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SOIL SCIENCE

Soil phosphorus availability and uptake by mycorrhizal and non-mycorrhizal plants in an onion no-tillage system

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ABSTRACT: *Onion is an important vegetable crop, predominantly grown under conventional tillage system management. Alternatively, the vegetable no-tillage system uses cover crops to form a residue layer, which improves soil physical, chemical, and biological attributes. Aiming to understand the interaction of mycorrhizal and non-mycorrhizal cover crops, phosphatase activity, and soil phosphorus availability and uptake by plants, a no-tillage vegetable production system experiment with onion was carried out in Ituporanga, Southern Brazil. The treatments were black oats (Avena strigosa); rye (Secale cereale); oilseed radish (Raphanus sativus);* rye + *oilseed radish;* black oats + *oilseed radish, and a control with spontaneous plants. Additionally, two plots, a conventional tillage system area and a forest, both adjacent to the experiment, were evaluated. We measured cover crop biomass, onion yield, acid phosphatase activity, and resin-extracted phosphorus in the soil, shoot and root phosphorus content, and root colonization in cover crops, spontaneous plants, and onions. The treatments with cover crops had the highest plant biomass in winter and onion yield. Available soil phosphorus and acid phosphatase activity were higher in no-tillage plots than in the conventional tillage system area. The presence of non-mycorrhizal oilseed radish was associated with decreased colonization of rye and onion roots by arbuscular mycorrhizal fungi. No-tillage areas with cover crops or spontaneous plants in winter accumulated more phosphorus than conventional tillage system areas. The conventional tillage system showed adverse effects on most soil attributes, as shown by a Principal Component Analysis.*

Key words: *Allium cepa, Acid phosphatase, resin-extracted phosphorus, conventional tillage, cover crops.*

Disponibilidade e absorção de fósforo no solo por plantas micorrízicas e não micorrízicas em sistema de plantio direto de cebola

RESUMO: *A cebola é uma importante cultura vegetal, cultivada predominantemente sob sistema de preparo convencional. Como alternativa, o sistema de plantio direto de hortaliças utiliza culturas de cobertura para formar uma camada de biomassa, o que melhora os atributos físicos, químicos e biológicos do solo. Com o objetivo de entender a interação de culturas de cobertura micorrízicas e não-micorrízicas, atividade da fosfatase ácida e disponibilidade e absorção de fósforo do solo pelas plantas, foi realizado um experimento em sistema de plantio direto de hortaliças com a cultura da cebola em Ituporanga, sul do Brasil. Os tratamentos foram: aveia preta (Avena strigosa); centeio (Secale cereale); nabo forrageiro (Raphanus sativus); centeio + nabo forrageiro; aveia preta + nabo forrageiro e um controle com vegetação espontânea. Além disso, duas outras parcelas, uma área em sistema de preparo convencional e uma floresta, ambas adjacentes ao experimento, foram avaliadas. Medimos a biomassa da cultura de cobertura, o rendimento de cebola, a atividade de fosfatase ácida e o fósforo extraído por resina no solo, bem como o conteúdo de fósforo da parte aérea e da raiz e a colonização das raízes em plantas de cobertura, plantas espontâneas e cebolas. Os tratamentos com plantas de cobertura apresentaram a maior biomassa de culturas de cobertura e rendimento de cebola. A atividade de fosfatase ácida e fósforo disponível no solo foram maiores nas parcelas de plantio direto do que na área convencional. A presença de nabo forrageiro, uma planta não micorrízica, foi associada a reduções na colonização de raízes de centeio e cebola por fungos micorrízicos arbusculares. As áreas de plantio direto com plantas de cobertura ou plantas espontâneas no inverno acumularam mais fósforo do que as áreas com preparo convencional. O sistema convencional de lavoura mostrou efeitos adversos para a maioria dos atributos do solo, como mostra a Análise de Componentes Principais.*

Palavras-chave: *Allium cepa, fosfatase ácida, fósforo extraído por resina, preparo convencional do solo, plantas de cobertura.*

INTRODUCTION

Onions are among the most commonly grown vegetables worldwide. Crop management predominantly uses conventional tillage systems, with intensive use of pesticides and highly soluble fertilizers (FERREIRA et al., 2018). Successive plowing, harrowing, or scarification lead to soil, water, and nutrient losses due to erosion (PANACHUKI et al., 2011; SANTOS et al., 2017; FERREIRA et al.,

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2018). Consequently, soils in the producing regions, such as the Upper Itajaí Valley in southern Brazil, are heavily degraded (COMIN et al., 2018a).

As an alternative to the conventional tillage system, our team has been developing a vegetable no-tillage system for different plant species (FAYAD et al., 2019). It follows ecology-based principles, such as reduced soil tillage, split fertilizer application, and avoidance of highly soluble fertilizers. The system relies on the use of cover crops, aiming to eliminate herbicides for the management of spontaneous plants (FAYAD et al., 2019; MÜLLER JÚNIOR et al., 2019). The vegetable no-tillage system uses cover crops to form a layer of plant residues on the soil surface and minimize tillage. Cover crops can uptake minerals from deeper soil layers, accumulate them in their roots and shoots, thus improving nutrient supply and cycling (OLIVEIRA et al., 2017). After residue decomposition, nutrients in the plant tissues are released and can be uptaken by cash crops (WHITE & WEIL, 2011). In addition to changes in nutrient concentration in the upper layers, cover crops promote changes in soil structure (SANTOS et al., 2018), reduction of erosion processes (COMIN et al., 2018a), as well as increased soil biological activity and organic matter concentration (DE PONTES et al., 2017). Increased plant diversity should result in higher soil microbiological activity, but studies evaluating soil quality with microbiological indicators are still scarce (MENDES et al., 2019). The selection of biological indicators is vital for the development and scaling up of the vegetable no-tillage system since they help to understand the underlying biochemical and ecological interactions. Biological indicators can also be used by technicians and farmers to monitor soil quality.

Management practices used in conventional tillage system and vegetable no-tillage system affect soil microbial communities, such as arbuscular mycorrhizal fungi, which have their diversity affected by soil tillage (CARNEIRO et al., 2019). Arbuscular mycorrhizal fungi associated with plant roots provide an increased area for nutrient uptake, especially phosphorus, through their network of hyphae (SMITH & READ, 2008). Mycorrhizal colonization is affected by soil available phosphorus content, but previous studies on the same area did not show differences in Mehlich 1-extracted soil phosphorus (SOUZA et al., 2013, SANTOS et al., 2017), showing the need to use other extractor, such as resin. Besides, arbuscular mycorrhizal fungi promote soil aggregation, due to the action of their external mycelia and the production of glomalin, a cementing agent (RILLIG & MUMMEY, 2006). Mycorrhizas have also been linked to increased phosphatases activity in the soil (TAWARAYA et al., 2006).

