1} leached from agricultural soils in Quebec (Messiga et al., 2012) computed as the sum of runoff P and leached P from top soil (0-20 cm). The sum of simulated P runoff and P leaching for the 0-20 cm layer were 1.36 and 1.50 kg P ha^{-1} for the model.

7.6.2. Soil P status evolution in NT

The simulated ***Cp*** change under NT and three P doses are presented in **Fig. 7-3** for the 0-5, 5-10, 10-20, 20-30 and 30-40 cm layers. The model simulates P stratification at high P level (***Cp*** values) in surface layers as usually found for NT systems (Calegari et al., 2013; Lupwayi et al., 2006; Messiga et al., 2010). In [NT, 0P], P stratification is due to P restitution from crop residues left on soil surface under NT (Scopel et al., 2013). The P restitution could increase, maintain or mitigate its decrease soil P status near soil surface. The P uptake contribution from subsoil averaging 31% and 25% for corn and soybean under [NT, 0P], respectively over 24 years, decreased ***Cp*** values in the 5-10, 10-20, 20-30 and 30-40 cm layers to build P stratification over time. The P stratifications under [NT, 0.5P] and [NT, 1P] are caused by the P fertilization. The P fertilizers banded 5-cm deep increased the ***Cp*** values in the 0-5 and 5-10 cm layers, while P uptake decreased the ***Cp*** values in the 10-20, 20-30 and 30-40 cm layers leading to P stratification. The P stratifications under [NT, 0.5P] and [NT, 1P]

developed in different ways at different distances from row. The ***Cp*** in each soil grid unit under [NT, 1P] is presented every five years in **Fig. 7-4**. Because the P fertilizer was constrained to the band, P stratification 5 cm from row was caused by P fertilization. The P stratifications at other distances (-25, -15, -5, 15 and 25 cm) is similar to [NT, 0P]. The simulated results for [NT, 0.5P] show the similar patterns as [NT, 1P].

7.6.3. Comparison with measured data

The comparisons between simulated and measured data under NT are presented in **Fig. 7-5**. The soil profiles with banded P fertilizer under [NT, 0.5P] and [NT, 1P] showed simulated ***Cp*** values much higher than observed ***Cp*** values in the 0-5 and 5-10 cm layers. Where P fertilizer input was zero, simulated ***Cp*** values tended to be smaller compared to the ***Cp*** values observed in the 0-5 and 5-10 cm layers. There are three possible reasons for this.

First, higher observed ***Cp*** values in the inter-row (15-, 25-cm distances) may indicate higher P restitution from shoot residues. Model ratios of P in shoot (***a*** in **Eq 7-5**) to P in grain were set at 0.4 for corn and 0.125 for soybean. However, Latati et al. (2014) reported corn shoot biomass of 21.75 t ha^{-1} and root mass of 2.25 t ha^{-1} at the R1-R2 stage at grain yield of 4 t ha^{-1}. Assuming P concentrations for corn roots, shoot P were about 30% grain P reported by Parentoni and Souza Júnior (2008), the values of ***a*** for corn could be 1.63 in Latati et al. (2014) rather than 0.4 in our model. With more P in residues, especially shoot residues, dispersed in inter-row, simulated ***Cp*** 15 and 25 cm from row would increase considerably. On the other hand, observed ***Cp*** values for the 20-30 cm layer were smaller than simulated values. It might indicate that more P was taken from subsoil in practice than in simulation and finally restituted to soil surface through crop residues under NT. The possible cause of this difference was that in the model, we did not consider soil water availability to estimate P uptake weight as in Daroub et al. (2003). Although the surface soil layer is high in P under NT, it also tends to be drier than deeper layers in absence of precipitation (Richards and Caldwell, 1987). Where water availability is low in the surface layer, nutrient uptake decreases (Farmaha et al., 2012) and the plant rely more on subsoil P (Kautz et al., 2013).

The second reason is lateral movement of soil P. The lateral diffusion of phosphate ions was ignored in the model. But the micro-fauna (>1 cm) could mix the soil in their gut (Lavelle, 1996; Lavelle and Blanchart, 1992), especially under NT where soil biodiversity is enhanced (Scopel et al., 2013). Chapuis-Lardy et al. (2009) reported that earthworms could roughly ingest 250 t of soil (casts) per year and hectare in a Ferralsols while Blouin et al. (2013) mentioned that earthworms could transport 40 t casts ha^{-1} $year^{-1}$ to soil surface under temperate climate conditions in Europe (based on 19 studies). Even though soil is likely to be ingested more than once (Blouin et al., 2013), annual amount of soil transported by bioturbation is considerable. Compared to bulk soil, earthworm casts have higher levels of available P (Vos et al., 2014). Blouin et al. (2013) estimated that the amount of P in casts was equivalent to 50% of the annual uptake by plants. The most probable earthworm species causing such a dispersion of soil P are the epigeic species living on soil surface, rarely digging burrows and leaving small casts on soil surface, and the anecic species living in permanent burrows and leaving casts mostly on surface and burrow walls (Piron et al., 2012). Besides bioturbation, horizontal flow in soil, suggested in hydraulic models (Beckers and Degré, 2011), could redistribute P, but this was ignored in our model.

At last, the P banding could be more dominant. In our model, P fertilizer was banded approximately 5 cm deep and 5 cm from row. Considering 5 cm error, the fertilizer band would move from 5-cm deep and 5-cm laterally to 5-cm deep, 10-cm laterally. The P fertilizer assumed to be distributed uniformly in 1-1 and 2-1 soil grids (**Fig. 7-1**) could affect four soil grid units (1-1, 1-2, 2-1 and 2-2 grids) and average P input would be two times smaller than predicted by the model because of P dilution in the soil mass. Over a long-term, total amount of P would be diluted into a larger soil volume than expected. The P dilution would decrease

Cp values in upper soil layers (0-5 and 5-10 cm layers) at 5-cm distance in the simulation model and increase the ***Cp*** values at the other distances.

