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Release of Phosphorus Forms from Cover Crop Residues in Agroecological No-Till Onion Production

Rodolfo Assis de Oliveira[(1)], Jucinei José Comin[(2)], Tales Tiecher[(3)], Rogério Piccin[(4)], Luiza Michelon Somavilla[(5)], Arcângelo Loss[(2)], Cledimar Rogério Lourenzi[(2)], Claudinei Kürtz[(5)] and Gustavo Brunetto[(6)*]

[(1)] Universidade Federal de Santa Catarina, Centro de Ciências Agrárias, Departamento de Engenharia Rural, Programa de Pós-graduação em Agroecossistemas, Florianópolis, Santa Catarina, Brasil.
[(2)] Universidade Federal de Santa Catarina, Centro de Ciências Agrárias, Departamento de Engenharia Rural, Florianópolis, Santa Catarina, Brasil.
[(3)] Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Departamento de Solos, Programa de Pós-Graduação em Ciência do Solo, Porto Alegre, Rio Grande do Sul, Brasil.
[(4)] Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Solos, Programa de Pós-graduação em Ciência do Solo, Santa Maria, Rio Grande do Sul, Brasil.
[(5)] Empresa de Pesquisa e Extensão Rural de Santa Catarina, Ituporanga, Santa Catarina, Brasil.
[(6)] Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Solos, Santa Maria, Rio Grande do Sul, Brasil.

ABSTRACT: Cover crops grown alone or in association can take up different amounts of phosphorus (P) from the soil and accumulate it in different P-forms in plant tissue. Cover crop residues with a higher content of readily decomposed forms may release P more quickly for the next onion crop. The aim of this study was to evaluate the release of P forms from residues of single and mixed cover crops in agroecological no-till onion *(Allium cepa* L.) production. The experiment was conducted in Ituporanga, Santa Catarina (SC), Brazil, in an Inceptisol, with the following treatments: weeds, black oat (*Avena sativa* L.), rye (*Secale cereale* L.), oilseed radish (*Raphanus sativus* L.), oilseed radish + black oat, and oilseed radish + rye. Cover crops were sown in April 2013. In July 2013, plant shoots were cut close to the soil surface and part of the material was placed in litterbags. The bags were placed on the soil surface and residues were collected at 0, 15, and 45 days after deposition (DAD). Residues were dried and ground and P in the plant tissue was determined through chemical fractionation. The release of P contained in the tissue of cover crops depends not only on total P content in the tissue, but also on the accumulation of P forms and the quality of the residue in decomposition. The highest accumulation of P in cover crops occurred in the soluble inorganic P fraction, which is the fraction of fastest release in plants. Black oat had the highest initial release rate of soluble inorganic P, which became equal to the release rate of other cover crop residues at 45 DAD. Weeds released only half the amount of soluble inorganic P in the same period, despite accumulating a considerable amount of P in their biomass. The mixtures of oilseed radish + rye and oilseed radish + black oat showed higher release of P associated with RNA at 45 DAD in comparison to the single treatments.

Keywords: Green manure crops, P cycling, *Allium cepa* L.

*** Corresponding author:**
E-mail: brunetto.gustavo@gmail.com

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INTRODUCTION

Phosphorus (P) is an essential macronutrient for molecular and physiological plant functions, including energy transfer, photosynthesis, and carbohydrate metabolism. Phosphorus is also a component of nucleic acids and phospholipids (Niu et al., 2015). Low concentrations of P, typically less than 0.3 mg L^{-1} P, and often less than 0.001 mg L^{-1} P, are found in the soil solution, while concentrations of up to 3000 mg kg^{-1} P can be found in plant tissue (Bieleski 1973, 1976). Low P availability in the soil is a result of its sorption capacity in highly weathered soils, rich in 1:1 clays and Fe and Al oxides (Fink et al., 2016; Gérard, 2016). Therefore, P is one of the nutrients that most often limits yield in Brazilian agricultural systems.