Enzymes are good indicators of soil quality since they catalyze different reactions in soils (MENDES et al., 2019), involving processes such as organic waste decomposition and nutrient mineralization (BOWLES et al., 2014). Soil enzyme activity is affected by the microbiota, plant cover, and plant and soil management. Arbuscular mycorrhizal fungi can produce enzymes, especially phosphatases, which promote phosphorus mineralization and may act in the solubilization for inorganic phosphate (TARAFDAR, 1995; QIU et al., 2018). Non-mycorrhizal plants have developed strategies to obtain organic phosphorus, which include increased phosphatase activity around their roots (DALLA COSTA & LOVATO, 2004; KUNZE et al., 2011). However, there is limited information on the relationship between enzyme activity, especially phosphatases, and the presence of mycorrhizal and non-mycorrhizal plants on the tillage system. Our main hypothesis is that plant species, along with soil phosphorus availaibility, can differentially affect phosphatase activity in soils.

This study aims to evaluate the effect of mycorrhizal and non-mycorrhizal cover crops on soil phosphatase activity and phosphorus availability and uptake in a no-tillage onion production system.

MATERIALS AND METHODS

Location and experimental design

The study was carried out in Ituporanga (Santa Catarina State, Brazil) (27º 24 ‘52 “S, 49º 36’ 9 “W, 475 m altitude). The region climate, according to the Köeppen classification, is subtropical humid (Cfa), with a mean temperature of 17.6 °C and 1400 mm yearly rainfall. The soil in the area is classified as Cambissolo Húmico (EMBRAPA, 2013), equivalent to Humic Distrudept (SOIL SURVEY STAFF, 2010). The area had a history of twenty years of onion cultivation in conventional tillage system (plowing, harrowing, and scarification) until 1996 when a minimum tillage system with onion and cover crop rotation was adopted. The rotation included oats (*Avena strigose* Schreb), velvet bean (*Mucuna aterrima* Piper & Tracy), millet (*Pennisetum glaucum* L.), crotalaria (*Crotalaria juncea* L.), and vetch (*Vicia sativa* L.). In 2007, sweet potato (*Ipomoea patatas* (L.) Lam.) was planted and grown until 2009, when the vegetable no-tillage system experiment with onion was established. Spontaneous plants were desiccated with glyphosate, and afterward, no pesticide was used in the area. In the 0-10 cm layer, the soil had 380 g kg^{-1} clay, 40 g kg^{-1} total organic carbon, 6.2 pH in water, 26.6 mg dm^{-3} available phosphorus, and 145.2 mg dm^{-3} exchangeable potassium (the latter two extracted by Mehlich-1 solution), 0.0 $mmol_c$ kg^{-1} exchangeable aluminum, 7.2 $cmol_c$ kg^{-1} exchangeable calcium, and 3.4 $cmol_c$ kg^{-1} exchangeable magnesium (extracted by 1 mol L^{-1} KCl) (TEDESCO et al., 1995).

The treatments were: 100% black oats (*Avena strigosa* Schreb), with sowing mass of 120 kg ha^{-1}; 100% rye (*Secale cereale* L.), with sowing mass of 120 kg ha^{-1}; 100% oilseed radish (*Raphanus sativus* L.), with 20 kg ha^{-1}; intercropping of rye (86%) and oilseed radish (14%), with 60 and 10 kg ha^{-1}, respectively; intercropping of black oats (86%) and oilseed radish (14%), with 60 and 10 kg ha^{-1}, respectively. The sixth treatment was a fallow control with spontaneous plants, with the predominance of *Amaranthus lividus* L., *Oxalis* spp., *Cyperus* spp., *Stachys arvensis* L. *Cynodon* spp., and *Rumex obtusifolius* L. (SOUZA et al., 2018). Seed densities for cover crops were defined based on the recommendation of MONEGAT (1991) and increased by 50%, aiming a suitable dry mass production for the system, around 5.0 Mg ha^{-1}. The cover crops are winter species and were sown in early Autumn (April) each year.

In July of every year (2009 to 2017), all winter cover crops were rolled down with a knife roller (model RF240, MBO Ltda., Erechim, Brazil). We used fertilizer doses and applicartion procedures usually adopted by onion farmers in the region: Gafsa natural phosphate at a rate of 96 kg P_2O_5 ha^{-1}, and poultry litter, corresponding to 175 kg ha^{-1} of P_2O_5, 125 kg ha^{-1} of K_2O and 160 kg ha^{-1} of N. One half of the poultry litter was applied at onion seedling planting, and the remainder was applied 45 days later. In 2011, no natural phosphate was applied, as soil analysis showed that levels were very high (CQFS-RS / SC, 2004). Furrows were dug with a sowing planter adapted for direct onion planting, and onion seedlings (cultivar Empasc 352 - Bola Precoce) were manually transplanted. Each experimental unit had 25 m^2 (5.0 × 5.0 m), with 0.40 m between rows and 0.10 m between plants, with ten onion rows per plot. Weeding was done by hoeing, 60 and 90 days after the onion seedlings were transplanted. After the onion harvest, in November or December of each year, velvet bean (*Mucuna aterrima* Piper & Tracy) was sown (120 kg ha^{-1}) on the cultivated area. The velvet bean plants were rolled down each April, before sowing the cover crops. The experiment was arranged in a randomized block design with eight replicates. The soil apparent density in the area ranges between 1.28 and 1.32 Mg m^{-3} (LOSS et al., 2017).

Additionally, two more plots were evaluated, both adjacent to the experiment. The conventional tillage system plot is a 41-year-old onion field. Onions were grown under conventional tillage system for 31 years from 1976 until 2007. Since 2007, onion has been grown in rotation with millet in the summer. Millet was rolled down at flowering with a knife roller, and after 30-60 days, the area was plowed and harrowed to establish the onion crop. The soil (0-10 cm layer) had 420 g kg^{-1} clay, 5.8 pH in water, 17.1 mg dm^{-3} available phosphorus, and 80.0 mg dm^{-3} exchangeable potassium (the latter two extracted by Mehlich-1 solution), 0.0 $mmol_c$ kg^{-1} exchangeable aluminum, 7.3 $cmol_c$ kg^{-1} exchangeable calcium, and 3.0 $cmol_c$ kg^{-1} exchangeable magnesium (extracted by 1 mol L^{-1} KCl). Soil apparent density is 1.40 Mg m^{-3} (LOSS et al., 2017). Fertilizer was applied according to regional recommendations (CQFS-RS / SC, 2004). Also, soil samples were taken in a secondary forest used as a reference to represent an environment without management stress. That area, at a 550-meter distance from the experimental area, has 35 years of regeneration, and soil apparent density is 0.98 Mg m^{-3} (LOSS et al., 2017).