The P stock model estimation (**Eq 7-1, 7-2**) could be further improved. For phosphate ion transfer at the interface between soil solid and liquid phases, the fitted parameters (***v***, ***w*** and ***p***) for the 0-5 cm layer were assumed to be valid for the soil profile across treatments. However, Messiga et al. (2012) pointed out that parameters could vary with soil properties. Moreover, time (***t***) to reach sorption-desorption equilibrium could be adjusted to P stock variation in response to the P budget.

7.7. Conclusions

The simulation model based on P budget discretized into soil grid units to simulate phosphate ions dynamics in a 24 year experiment with NT where P dosage was varied. Simulation showed gradual P stratification along soil profile under NT. Model parameters could be re-adjusted to match observed and simulated data more closely.

7.8. Acknowledgement

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Table 7-1 Initial phosphate ion concentration in soil solution (***Cp***) for soil units in the P model.

Cp (mg P L^{-1})	Lateral distances					
	(-30)-(-20) cm	**(-20)-(-10) cm**	**(-10)-0 cm**	**0-10 cm**	**10-20 cm**	**20-30 cm**
Depths						
0-5 cm	0.0916	0.0916	0.0916	0.0916	0.0916	0.0916
5-10 cm	0.0916	0.0916	0.0916	0.0916	0.0916	0.0916
10-20 cm	0.0916	0.0916	0.0916	0.0916	0.0916	0.0916
20-30 cm	0.0522	0.0522	0.0522	0.0522	0.0522	0.0522
30-40 cm	0.0145	0.0145	0.0145	0.0145	0.0145	0.0145

Table 7-2 Simulated P stock and flux (P fertilization and uptake, shoot P restitution, root P restitution, P runoff and P leaching) in each soil grid unit of the soil zone (60 cm × 40 cm) under NT1P in 2014 (corn year)

	P stock	P dose	P uptake	Shoot P	Root P	P runoff	P leaching
				kg P ha^{-1}			
Distance: -25 cm							
0-5 cm	45.43	0	0.49	0.71	0.014	0.029	0.106
5-10 cm	41.08	0	0.27	0	0.011	0	-0.037
10-20 cm	70.98	0	1.05	0	0.025	0	-0.021
20-30 cm	71.92	0	0.44	0	0.010	0	-0.009
30-40 cm	43.16	0	0.33	0	0.013	0	-0.029
Distance: -15 cm							
0-5 cm	48.86	0	0.27	0.71	0.008	0.035	0.126
5-10 cm	41.26	0	0.15	0	0.007	0	-0.056
10-20 cm	70.38	0	0.61	0	0.015	0	-0.023
20-30 cm	70.45	0	0.31	0	0.007	0	-0.010
30-40 cm	45.05	0	0.20	0	0.007	0	-0.026
Distance: -5 cm							
0-5 cm	43.25	0	0.54	0.71	0.052	0.026	0.094
5-10 cm	37.89	0	0.30	0	0.036	0	-0.037
10-20 cm	55.27	0	1.26	0	0.078	0	-0.030
20-30 cm	56.07	0	0.96	0	0.044	0	-0.005
30-40 cm	37.70	0	0.51	0	0.029	0	-0.014
Distance: 5 cm							
0-5 cm	196.35	17.5	2.44	0.71	0.052	0.922	3.317
5-10 cm	224.39	17.5	1.75	0	0.036	0	0.439
10-20 cm	74.09	0	1.69	0	0.078	0	-3.703
20-30 cm	56.18	0	0.96	0	0.044	0	-0.031
30-40 cm	37.70	0	0.51	0	0.029	0	-0.014
Distance: 15 cm							
0-5 cm	48.86	0	0.27	0.71	0.008	0.035	0.126
5-10 cm	41.26	0	0.15	0	0.007	0	-0.056
10-20 cm	70.38	0	0.61	0	0.015	0	-0.023
20-30 cm	70.45	0	0.31	0	0.007	0	-0.010
30-40 cm	45.05	0	0.20	0	0.007	0	-0.026
Distance: 25 cm							
0-5 cm	45.43	0	0.49	0.71	0.014	0.029	0.106
5-10 cm	41.08	0	0.27	0	0.011	0	-0.037
10-20 cm	70.98	0	1.05	0	0.025	0	-0.021
20-30 cm	71.92	0	0.44	0	0.010	0	-0.009
30-40 cm	43.16	0	0.33	0	0.013	0	-0.029
Total	1916.03	35	19.12	4.25	0.71	1.08	0.06

Table 7-3 Simulated P stock and flux (P fertilization and uptake, shoot P restitution, root P restitution, P runoff and P leaching) for each soil grid unit of the soil zone (60 cm × 40 cm) under NT1P in 2015 (soybean year)