Faced with a growing global demand for food and an expected shortage of phosphate fertilizer in the first half of this century (Grantham, 2012), the efficient use of P has become a key issue for humanity. Therefore, we must increase agricultural production in the most sustainable way possible (Worstall, 2013) and reduce the risk of environmental contamination caused by indiscriminate use of phosphate fertilizers (Broetto et al., 2014). Thus, strategies of nutrient management in soils, such as the use of cover crops to maintain and increase crop yield and also to reduce the demand for mineral fertilizer, need to be developed in order to more efficiently use naturally occurring and applied P in soils (Tiecher et al., 2015).

Phosphorus dynamics in Brazilian soils are often the subject of studies that address the influence of soil mineralogy (Eberhardt et al., 2008; Fink et al., 2014) and mineral (Schmitt et al., 2013) and organic fertilizer use (Tiecher et al., 2015). Furthermore, there have been studies evaluating the effects of the use of cover crops and soil management systems (Bezerra et al., 2015), as well as the influence of biotic and abiotic environmental factors (Resende et al., 2011). However, few studies have evaluated the accumulation and release of P forms from crop residues and they generally only evaluate total P accumulation and P release throughout crop cycles (Viola et al., 2013). According to Lajtha and Harrison (1995), plants may have mechanisms that help acquire P, such as increased root/shoot ratio and root surface, higher uptake rate per root unit, increased root exudation of phosphatases and organic complexing, and mycorrhizal association. Therefore, plants can take up varying amounts of P in the same soil, resulting in higher or lower P cycling by the deposition of crop residues on the soil surface.

Average total P content in plants ranges from 0.05 to 0.50 % (Marschner, 2012) and P content associated with RNA and lipids may vary by a factor of up to five times, whereas soluble inorganic P may vary up to 50 times (Bieleski, 1973). According to Bieleski and Ferguson (1983), this occurs because the vacuole of higher plant cells acts as a non-metabolic soluble inorganic P reservoir, responsible for providing 85-95 % of inorganic P to plants. Nearly all P in the vacuole is found in soluble inorganic forms, and part of it in soluble organic forms, mainly as monoesters. Diester forms of organic P are mainly found in nucleic acids (DNA and RNA) and water-insoluble phospholipids. Thus, in addition to total P content in plant tissue, it is important to know the forms of P that plants accumulate in their tissues, as this will determine the rate of P release from plant tissues for subsequent crops (Casali et al., 2011). To acquire this information, a sequential chemical fractionation technique originally proposed by Bieleski (1973) can be used. This technique separates P from plant tissue into six groups: (*i*) soluble inorganic, (*ii*) soluble organic, (*iii*) lipidic, (*iv*) DNA, (*v*) RNA, and (*vi*) residual fraction.

In an agroecological no-tillage onion (*Allium cepa* L.) production system, cover crop residues are deposited between onion rows, especially in late winter and early spring. This provides greater protection of the soil surface against the impact of raindrops, which increases the stability of aggregates and reduces soil erosion, suppresses the incidence of weeds, and increases water storage in the soil profile (Loss et al., 2015). Part of the nutrients taken up by the cover crops are accumulated in roots and shoots, which reduces

the likelihood of leaching in the soil profile (e.g., nitrate) or loss by specific adsorption of high energy by the colloidal fraction of the soil (e.g., phosphate). This increases the residence time of these nutrients in available forms in the soil and thus favors uptake by the subsequent crop. However, there are few studies addressing the dynamics of forms of P accumulated in species of single or mixed cover crops, or the release rate of forms of P to the soil throughout residue decomposition.

Residues of cover crops alone or in association in decomposed, release different amounts of P in soil in readily available or more recalcitrant forms to the next onion crop. However, studies addressing the dynamics of accumulation of P forms and their rate of release from cover crops grown alone or in association are still lacking. For this reason, the aim of this study was to evaluate the release of forms of P from residues of single and mixed cover crops in agroecological no-tillage onion production.