Sampling and measurements for area characterization

The Ituporanga EPAGRI weather station, maintained by the Centro de Informações de Recursos Ambientais e de Hidrometeorologia de Santa Catarina (CIRAM), provided the information on weather conditions (temperature and rainfall daily value) in the period during which the experiment was carried out.

In June and July 2017, at 58, 80, and 99 days after sowing of winter cover crops, three subsamples were collected from each plot, using a 0.5m x 0.5m frame. The plants were cut, stored in paper bags, oven-dried at 65 °C until constant mass, and weighed.

In November, onion bulbs were harvested manually in six central lines of each plot. left on the soil surface for ten days, sorted by size class (HORTIBRASIL, 2010), and weighed.

Soil phosphatase activity and phosphorus availability

Acid phosphatase activity and resin-extracted phosphorus were measured in soil samples from all treatments during the cover crop cycle at 58, 80 and 99 days after sowing, and at 40 and 99 days after seedling planting during the onion cycle. In each plot of the experiment and adjacent areas, approximately 300 grams of soil were collected at a depth of 0 to 10 cm. The samples were transported to the laboratory with ice and kept at 4 °C until the analyses were performed within two months after collecting.

For acid phosphatase activity analysis, 1.0 gram of soil was mixed with p-nitrophenyl phosphate (PNP) in a modified universal buffer (MUB pH 6.5), incubated at 37 °C, and hydrolysis was stopped after one hour with 0.5 mol L^{-1} calcium chloride. ($CaCl_2$) and 0.5 mol L^{-1} NaOH. Absorbance (410nm) of the centrifuged and filtered extract was measured in a UV-visible spectrophotometer. Controls were performed using the same procedures with the enzyme substrate (p-nitrophenyl phosphate) added at the end of the incubation period. The standard curve was obtained with p-nitrophenol in $CaCl_2$ and NaOH (TABATABAI, 1994).

Soil resin-extracted phosphorus was measured using Anion Exchange Resins (RAIJ et al., 1987) after stirring for 16 hours. Subsequently, the resins were removed from the solution, and the phosphate ions were extracted with a 0.5 mol L^{-1} HCL solution. Phosphorus was quantified by UV-vis spectrometry (MURPHY & RILEY, 1962).

Mycorrhizal root colonization, and shoot phosphorus concentration and uptake

Root colonization by arbuscular mycorrhizal fungi was measured in rye, oilseed radish, rye + oilseed radish, and spontaneous plants treatments at 80 days after sowing during the winter cover crop cycle, and at 40 and 99 days after seedling planting during the onion cycle. We decided to evaluate only rye because the behavior of grasses is similar, and previous studies in the same experiment showed that rye had better results in soil quality improvement (OLIVEIRA et al., 2016). In the first sampling, three rye and three oilseed radish plants were collected from the single-crop plots and the intercropped rye and oilseed radish plots. In each spontaneous plants plot, three *Rumex obtusifolius* plants and three *Amaranthus lividus* plants were collected because those species were present in all treatments. *R. obtusifolius* has a dominance index of 20 to 33% during winter and onion cycles, respectively, and *A. lividus* indexes are 17 to 61% in the same cycles (COMIN et al., 2018b). In the other two sampling times, during the onion cycle, three onion plants were collected in each plot. Onion plants were also collected in the adjacent area with conventional tillage system.

Shoots of three plants of each treatment were oven-dried at 65 ºC until constant mass, weighted, and ground to determine phosphorus concentration and total uptake. Plant material was subjected to digestion in H_2SO_4 and H_2O_2 (TEDESCO et al., 1995), and phosphorus was determined by spectrometry (MURPHY & RILEY. 1962).

Roots were transported in ice coolers and stored at 4 °C for further analysis. Root samples were cleared with KOH and stained with trypan blue (KOSKE & GEMMA, 1989), and colonization was measured according to MCGONIGLE et al. (1990). Stained root fragments of 1.0 cm were mounted on slides with polyvinyl lactoglycerol and examined under a microscope at 200x magnification, and 100 intersections were examined, counting the presence of hyphae, arbuscles, and vesicles.

Statistical analysis

Data on biomass, yield, mycorrhizal colonization, and phosphorus availability in soil and plants were tested for homogeneity (Bartlett) and normality (Shapiro-Wilk). Mycorrhizal colonization data (hyphae only, arbuscles, vesicles, and total colonization) were transformed using $(x + 1)^{0.5}$. Data were subjected to analysis of variance, and when there were significant effects, means were separated by the Skott Knott test at 5% probability.

Analyses of regression were performed, followed by analyses of variance at 5% probability. A principal component analysis was performed using the vegan package (OKSANEN et al., 2013). The software R v. 3.6 (R Development Core Team, 2008) for statistical analysis and the Sigma Plot 12.3 (Systat Corp.) software for graphs were used.

RESULTS

Winter cover crop biomass and onion yield

Winter cover crop produced more above-ground biomass than spontaneous plants, in all sampling times (Table 1). At 58 and 99 days after sowing, shoot biomass of intercropped or single-species winter cover crops was three times higher than plant biomass in plots with spontaneous plants. In the adjacent area under conventional tillage system, millet biomass production in summer was double the biomass of winter cover crops.

The total yield of onion bulbs ranged from 6.9 Mg ha^{-1} for spontaneous plants to 13.9 Mg ha^{-1} for black oats (Table 1). The treatments previously sown with black oats and black oats + oilseed radish had higher onion yields than the other treatments. In general, onion yields in treatments with cover crops were 32 to 50% higher than in the plots with

Table 1 - Winter cover crops biomass in three sampling times, and onion bulb yield in a no-tillage system with black oats (BO), rye (RY), oilseed radish (OR), as single or combined cover crops or with spontaneous plants (SP), with an adjacent conventional tillage system (CTS) having millet as summer cover crop takin as reference. DAS = days after sowing.