	P stock	P dose	P uptake	Shoot P	Root P	P runoff	P leaching
				kg P ha^{-1}			
Distance: -25 cm							
0-5 cm	45.46	0	0.13	0.20	0.027	0.030	0.108
5-10 cm	40.88	0	0.09	0	0.011	0	-0.039
10-20 cm	70.05	0	0.27	0	0.026	0	-0.022
20-30 cm	71.74	0	0.15	0	0.008	0	-0.008
30-40 cm	43.07	0	0.12	0	0.016	0	-0.029
Distance: -15 cm							
0-5 cm	49.09	0	0.10	0.20	0.018	0.036	0.129
5-10 cm	41.20	0	0.23	0	0.034	0	-0.059
10-20 cm	69.89	0	0.77	0	0.066	0	-0.023
20-30 cm	70.41	0	0.39	0	0.028	0	-0.009
30-40 cm	45.10	0	0.13	0	0.015	0	-0.026
Distance: -5 cm							
0-5 cm	43.29	0	0.30	0.20	0.069	0.027	0.096
5-10 cm	37.69	0	0.32	0	0.072	0	-0.039
10-20 cm	54.17	0	0.81	0	0.134	0	-0.031
20-30 cm	55.36	0	0.50	0	0.051	0	-0.004
30-40 cm	37.42	0	0.25	0	0.038	0	-0.014
Distance: 5 cm							
0-5 cm	209.07	0	1.44	0.20	0.069	1.083	3.895
5-10 cm	240.16	0	2.03	0	0.072	0	0.529
10-20 cm	74.80	0	1.12	0	0.134	0	-4.370
20-30 cm	55.49	0	0.50	0	0.051	0	-0.033
30-40 cm	37.42	0	0.25	0	0.038	0	-0.014
Distance: 15 cm							
0-5 cm	49.09	0	0.10	0.20	0.018	0.036	0.129
5-10 cm	41.20	0	0.23	0	0.034	0	-0.059
10-20 cm	69.89	0	0.77	0	0.066	0	-0.023
20-30 cm	70.41	0	0.39	0	0.028	0	-0.009
30-40 cm	45.10	0	0.13	0	0.015	0	-0.026
Distance: 25 cm							
0-5 cm	45.46	0	0.13	0.20	0.027	0.030	0.108
5-10 cm	40.88	0	0.09	0	0.011	0	-0.039
10-20 cm	70.05	0	0.27	0	0.026	0	-0.022
20-30 cm	71.74	0	0.15	0	0.008	0	-0.008
30-40 cm	43.07	0	0.12	0	0.016	0	-0.029
Total	1938.60	0	12.27	1.23	1.23	1.24	0.06

Fig. 7-1

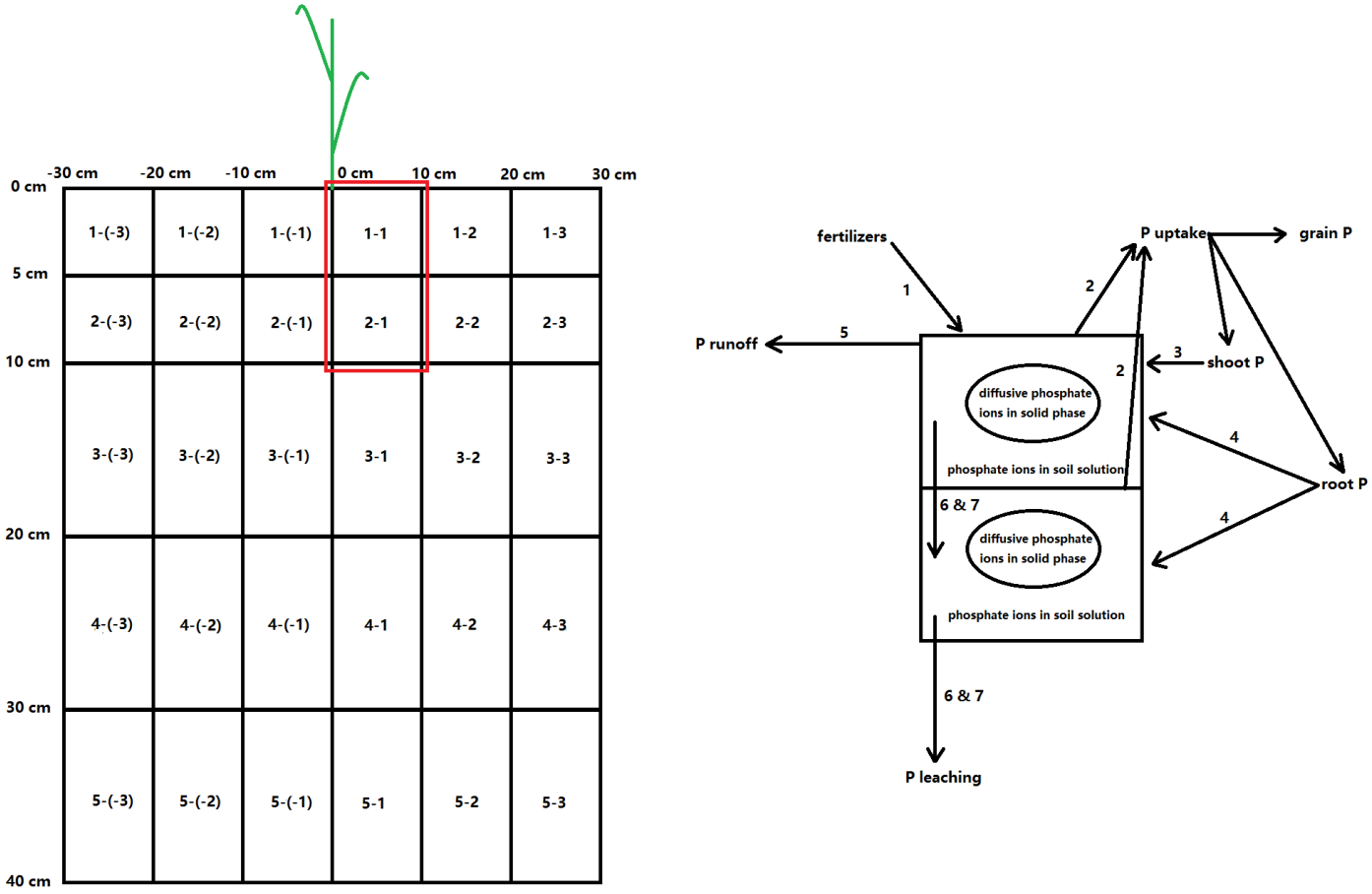
-30 cm
-20 cm
-10 cm
0 cm
10 cm
20 cm
30 cm
0 cm
5 cm
10 cm
20 cm
30 cm
40 cm
1-(-3)
1-(-2)
1-(-1)
1-1
1-2
1-3
2-(-3)
2-(-2)
2-(-1)
2-1
2-2
2-3
3-(-3)
3-(-2)
3-(-1)
3-1
3-2
3-3
4-(-3)
4-(-2)
4-(-1)
4-1
4-2
4-3
5-(-3)
5-(-2)
5-(-1)
5-1
5-2
5-3
fertilizers
1
2
P uptake
grain P
P runoff
5
3
shoot P
2
diffusive phosphate ions in solid phase
phosphate ions in soil solution
4
root P
6 & 7
4
diffusive phosphate ions in solid phase
phosphate ions in soil solution
6 & 7
P leaching