MATERIALS AND METHODS

Experimental area and treatments

The experiment was carried out from July to November 2013 in Ituporanga, in the Upper Itajai Valley region, state of Santa Catarina, Brazil (27° 22' S, 49° 35' W, and 475 m altitude). According to the Köppen classification system, the climate is humid subtropical (Cfa) (Kottek et al., 2006). Climate data collected during the experiment in 2013 are shown in figure 1. There are hot summers and infrequent frosts and no defined dry season. The soil was classified as a *Cambissolo Húmico* according to the Brazilian System of Soil Classification, and as an Inceptisol, according to the Soil Taxonomy System (Soil Survey Staff, 2006), with 380, 200, and 420 g kg^{-1} of clay, silt, and sand (clay loam texture), respectively (Claessen, 1997). At the beginning of the experiment (April 2009), the soil from the 0.00-0.10 m layer had the following chemical properties (Tedesco et al., 1995): organic matter, 40 g kg^{-1}; pH in water, 6.0; available P and K, 26.6 and 145.2 mg kg^{1}, respectively (extracted by Mehlich-1); exchangeable Al, Ca, and Mg, 0.0, 7.2, and 3.4 $cmol_c$ kg^{-1}, respectively (extracted by 1 mol L^{-1} KCl); $CECpH_{7.0}$, 14.32 $cmol_c$ kg^{-1}, base saturation of $CECpH_{7.0}$, 76 %; and Al saturation, 0.00 %.

The experiment was established in an area with a 20-year history of onion growth under conventional tillage (plowing and harrowing) until 1996. From that year on, a minimum-tillage system of onion was established with crop rotation and cover crops (black oat - *Avena strigosa*, velvet bean - *Mucuna aterrima*, pearl millet - *Pennisetum glaucum*, sunn hemp - *Crotalaria juncea*, common vetch - *Vicia sativa*). This system continued from 1996 to 2007, and then sweet potato (*Ipomoea batatas*) was grown until 2009. Since that time, a no-tillage system experiment of onion has been carried out. In April 2009, the weeds were desiccated, and lime was applied and incorporated to raise pH in water to 6.0.

Treatments consisted of sowing single or mixed cover crops, and fallow treatment used as a control was dominated by weeds (WD) comprising 20 botanical families, mostly bermuda grass (*Cynodum* sp.), bitter dock (*Rumex obtusifolius*), staggerweed (*Stachys arvensis*), purple amaranth (*Amaranthus lividus*), flatsedge (*Cyperus* spp.), radishroot woodsorrel (*Oxalis corniculata*), hairy beggarticks (*Bidens pilosa*), and gallant soldier (*Galinsoga parviflora*). Cover crops were black oat (*Avena strigosa* Schreb - 120 kg ha^{-1} of seeds) (BO), rye (*Secale cereale* L. - 120 kg ha^{-1} of seeds) (RY), oilseed radish (*Raphanus sativus* - 20 kg ha^{-1} of seeds) (RD), oilseed radish (10 kg ha^{-1} of seeds) + rye (60 kg ha^{-1} of seeds) (RD + RY), and oilseed radish (10 kg ha^{-1} of seeds) + black oat (60 kg ha^{-1} of seeds) (RD + BO). At the time of setting up the experiment in April 2009 and during the following years, the cover crops were sown by broadcasting on the soil surface without the use of soluble fertilizers. The quantity of seeds used per hectare was 50 % of the highest values recommended by Monegat (1991). The proportion of seeds of winter species in the RD + RY and RD + BO treatments was 86 % for the cruciferous and 14 %