Treatments	----------Cover crop biomass----------			Onions bulb yield
	58 DAS	80 DAS	99 DAS	
	----------Mg ha^{-1}----------			
Black oats (BO)	1.5 a*	3.1 a	4.5 a	13.9 a
Rye (RY)	1.4 a	3.2 a	4.3 a	10.3 b
Oilseed radish (OR)	1.5 a	3.8 a	4.1 a	10.2 b
RY + OR	1.7 a	3.4 a	4.6 a	10.2 b
BO + OR	1.6 a	3.8 a	4.5 a	12.6 a
Spontaneous Plants	0.4 b	1.7 b	1.5 b	6.9 c
CTS			9.0	25.0

*Means followed by the same lowercase letter in each column do not differ according to Scott-Knott test ($P \leq 0.05$).

spontaneous plants (Table 1), which also had smaller bulbs (Table 2). Bulb yield in conventional tillage system was 1.7 to 3.6 times higher than in vegetable no-tillage system treatments. There was a significant regression coefficient (r^2 = 0.83; p-value < 0.001)) between cover crop biomass and onion yield.

Mycorrhizal colonization and shoot phosphorus concentration and uptake

During the winter cover cycle, there was mycorrhizal colonization in the roots of rye (Figure 1), a mycorrhizal plant. No mycorrhizal structures were found in oilseed radish, *R. obtusifolius,* or *A. lividus* roots, as expected since those plant species belong to typically non-mycorrhizal families (BRUNDRETT, 2017). Total root colonization in treatments containing only rye and intercropped rye species were 20% and 12%, respectively, but vesicle and arbuscule indexes did not differ between treatments.

Onion roots were colonized by arbuscular mycorrhizal fungi in both sampling times (Table 3). There were differences among treatments only for total colonization in the first sampling, and for vesicles in the second sampling. In the first sampling time, total root colonization in the treatments with oilseed radish was lower than in the treatments with rye only, with spontaneous plants, or under conventional tillage system. In the second sampling, the highest rate of vesicle formation occurred in the spontaneous plant treatment.

During the cover crop cycle, shoot phosphorus concentration in cover crops and spontaneous plants varied from 4.3 to 5.7 g kg^{-1}, at 99 days after sowing (Table 4). Those concentrations

Table 2 - Yield of onion bulbs, separated by commercial class, in a no-tillage onion production system.

Treataments	----------Onion bulb production by commercial class----------				
	Class 1	Class 2	Class 3	Rotten	Total
	----------Mg ha^{-1}----------				
Black oats (BO)	0.4 b	10.3 a	2.9 a	0.1 ^{ns}	13.9 a
Rye (RY)	0.4 b	7.1 b	2.1 a	0.1 ^{ns}	10.3 b
Oilseed radish (OR)	0.4 b	7.0 b	2.4 a	0.1 ^{ns}	10.2 b
RY + OR	0.4 b	7.8 b	2.1 a	0.2 ^{ns}	10.2 b
BO + OR	0.5 b	9.2 a	2.7 a	0.3 ^{ns}	12.6 a
Spontaneous Plants	1.6 a	4.9 c	0.3 b	0.3 ^{ns}	6.9 c

*Means followed by the same letter in each column do not differ according to Scott-Knott test (P <0.05). ns = not significant by the F test (p <0.05). Class 1 (> 15 ≤ 35 mm), Class 2 (> 35 ≤ 50 mm), Class 3 (> 50 ≤ 60 mm).

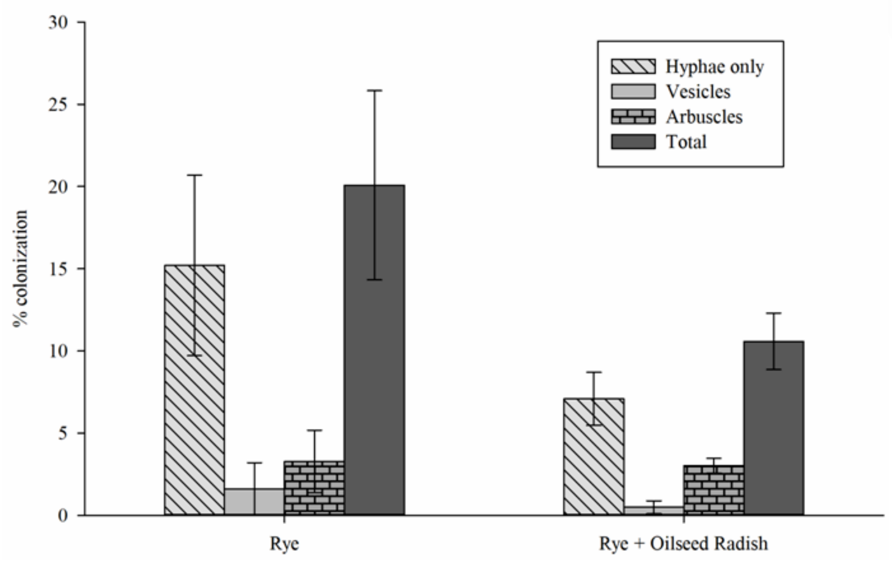

Figure 1 - Root mycorrhizal colonization (%), of rye (RY) grown singly or intercropped with oilseed radish (OR), 80 days after sowing. * Means followed by the same letter do not differ between the RY and RY + OR treatments according to Scott-Knott test at P ≤ 0.05.

are probably linked to the availability of phosphorus from rock phosphate (96 kg P_2O_5 ha^{-1}) and poultry litter (175 kg P_2O_5 ha^{-1}). Rye and spontaneous plants had the highest shoot phosphorus concentration, while phosphorus uptake was greater in cover crops than in spontaneous plants, mainly due to the difference in biomass production (Table 1). During the onion crop cycle, shoot phosphorus concentration did not differ among treatments, while the highest phosphorus uptake occurred in conventional tillage system plants, and oilseed radish and rye + oilseed radish treatments had the lowest phosphorus uptake rates.

Soil acid phosphatase activity and resin-extracted phosphorus

During the cover crop cycle, acid phosphatase activity ranged from 434 to 1031 ug PNP g dry $soil^{-1}$ h^{-1} in the plots in vegetable no-tillage system and in the adjacent production area under conventional tillage system, while it ranged from 1085 to 2297 ug PNP g dry $soil^{-1}$ h^{-1} in the forest area,

Table 3 - Mycorrhizal colonization of onion in summer, with onion under conventional tillage system (CTS) taken as references. Hy: hyphae only; Ves: vesicles; Arb: arbuscules. DAS: days after cover crop sowing; DAP: days after onion planting.