Fig. 7-2

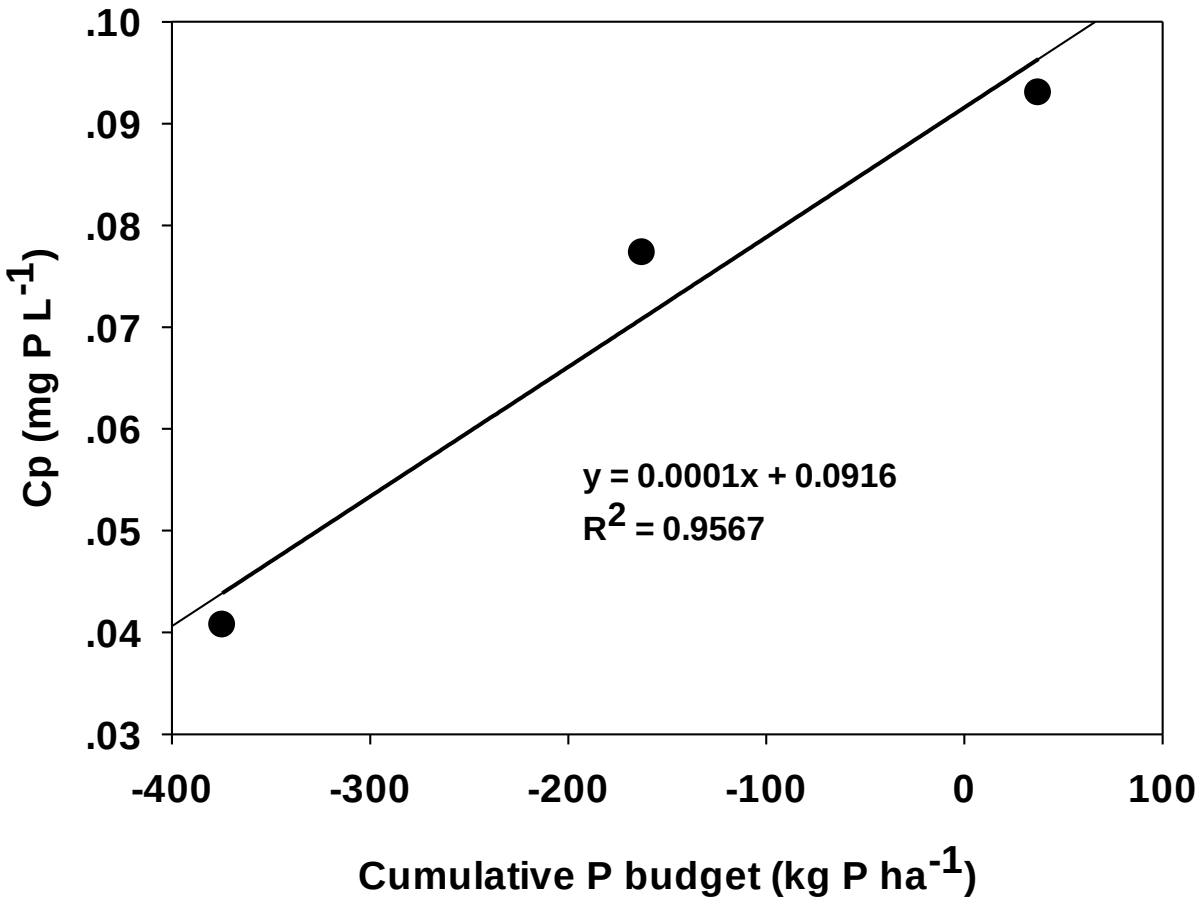
.10
.09
.08
.07
.06
.05
.04
.03
Cp (mg P L-1)
y = 0.0001x + 0.0916
R2 = 0.9567
-400
-300
-200
-100
0
100
Cumulative P budget (kg P ha-1)

Fig. 7-3

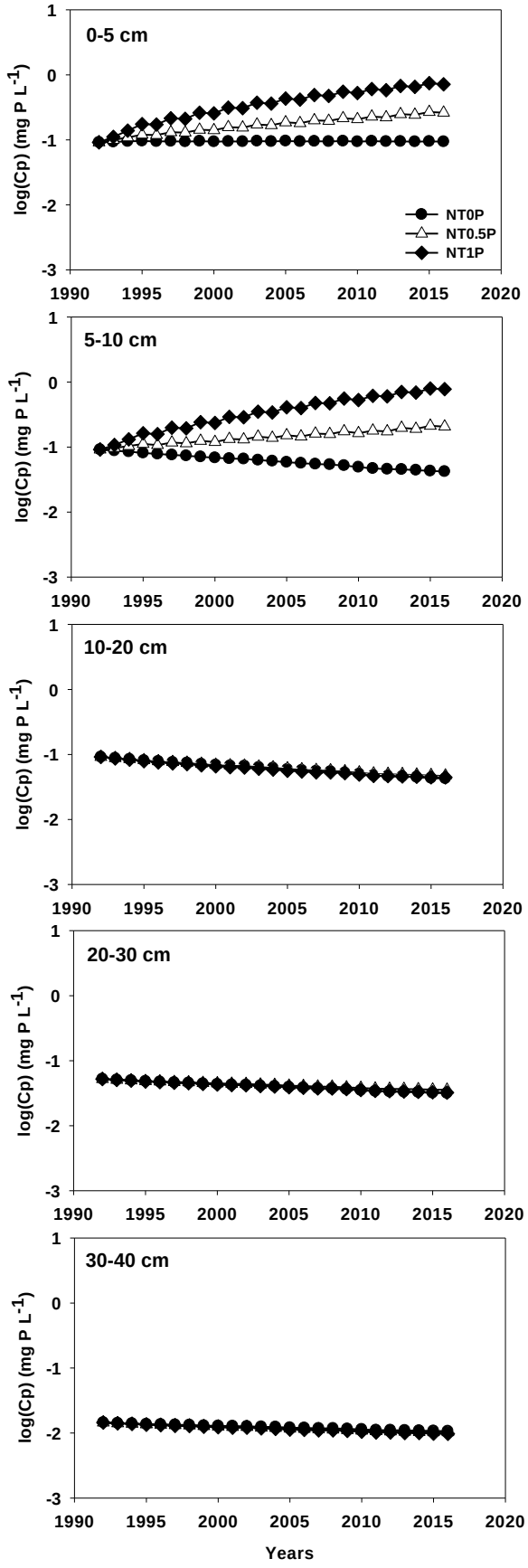
0-5 cm
5-10 cm
10-20 cm
20-30 cm
30-40 cm
log(Cp) (mg P L-1)
NT0P
NT0.5P
NT1P
1
0
-1
-2
-3
1990
1995
2000
2005
2010
2015
2020
Years