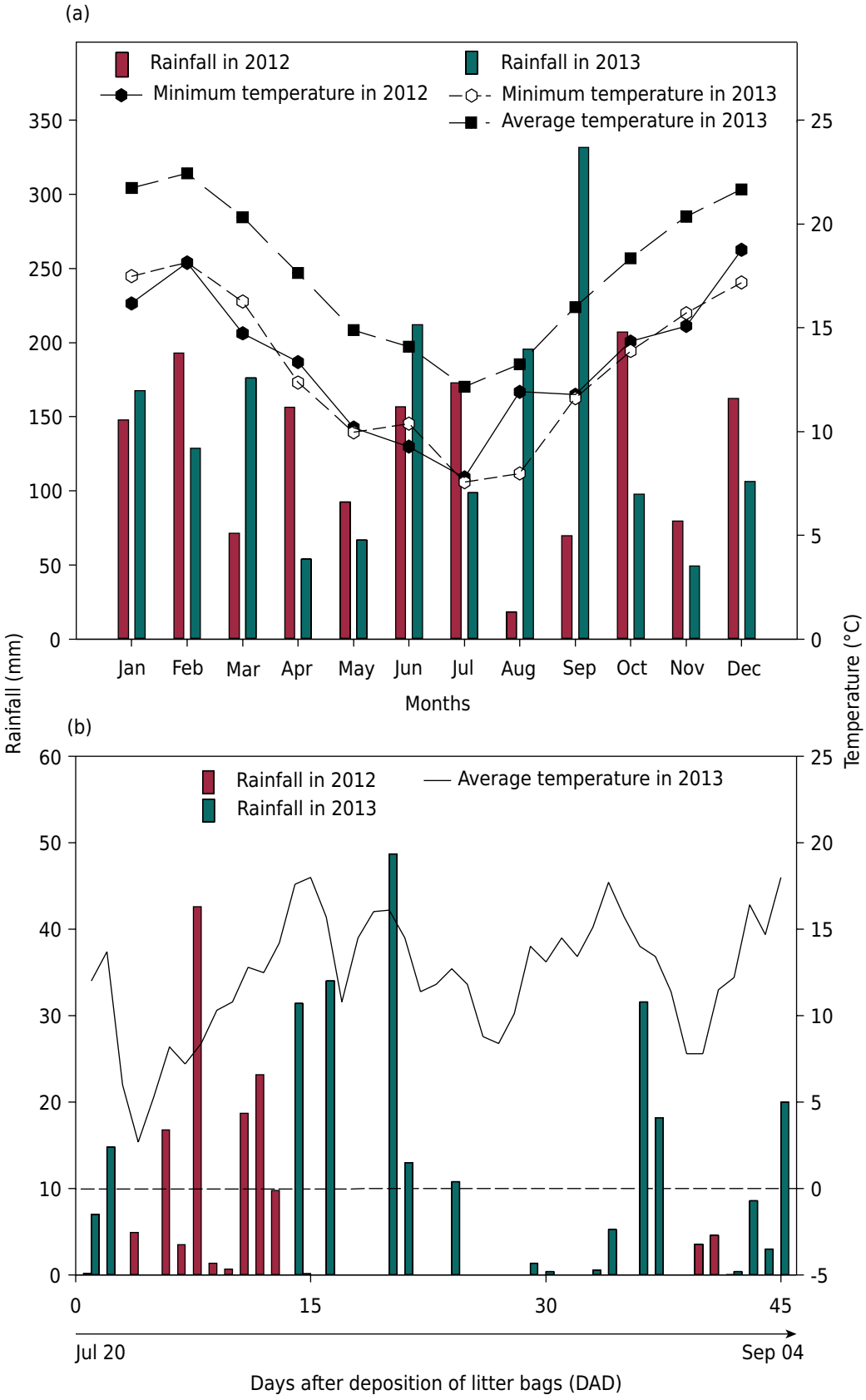

Figure 1. Monthly average rainfall and monthly average and minimum temperature in 2013 (a); Rainfall and daily average temperature in 2013 at 0, 30, and 45 DAD in the experimental area (b).

for the grass species. In the summer of each year, all plots were sown with velvet bean (*Mucuna aterrima*). A randomized block experimental design was used, with eight replicates. Each experimental unit was 5 × 5 m (25 m²).

In July of each year, all winter species and weeds were rolled down with a knife roller. Subsequently, the total amount of 42 kg ha^{-1} P (Gafsa rock phosphate) was broadcast on the soil surface. In addition, 104 kg ha^{-1} K and 160 kg ha^{-1} N in the form of poultry manure were broadcast. Half of the fertilizer was applied at transplanting of seedlings and half 30 days after transplanting. In the 2011 crop, rock phosphate was not applied because P content was very high according to regional soil fertility parameters (CQFSRS/SC, 2004). Plant furrows were opened with a machine adapted for no-tillage, and onion seedlings (cv. EMPASC 352 - Bola Precoce) were manually transplanted. Plants were placed in rows 0.40 m apart, with 0.10 m between plants. Each plot had 10 rows, for a total of 500 onion plants per plot. Weeding was done at 60 and 90 days after seedling transplanting. The procedures were repeated each year.