Treatments	Hy	Ves	Arb	Total	Hy	Ves	Arb	Total
	40 DAP				99 DAP			
Rye (RY)	6.3 ^{ns}	7.3^{ns}	26.8^{ns}	39.3 a	9.5^{ns}	2.0 b	24.0^{ns}	33.8^{ns}
Oilseed radish (OR)	5.0	4.8	22.0	25.8 b	7.8	4.8 b	21.8	25.8
RY + OR	7.0	2.8	23.5	27.8 b	7.0	2.0 b	22.0	29.8
Spontaneous plants	7.3	6.8	34.3	43.3 a	11.0	10.0 a	17.3	30.8
CV %	24.1	43.3	19.6	10.5	16.6	27.7	11.7	7.6
CTS (mean±CI)	7.8±4.0	9.0±5.7	37.3±11.9	46.0±13.2	7.8±5.3	3.3±1.5	20.8±7.3	30.3±5.7

*Means followed by the same lowercase letter in each column do not differ according to Scott-Knott test at P < 0.05. ns = not significant; DAP = Days after onion planting; CV = coefficient of variation. CI = confidence interval.

Table 4 - Phosphorus concentration (PC) (g kg^{-1}) and total uptake (PU) (kg ha^{-1}) in cover crops and spontaneous plants (SP), 99 days after sowing, and in onion plants, 99 days after planting. CTS: Conventional tillage system.

Treatments	-------------------Cover crops cycle-------------------		---------------------Onion cycle--------------------	
	PC (g kg^{-1})	PU (kg ha^{-1})	PC (g kg^{-1})	PU (kg ha^{-1})
Rye (RY)	5.7 a[*]	23.2 a	3.4[ns]	9.1 a
Oilseed radish (OR)	4.3 b	17.6 a	3.3	6.8 b
RY + OR	4.9 b	22.5 a	3.5	6.3 b
Spontaneous plants	5.4 a	8.1 b	3.1	9.0 a
CV %	nd	nd	18.6	20.0
CTS (mean±CI)	nd	nd	3.5±0.3	15.2±1.8

[*]Means followed by the same lowercase letter do not differ from each other in the column according to Scott-Knott test (P≤ 0.05), ns = not significant, nd = not determined.

used as a reference (Table 5). There was an increase in enzyme activity throughout the three sampling times in all treatments. Acid phosphatase activity in all samplings times during the winter cover crop cycle was higher in black oats and black oats + oilseed radish treatments than in spontaneous plants. In the treatments with single oilseed radish or rye + oilseed radish, acid phosphatase activity only differed from the spontaneous plants in the last sampling time, at 99 days.

During the onion cycle, acid phosphatase activity ranged from 638 to 1230 ug PNP g dry $soil^{-1}$ h^{-1} in the areas with vegetable no-tillage system and conventional tillage system (Table 5). In both onion cycle samplings, there were no differences among treatments in vegetable no-tillage system, and all values were higher than those found in the area in conventional tillage system. In the forest area, acid phosphatase activity remained higher than in cultivated areas, ranging from 1623 to 1712 ug PNP g dry $soil^{-1}$ h^{-1}.

During the cover crop cycle, resin-extracted soil phosphorus (Table 6) ranged from 9.7 to 147 mg kg^{-1} in the vegetable no-tillage system and conventional tillage system areas, and from 11.9 at 14.5 mg kg^{-1} in the forest area. In the three sampling times, there were no differences in soil phosphorus among cover crops, except for oilseed radish and rye

Table 5 - Acid phosphatase (AP) activity (ug PNP g dry $soil^{-1}$ h^{-1}) in soil under winter cover crop cycle and during summer onion cultivation, in areas with cover crops and subsequent onion cycle, area with onion under conventional tillage system (CTS) and in a forest taken as reference.

Treatments	--------------AP activity during cover crop cycle-------------			----AP activity during onion cycle---	
	58 DAS	80 DAS	99 DAS	40 DAP	99 DAP
Black oats (BO)	757 aB[*]	990 aA	1031 aA	994 a[ns]	1230 a
Rye (RY)	770 aB	928 bA	987 aA	969 a	1187 a
Oilseed radish (OR)	689 bB	884 bA	936 aA	953 a	1213 a
RY + OR	642 bB	905 bA	958 aA	1021 a	1171 a
BO + OR	849 aB	983 aA	987 aA	997 a	1182 a
Spontaneous Plants	719 bB	851 bA	848 bA	926 a	1053 a
CV%	14.0	10.7	15.8	13.0	17.4
CTS (mean±CI)	434±48	690±62	670±76	638 ± 103	680 ±110
Forest (mean±CI)	1085 ±315	2140 ±203	2297±792	1623 ±377	1712±386

[*]Means followed by the same letter, lowercase in each column and uppercase in each row, do not differ according to Scott-Knott test (P < 0.05). ns = not significant for analysis between collections during onion cycle; DAS = Days after sowing; DAP = Days after seedling planting; CV = coefficient of variation. CI = confidence interval.

Table 6 - Resin-extracted soil phosphorus (mg kg^{-1}) in the soil during the winter cover crop cycle and summer onion cultivation, in plots with cover crops and subsequent onion cycle, in areas with onion under conventional tillage system (CTS), and a forest taken as reference.

Treatments	------------------------Cover crop cycle--------------------------			-------------Onion cycle------------	
	58 DAS	80 DAS	99 DAS	40 DAP	99 DAP
	-----------------------------------mg kg^{-1}---------------------------------				
Black oats (BO)	145 aA*	110 aB	104 aB	144 a	162 a
Rye (RY)	147 aA	111 aB	95 aB	145 a	166 a
Oilseed radish (OR)	123 bA	119 aA	116 aA	139 a	155 a
RY + OR	107 bB	127 aA	91 aB	137 a	184 a
BO + OR	141 aA	132 aA	95 aB	178 a	173 a
Spontaneous Plants	135 aA	125 aA	108 aA	174 a	173 a
CV %	17.07	23.8	13.3	18.8	27.5
CTS (mean±CI)	9.7 ± 5.2	51 ± 8.9	53 ± 10.8	57 ±7.2	64 ±15.7
Forest (mean±CI)	12.9 ± 1.2	11.9 ± 2.0	14.5 ± 2.1	14.2± 2.0	15.4 ±1.4

*Means followed by the same letter, lowercase in each column, and uppercase in each row, do not differ according to Scott-Knott test ($P < 0.05$). DAS = Days after sowing; DAP = Days after seedling planting; CV = coefficient of variation. CI = confidence interval.