Fig. 7-4

NT1P

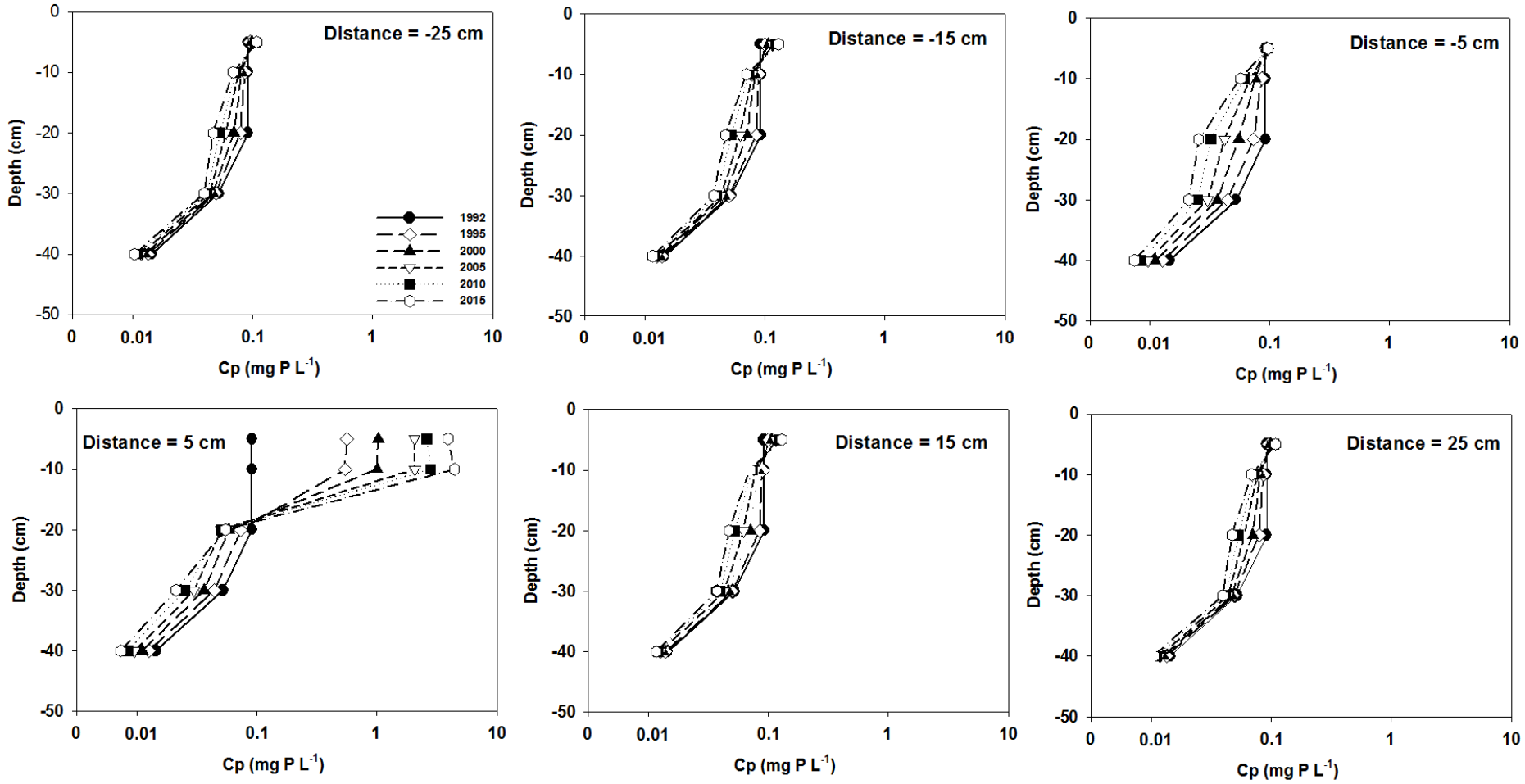
Distance = -25 cm
Distance = -15 cm
Distance = -5 cm
Distance = 5 cm
Distance = 15 cm
Distance = 25 cm
Depth (cm)
Cp (mg P L-1)
0
-10
-20
-30
-40
-50
0
0.01
0.1
1
10
1992
1995
2000
2005
2010
2015

Fig. 7-5

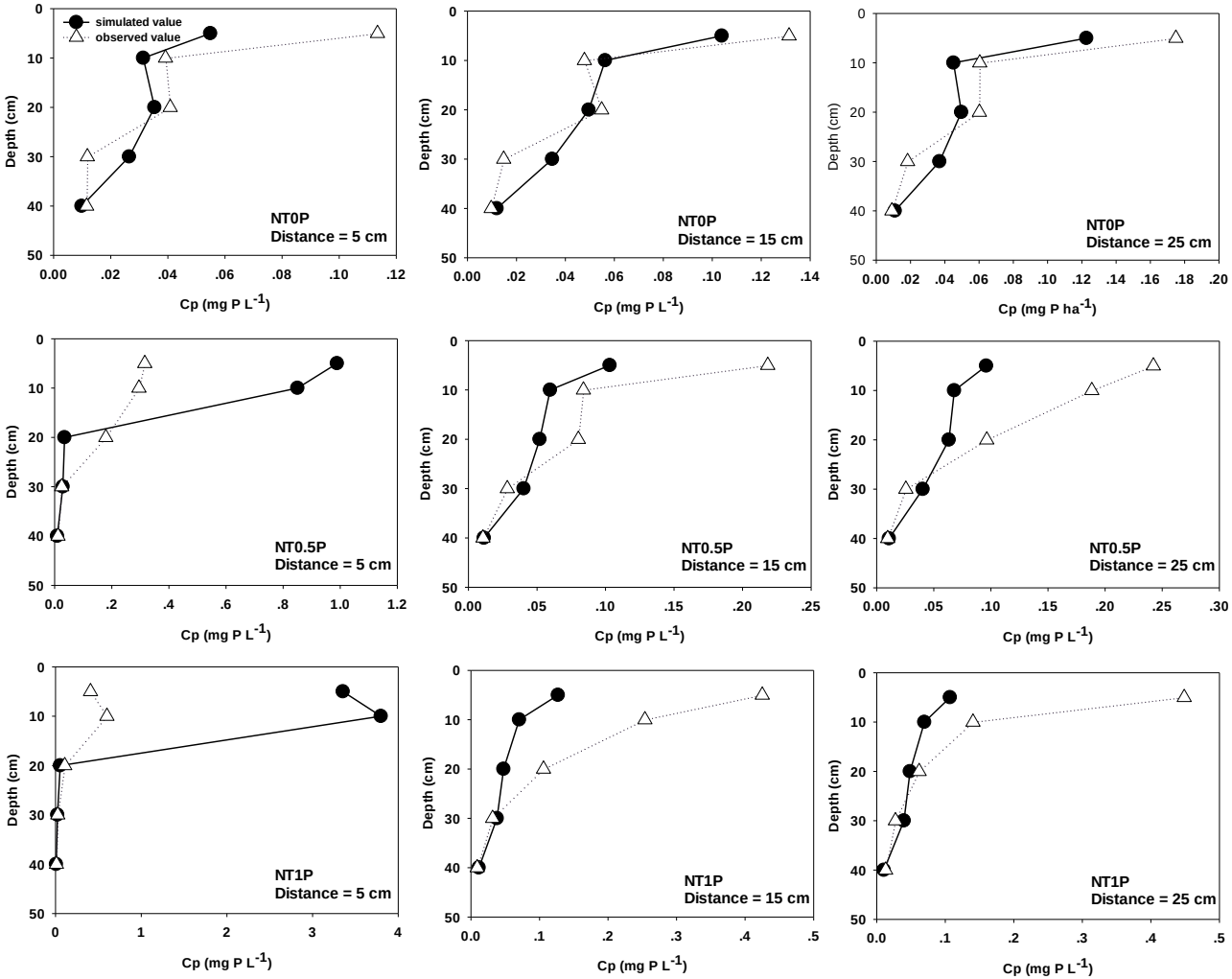
simulated value
observed value
NT0P
Distance = 5 cm
NT0P
Distance = 15 cm
NT0P
Distance = 25 cm
NT0.5P
Distance = 5 cm
NT0.5P
Distance = 15 cm
NT0.5P
Distance = 25 cm
NT1P
Distance = 5 cm
NT1P
Distance = 15 cm
NT1P
Distance = 25 cm
Depth (cm)
Cp (mg P L-1)
Cp (mg P ha-1)

Fig. captions

Fig. 7-1: Schematic structure of the P model. A soil profile 40 cm deep and 30 cm wide is divided into 15 soil grid units by five depths (0-5, 5-10, 10-20, 20-30 and 30-40 cm) and six distances [(-30)-(-20), (-20)-(-10), (-10)-0, 0-10, 10-20 and 20-30 cm]. The model comprises two P pools and seven fluxes (in form of phosphate ions) in each soil grid unit. The two P pools are the amount of phosphate ions in soil solution and the amount of diffusive phosphate ions in soil solid phase buffering the soil solution over time. The two pools add up to soil P stock in each soil grid unit. The seven P fluxes are: 1. Attributed input of mineral P fertilizer; 2. Attributed Output of P uptake by crops. The P uptake is divided into three P fluxes as crop grains, shoots and roots, but only P in grains is exported; 3. Attributed input by P restitution with shoot residue; 4. Attributed input of P restitution from root residue; 5. Attributed output of runoff P; 6. Input of P leaching from upper grid unit. Soil grid units on surface do not receive P leaching input; 7. Output of P leaching to lower grid units.

Fig. 7-2: Correlation between cumulative P budget (kg P ha^{-1}) and phosphate ion concentration in soil solution (mg P L^{-1}) of 2014 in MP plots at three P doses.

Fig. 7-3: Simulated phosphate ion concentrations (***Cp***, mg L^{-1} in log) in the 0-5, 5-10, 10-20, 20-30 and 30-40 cm layers from 1992-2015 under NT and three P doses (0P, 0.5P and 1P).

Fig. 7-4: Simulated phosphate ion concentrations (***Cp***, mg L^{-1}) in the soil zone from 1992-2015 in NT under three P doses (0P, 0.5P and 1P) every five years.

Fig. 7-5: Comparison of simulated and measured phosphate ion concentrations (***Cp***, mg L^{-1}) in soil of 2014 under NT and three P doses (0P, 0.5P and 1P).

Chapter VIII Conclusion and perspectives

Phosphorus (P) plays an important role for crop growth in agriculture systems. Efficient P management could maintain crop yields with the most efficient P-use efficiency, avoiding P stress and over-fertilization. Most studies on P cycle focus on the upper layer (15-30 cm deep) in conventional agroecosystems, assuming that P uptake occur in plough soil layer where soil P status is thought to be homogeneous. However, this view was challenged by subsoil contribution to P uptake and conservation agriculture systems that result in heterogeneous soil P distribution.