+ oilseed radish treatments at 58 days after sowing, which had higher resin-extracted phosphorus than conventional tillage system and forest. In general, there was a decrease in soil phosphorus throughout the three sampling times in all treatments in the vegetable no-tillage system, except for oilseed radish and spontaneous plants (Table 4). During the onion cycle, resin-extracted phosphorus ranged from 57 to 184 mg kg^{-1} in the areas under vegetable no-tillage system and conventional tillage system, and from 14.2 to 15.4 mg kg^{-1} in the forest area. In the onion cycle samplings, resin-extracted phosphorus did not differ among vegetable no-tillage system treatments, which had higher values than the conventional tillage system and forest area.

Interactions among variables

The correlations between the variables resin-extracted phosphorus and acid phosphatase activity (Figure 2) show two different behaviors. During the winter cover crop cycle (58, 80, and 99 days after sowing), correlations are moderate and positive, while in the onion cycle (40 and 99 days after seedling planting), correlations are strong and negative.

The principal component analysis explained 75.4% of the data variance in the two main components. PC1 explained 57.9% of the variance and grouped the highest values of the attributes related to phosphorus availability (phosphorus concentration, acid phosphatase, resin-extracted phosphorus, and phosphorus uptake) with no-till treatments (Figure 3). The conventional production system correlated with the lowest values in soil attributes related to phosphorus availability and cycling. PC2 explained 17.5% of the variance and separated phosphorus availability attributes and onion colonization at 99 days after onion seedling planting.

DISCUSSION

Winter cover crop biomass and onion yield

Lower biomass production in the fallow control area (spontaneous plants) than in areas with cover crops is due to lower biomass production and slower growth by the spontaneous plants (HERTWIG BITTENCOURT et al., 2013), resulting in low availability of nutrients derived from plant residue decomposition (PISSINATI, MOREIRA, & SANTORO, 2016). When plant cover is scarce, the soil is also more exposed to rainfall, increasing the probability of particle and nutrient losses by erosion and runoff (PANACHUKI et al., 2011). That seems to have affected the onion yield, which had the lowest total bulb yield in the area without the use of cover crops. Onion yield was affected by a drought of approximately 40 days, which was associated with a mean temperature of 19.4 °C in September (Figure 4), which is above the September average, that ranges between 15 and 16 ° C (https://ciram.epagri.sc.gov.br/index.php/solucoes/climatologia/). The high thermal amplitude in this period, at the stage of bulb formation may also have resulted in smaller bulbs. In previous

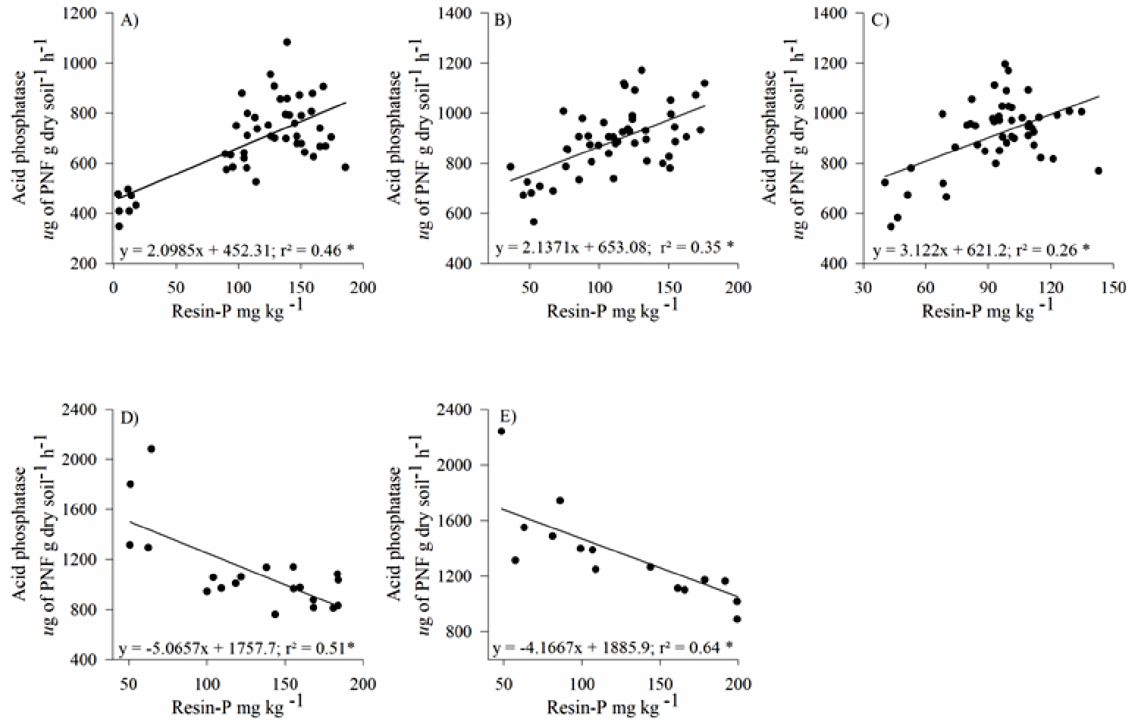

Figure 2 - Analysis of regression between resin-extracted soil phosphorus and acid phosphatase activity in areas with winter cover crops or onion. A: 58 days after sowing (DAS) cover crops; B: 80 DAS; C: 99 DAS; D: 40 days after onion seedling planting (DAP); E: 99 DAP. * Significant at $P \leq 0.05$.

years, the total yield of onion bulbs was 20 Mg ha^{-1}, values closer to the yield commonly found with the use of conventional management in the region, which is 25 Mg ha^{-1} (SOUZA, 2013; EPAGRI, 2019).

The high onion yield in treatments with single oilseed radish or black oats + oilseed radish can be due to the high efficiency of oats in nutrient cycling, especially for potassium (SANTOS et al., 2012). In the same experimental area, OLIVEIRA et al. (2016) found high amounts of potassium in residues of black oats and intercropped black oats and oilseed radish. Potassium is especially important for the development of onion plants, as it is uptaken from the soil and exported in higher amounts than any other nutrient (DESHPANDE et al., 2013; KHOKHAR, 2019.).