Our objectives were to: 1. Qualify and quantify the effects of tillage practices and P dosage on root distribution and morphology of corn (*Zea mays* L.) and soybean (*Glycine max* L.) in the long term; 2. Calculate the proportions of P uptake along soil profile including subsoil; 3. Combine the dynamic model of phosphate ion transfer at the soil solid and liquid interface and the P budget to evaluate soil P status time variations in the conventional tilled (CT) zone; 4. Simulate the dynamics of the P status along soil profile for CT and NT systems in the long run.

Hypothesis 1. Mineral P fertilization and tillage practices affect the P cycle in the long term by affecting the spatial distribution and morphology of roots and P availability in the soil.

We observed that root morphology and distribution along the soil profile changed under no-till (NT) compared to conventional tillage and with P fertilization rate. The no-till practice resulted in deeper compacted layer (20-40 cm) with higher soil bulk density and stratification of P and other nutrients (C, N and K) accumulating in the 0-20 cm layer compared to moldboard plough (MP). The NT made weed control more difficult, but only in the corn year (**Addendum 2**). The root systems of corn and soybean responded differently to soil alterations caused by tillage practice and P fertilization. The NT mainly reduced root biomass of corn roots by 35% compared to MP, especially the primary and secondary roots (-49 and -37%, respectively). The weed invasion in 2014 was main reason for corn root reduction due to severe weed competition for water and nutrient leading to late emergence of corn root radicle. In 2015, as the chemical control of weeds was more efficient, we did not observed weed competition with soybean. Soybean roots showed different root distribution along soil profile between NT and MP. Soybean roots under NT were located more in the 0-10 cm layer (46% of total root length) likely because of crop response to nutrient stratification. The different distributions of soybean roots caused small difference on soybean yields between NT and MP (**Addendum 3**). The NT treatment caused nutrient stratification on long term leading to shallower root system for soybean and higher sensitivity to dry spells. Thus, the incorporation of permanent soil cover, which helps to keep soil moisture, is strongly recommended for the implement of NT to maintain crop yield, especially in arid or semi-arid area. The drying of upper soil layers might reduce the soil P mobility and availability. So in this context a deeper P fertilization might be favorable.

Hypothesis 2. It is necessary to take into account of spatial location in soil profile of both soil P availability and root surface area to accurately model the P biogeochemical cycle in contexte of soil conservation agriculture.

The P budget depends on P fertilization and crop yield, impacting on soil test P over time. Most soil test P, especially chemical extraction methods, provide an elusive definition of soil P availability. The phosphate ion transfer kinetics model offers more opportunities to connect soil test P to P budget. The amount of exchangeable phosphate ions between solid and liquid phases computed using phosphate ion transfer kinetics could represent soil available P stock. At the l'Acadie experimental site, the period for phosphate ion transfer equilibrium was set at

one-year because estimated soil P stocks was related to measured cumulative P budgets for the plough soil layer (0-20 cm). Most of models assumed that soil P distribution was considered homogeneous and the P budget computed as the difference between P fertilization and P exportation. To take account of agricultural practices other than soil tillage and bulk soil P fertilization, there were at least three main points to consider: 1. subsoil P uptake contribution should not be ignored; 2. soil P distributed heterogeneously along soil profile under NT; and 3. other P inputs (P restitution from crop shoot and root) and outputs (P runoff and P leaching). An approach of P uptake proportion estimation along soil profile was thus elaborated based on measured root surface density and soil P availability within soil profile. These calculations confirmed that soil P stock in deep layers might significantly contribute to crop P nutrition. Accordingly, we developed a 2D model to simulate soil P availability changes as a functionof soil P stock using phosphate ion transfer kinetics, root surface density and the P budget assigned tor each soil grid units. This new model was able to simulate the establishment of soil P stratification in NT treatment on the long term. Moreover, it was possible to simulate the effects of localized P fertilization on soil P stocks distribution and its contribution to crop P uptake.

Hypothesis 3. The contribution of subsoil P to crop P uptake is substantial in the soil P cycle under both CT and NT.

The main factors influencing P uptake by crops are soil available P and the root system that explores soil P resource. The distribution of soil P status and roots allow weighting P uptake from layers along soil profile. The P uptake contribution of each soil layer ranged from 17% to 30% depending on crop species (corn and soybean), soil tillage (no-till and moldboard plough) and P dosage (0P, 0.5P and 1P). Corn showed higher contribution of subsoil P to total P uptake (29%) compared to soybean (23%). The MP had higher subsoil contribution (29%) compared to NT (22%). Compared to corn roots, soybean roots, that are fibrous, tended to proliferate in the upper layers. The NT produced P stratification in upper layers. The [soybean, NT] treatment thus showed the smallest subsoil contribution (20%) compared to the highest with [corn, MP] treatment (33%). The 0P treatment showed higher subsoil contribution (32%) compared to 0.5P and 1P (26% and 29%, respectively). Topsoil and subsoil P might benefit from P fertilization to similar degrees. Subsoil received P from topsoil by downward flow. Although subsoil P contribution to P uptake should not be ignored in field study, many other factors should be considered in P uptake, such as water availability, air-porosity for root respiration and fine root proportion for P uptake efficiency.

Hypothesis 4. The 2D model makes it possible to test different P fertilization scenarios depending on soil tillage under different soil and climatic contexts.

The 1D CycP model performed well in evaluating the soil P evolution in the tilled soil layer under MP and in different scenorios of P dosage.

The 2D P cycle model adapted to NT evaluated soil P (phosphate ion concentration in soil solution, ***Cp***) change 60-cm wide and 40-cm deep depending on P fertilization (0P, 0.5P and 1P) between 1992 and 2015 after dividing the soil profile into soil grid units. Soil P stock (total amount of exchangeable phosphate ions) and the P budget (difference of P inputs and outputs) were simulated for each grid unit at each time-step set at one year. The P stock and fluxes estimation should be further improved. Initial ***Cp*** was computed from the relationship between ***Cp*** and cumulative P budget of MP plots without considering P uptake from subsoil. The P fertilization was assumed to be distributed in the same soil grids at every time-step, while in practical operation, there might be operation error during a long run. In addition, ratios of P in shoot or root to P in grain, for phosphate ion transfer kinetic parameters and parameters for the water budget should be measured more precisely. And the role of bioturbation for soil P movement should probably be integrated into P cycle model.

Conclusion

Our research firstly confirmed that the long-term implementation of NT stratified the distribution of roots and soil P compared to conventional tillage (CT). The different root systems under NT and CT might cause small difference in crop yields regardless of weed control (mainly in the corn phase), which is one of the key problems under NT. The P uptake proportions along soil profile were related to root and soil P distributions. The NT showed higher P uptake in the upper soil layers. We showed the important contribution of subsoil P to P uptake both NT and CT, although it was always ignored in soil P cycle studies.

The P cycle model (CycP) could evaluate the evolution of soil P status in tilled soil layers over a long-term using phosphate ion transfer kinetics and the P budget. The model could be improved using root distribution and soil P distribution in NT. Other factors such as bioturbation should also be integrated into the model under NT in future studies. However, the models has integrated the spatial distribution of factors (i.e. plant roots and soil P) in to the soil-plant P cycle, which is rarely mentioned in actual P cycle study in conventional agri-systems, but is very important in conservation agriculture with high heterogeneity of soil properties. The model could help to guide P fertilization strategy in fields in context of conservation agriculture for both dosage and application mode (i.e. fertilizer phase, application depth and etc.) determination.

Perspectives

1. Crop roots follow a dynamic process generating old roots dying and young roots. Crop root distribution and morphology should thus be measured at several sampling dates to select a representative root system for long-term P cycle studies.

2. *In situ* P tracing method should be developed for P fertilizer localization and subsoil P uptake studies. We proposed an approach to assess the P uptake contribution across the soil zone. But this approach should be validated with precise measurements. In laboratory, isotope labeling method with ^{32}P could help to conduct this type of experiments, but it can not be applied under field conditions. At this moment, the preliminary exploration could be done by analyzing the degree of soil P variations in the P fertilized zone and subsoil, which could represent the amount of P uptake in each zone. However, variations caused by bioturbation are difficult to quantify.

3. In terms of phosphate ion transfer kinetics, advanced studies could be conducted on fitting parameters. The determinations of fitting parameters for the phosphate ion transfer kinetics equation are based on laboratory works, which are time-consuming. The fitting parameters are related to soil properties such as aggregate size, texture, Fe/Al content and organic material, which mainly influence P sorption-desorption in soil. Thus, relationships between fitting parameters and soil properties could facilitate modeling the kinetics processes involved in P transfer.

4. The simulation model should be tested in other scenarios with various tillage practices, P dosage, crop rotations and pedo-climate conditions. Those results may contribute to validate and optimize the operational models.

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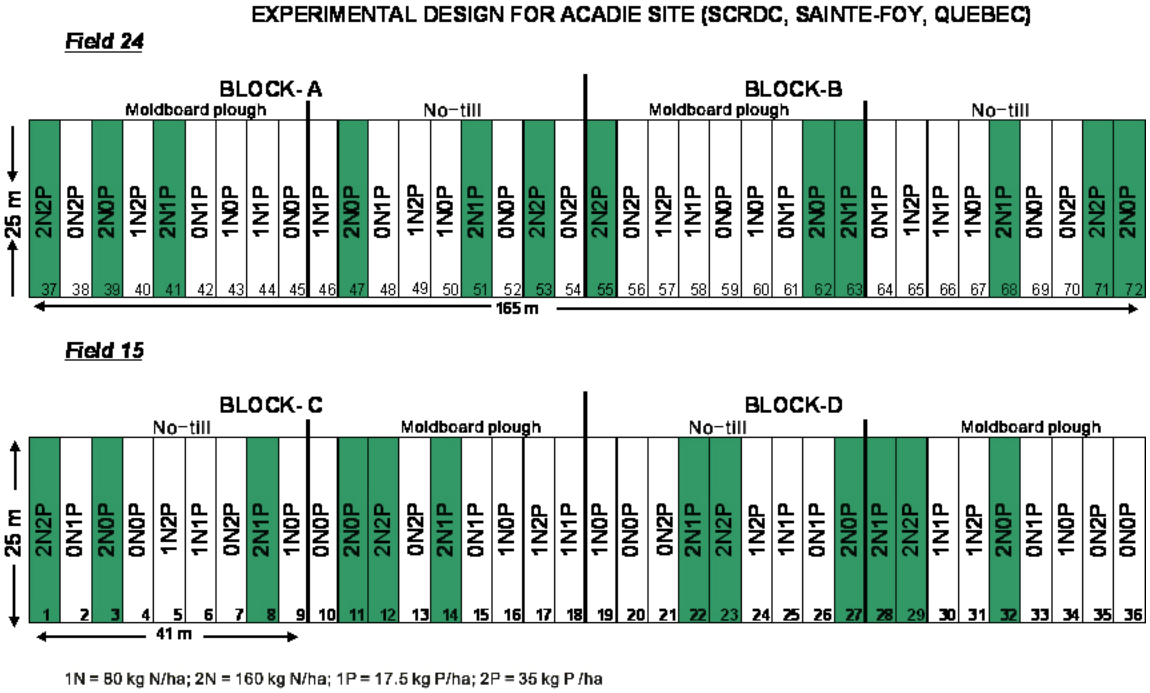
Addenda

Addendum 1 Experimental design for l'Acadie site



Addendum 2 Weed control situations of l'Acadie in 2014 for corn and in 2015 for soybean, respectively

2014 corn

2015 soybean

Addendum 3 Evolution of soybean yields (t ha^{-1}) under no-till (NT) and moldboard plough (MP) from 1995 to 2015 (different letters for a certain year indicate significant (P<0.05) difference between the yields of NT and MP)