The importance of winter cover crops is supported by the robust regression coefficient between winter plant cover biomass and onion yield ($r^2 = 0.83$). The mean yield of 4.5 Mg ha^{-1} of cover crop biomass contributed to nutrient cycling and kept the soil protected by decreasing soil and nutrient losses. A similar work found that *Brachiaria*, an aggressive weed, had an 80% reduction in biomass when the area was previously grown with oats yielding 2.6 Mg ha^{-1}, which also improved soil quality (THEISEN & VIDAL, 1999). WHITE & WEIL, (2011) evaluated cover crop biomass production and nutrient cycling, and found positive effects on maize yield, mainly due to the phosphorus cycling from high oilseed radish (4.2 Mg ha^{-1}) and rye (7.3 Mg ha^{-1}) biomass production.

Mycorrhizal colonization, and shoot phosphorus concentration and uptake

Root mycorrhizal colonization in rye (20% in single cultivation and 12% in consortium with oilseed radish) is low, as compared with values from previous studies (SATTELMACHER et al., 1991). This is possibly due to the high soil phosphorus content (BRAUNBERGER et al., 1991; NAHAR et al., 2020), indicated by high phosphorus concentration in plants from all treatments. Rye roots in the rye + oilseed radish treatment had reduced colonization, which may be linked to the simultaneous presence of

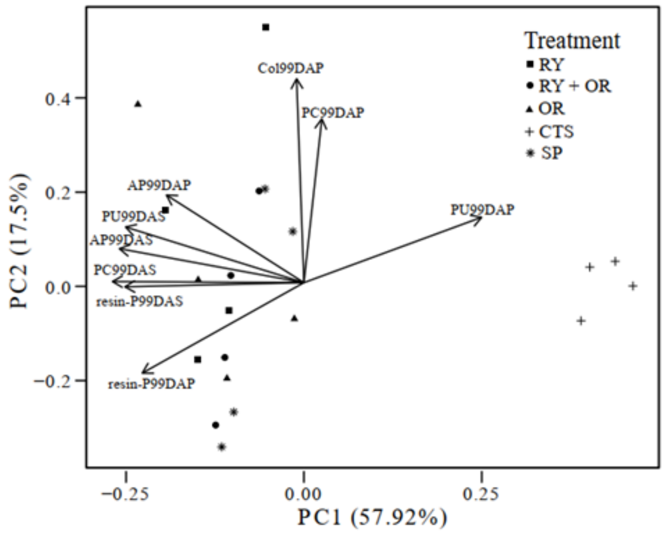

Figure 3 - Sorting diagram of principal component analysis of soil and plant attributes. DAS: Days after sowing; DAP: Days after seedling planting; PC: Phosphorus concentration; PU: Phosphorus uptake; AP: Acid phosphatase; resin-P: Resin extracted phosphorus; Col: Onion colonization; RY: rye; OR: oilseed radish; CTS: Conventional tillage system; SP: Spontaneous plants.

the non-mycorrhizal oilseed radish. Low arbuscular mycorrhizal root colonization also occurred in onion plants subsequently grown in the same areas. Reduced root colonization may be due to a decrease in the number of arbuscular mycorrhizal fungi propagules in the absence of arbuscular mycorrhizal association during cover crop growth (GALVEZ et al., 1995; DESSAI & RODRIGUES, 2012).

At 40 days after seedling planting, the percentages of onion root total colonization in the rye, spontaneous plants, and conventional tillage system plots were considerably high (ROZPADEK et al., 2016). In the oilseed radish and rye + oilseed radish treatments, the previous presence of the non-mycorrhizal plant species seems to have affected negatively the establishment of the mycorrhizal symbiosis in onion. That indicates that pre-cultivation with those plant species may lower the density of propagules needed to ensure adequate root colonization in the early stages of plant development. The higher percentage of vesicles in the spontaneus plants plots than in the other treatments may be due to stress conditions. Vesicles are formed by the fungus when it becomes nutritionally limited, as lipid accumulation in vesicles indicates that they are storage structures (PETERSON, 2004).

Cover plants in single rye and spontaneous plants in plots had higher phosphorus concentration in their tissues than those with oilseed radish or rye + oilseed radish. However, phosphorus uptake in plants from areas with cover crops was similar to, or higher than, those found in plants from the fallow areas with spontaneous plant. Inorganic phosphorus uptake by plants is enhanced by phosphatase-catalyzed mineralization of organic phosphorus (ADETUNJI et al., 2017). Besides, rye association with arbuscular mycorrhizal fungi may have contributed to the increases in phosphorus uptake. Those results highlight the importance of cover crops in nutrient cycling, affecting subsequent crops, such as onions.

Acid phosphatase activity and resin-extracted phosphorus

Acid phosphatase activity in treatments with cover crops was similar to results from previous studies (CONTE et al., 2002; DALLA COSTA & LOVATO, 2004; CASTRO LOPES et al., 2018). That demonstrates the role of acid phosphatase activity for the availability of organic phosphorus, and it also indicates that plants have different strategies to obtain that nutrient. The low acid phosphatase activity in the conventional tillage system may have been

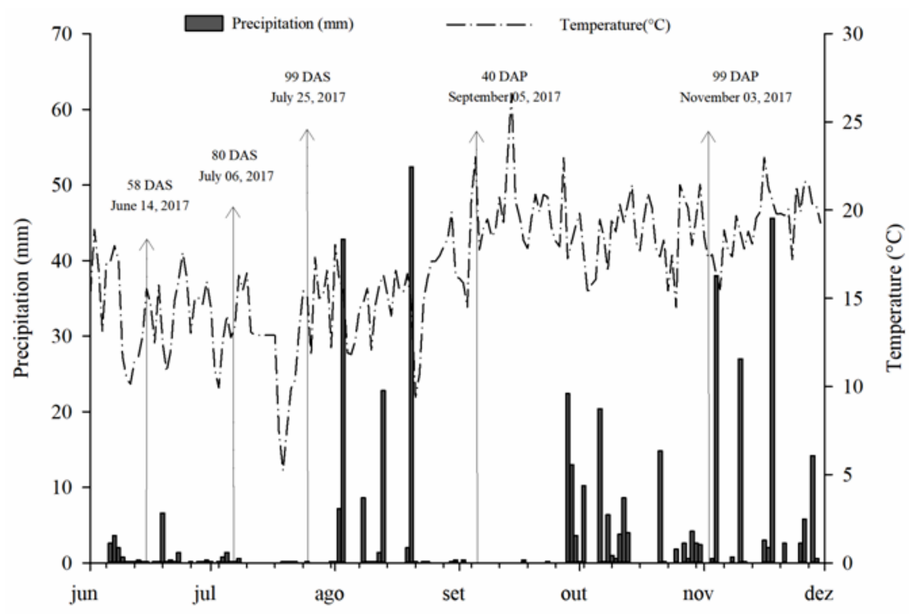

Figure 4 - Total precipitation (mm) and mean temperature (°C) from June to November 2017 in Ituporanga, Brazil (data from experimental station - EPAGRI). DAS = Days after sowing; DAP = Days after seedling planting).

caused by recurrent soil tillage, which accelerates decomposition of plant residues, resulting in lower levels of organic matter (SANTOS et al., 2018) and reduced sources of organic phosphorus (DICK, 1984). Higher acid phosphatase activity in no-tillage systems than in conventional tillage areas has been demonstrated in previous papers, like the one by LISBOA et al. (2012).

Acid phosphatase activity in the conventional tillage system was low, in comparison with the conservationist systems, as found in other studies (CONTE et al., 2002; BARBIERI et al., 2019). Although the conventional tillage system Mehlich-extracted soil phosphorus (17.1 mg dm^{-3}) is considered high according to the regional classification (CQFS RS / SC 2016), acid phosphatase activity may have been affected by the use of millet, which yields abundant biomass and a high C/N ratio, above 30 (CALVO et al., 2010). High C/N ratios delay microbial decomposition and increase the permanence of plant residues on the soil surface even after tillage. Those conditions elicit activity of microorganisms seeking phosphorus from organic sources (CARDOSO et al., 2013), such as the millet residues remaining in the conventional tillage system.

The high levels of acid phosphatase activity in the forest (Table 3), associated with low soil phosphorus concentration (Table 6), show that mineralization of organic phosphorus is an essential process for the availability of this element in natural areas. CONTE et al. (2002) measured phosphatase activity in a forest area adjacent to a no-till experiment and found that acid phosphatase activity in the soil under native forest was 2.5 times higher than in no-tillage plots. In natural systems, such as native forests and prairies, phosphorus supply to plants depends heavily on organic phosphorus cycling, especially in soils of tropical and subtropical regions (STEWART & TIESSEN, 1987).

We found a consistent pattern of higher acid phosphatase activity in the forest than in managed areas. The conventional tillage system area had the lowest enzymatic activity, while vegetable no-tillage system treatments had an intermediate activity, suggesting that acid phosphatase activity is a good indicator of soil quality (MAKOI & NDAKIDEMI, 2008; MANKOLO et al., 2012; ADETUNJI et al., 2017). That is even more relevant for management systems that use cover crops and have high shoot and root biomass accumulation during the year, in comparison to systems that keep the soil exposed in some periods (DODOR & TABATABAI, 2003).

Resin-extracted phosphorus was higher in the vegetable no-tillage system plots than in the conventional tillage system and the forest. Those high values are, in part, due to the fertilization used with Gafsa rock phosphate and poultry litter, and those levels are maintained over the years due to the use of cover crops that recycle the nutrient. The increase in resin-extracted phosphorus after the rolling of cover crop and spontaneous plant highlights the importance of soil-covering plants to phosphorus cycling. ALMEIDA et al. (2018) reported that cover crops increased resin-extractable phosphorus by up to 10% in a no-tillage system with soybeans and *Urochloa ruziziensis*. Such benefits did not happen in the conventional tillage system area, where this nutrient had to be supplied by chemical fertilizers. Phosphorus extraction with resin seeks to reproduce the process of plant uptake of the most labile phosphorus fractions in the soil. Our results suggest that it is a suitable method for systems increasing the addition of organic matter to the soil, as is the case of no-tillage associated with the use of cover crops. The positive correlation between acid phosphatase activity and resin-extracted phosphorus suggests that a significant part of the soil available phosphorus comes from enzyme action during the cover crop cycle. CUI et al. (2015) also found a positive correlation between enzyme activity and phosphorus availability in orchards with cover plants.

On the other hand, during the onion cycle, higher phosphorus levels appeared to inhibit phosphatase activity. Higher availability of soluble phosphorus in soil lowers acid phosphatase activity in soil with decreased phosphorus levels over successive years of cultivation (FUJITA et al., 2017; REDEL et al., 2019). The ability of plants to acquire inorganic phosphorus appears to be regulated by transcription of high-affinity phosphate transporters, many of them strongly induced during phosphate deficiency (RAGHOTHAMA & KARTHIKEYAN, 2005). Besides, altered root morphology, due to mycorrhizal symbiosis, increases the ability of plants to acquire inorganic phosphorus (MAI et al., 2019). Phosphorus supply to roots and uptake by the plant are complex processes that need to be approached with different techniques. Soil analysis procedures, such as extraction with resin, associated with measurements of soil biological activity, like enzyme activity, may offer valuable information on phosphorus dynamics in soil and plant.

The results of the principal component analysis show the effect of cover crops on soil chemical and biological quality. The inclusion of cover crops in different production systems favors nutrient cycling, as demonstrated for phosphorus in our study. In addition, the presence of different plants in the fallow, even yielding less plant biomass, reflected the ability of those spontaneous species to cycle nutrients, similarly to the sown cover crops, as previously demonstrated by OLIVEIRA et al. (2016) in the same area. Our results also reaffirmed the negative effect of conventional tillage system on the evaluated attributes, mainly acid phosphatase and available phosphorus, as indicated by resin-extracted forms.

The differences between the management systems demonstrate that crop systems minimizing soil tillage and maximizing plant biomass input increase the overall quality of the environment while avoiding heavy application of highly soluble fertilizers. Although there were lower yields in the no-tillage system than in the conventional tillage system, there were yield increases in comparison with the fallow area, due to reduced tillage and increased biomass addition by cover crops. In the long term, conservation management procedures improve biological and chemical attributes through enhanced nutrient cycling, thereby ensuring production sustainability, while requiring less external inputs to maintain soil and environmental quality.

CONCLUSION

Available soil phosphorus and acid phosphatase activity are higher in no-tillage areas than in conventional tillage areas, and plants in no-tillage areas, with winter cover crops or spontaneous plants, accumulate more phosphorus than plants from areas with tillage. In contrast, the conventional tillage system negatively affects acid phosphatase and resin-extracted phosphorus, as shown by Principal Component Analysis.

Areas with cover crops have higher biomass production and increased onion yield and phosphorus uptake compared with fallow areas with spontaneous plants, and the presence of oilseed radish, a non-mycorrhizal plant, is associated with decreases in the colonization of rye and onion roots by arbuscular mycorrhizal fungi.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHORS' CONTRIBUTIONS

The authors also contributed to the manuscript.

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