Cover crop sampling and litterbag preparation

On July 11, 2013, samples were taken of cover crop shoots from each treatment. Plants were cut at ground level in a 0.5 × 0.5 m (0.25 m^2) area for each subsample. Five fresh sub-samples were collected in each plot and dried at 65 °C until constant weight, and plant dry matter (DM) yield was measured. On July 22, 2013, shoots of the single and mixed species of winter cover crops remaining in the field were cut at ground level and left in the area. Samples of the plant shoot matter were dried at 65 °C. After drying, the DM was ground, and chemical composition was determined (Table 1). The other part of fresh shoot matter was homogenized, weighed, and placed in nylon fabric litterbags (0.40 × 0.40 m, with 2 mm mesh) (Tagliavini et al., 2007). The following amounts of fresh shoot matter were placed in separate litterbags: 235.13 g of WD, 767.99 g of BO, 434.50 g of RY, 460.46 g of RD, 570.67 g of RD + BO, and 416.82 g of RD + RY. These

Table 1. Initial chemical characterization of weed (WD), black oat (BO), rye (RY), oilseed radish (RD), oilseed radish + black oat (RD + BO), and oilseed radish + rye (RD + RY) residues and added amounts of dry matter and nutrients

Parameter	WD	BO	RY	RD	RD + BO	RD + RY
	g kg^{-1}					
TOC	409.4 ± 1.02[(1)]	375,0 ± 0.40	401.0 ± 2.06	365.2 ± 1.21	369.7 ± 0.80	366.8 ± 1.50
N	26.2 ± 0.17	19.8 ± 0.21	18.0 ± 0.19	22.3 ± 0.37	21.9 ± 0.34	24.2 ± 0.22
P	5.5 ± 0.12	6.0 ± 0.02	5.7 ± 0.02	6.5 ± 0.06	6.6 ± 0.01	6.6 ± 0.01
K	21.5 ± 0.10	42.9 ± 0.59	24.9 ± 0.30	33.4 ± 0.45	36.3 ± 0.32	33.0 ± 0.81
Ca	4.9 ± 0.10	3.5 ± 0.04	6.4 ± 0.12	6.9 ± 0.06	10.4 ± 0.11	8.0 ± 0.16
Mg	1.9 ± 0.05	1.6 ± 0.03	2.1 ± 0.11	2.1 ± 0.01	4.6 ± 0.05	2.6 ± 0.04
Cel	182.3 ± 1.02	239.6 ± 7.21	161.8 ± 7.59	211.0 ± 2.87	220.7 ± 2.34	192.9 ± 1.12
Lig	69.2 ± 0.77	86.1 ± 2.23	102.8 ± 5.33	71.7 ± 1.63	93.5 ± 2.41	80.6 ± 0.63
Bio	748.4 ± 1.28	674.3 ± 5.78	735.5 ± 9.01	717.3 ± 4.09	685.6 ± 4.19	726.5 ± 1.57
C/N	16 ± 0.99	19 ± 1.93	22 ± 2.32	17 ± 2.72	17 ± 2.50	15 ± 1.53
Lig/N	3 ± 0.17	4 ± 0.21	6 ± 0.19	3 ± 0.37	4 ± 0.34	3 ± 0.21
C/P	75 ± 14.82	62 ± 2.61	70 ± 5.36	56 ± 4.91	56 ± 6.28	56 ± 7.59
Cel/Lig	3 ± 0.17	3 ± 0.21	2 ± 0.19	3 ± 0.37	2 ± 0.34	2 ± 0.22
			Added amount (kg ha^{-1})			
DM	4620.0	5263.1	5060.0	3640.0	4030.0	3730.0
TOC	1891.6	1973.7	2029.0	1329.4	1490.1	1368.0
N	120.9	104.4	91.1	81.1	88.3	90.2
P	25.2	31.7	28.8	23.9	26.6	24.5
K	99.2	225.8	126.0	121.5	146.2	123.1
Ca	22.4	18.6	32.2	25.0	42.0	29.8
Mg	8.8	8.4	10.9	7.7	18.7	9.8

[(1)] Mean ± standard deviation (n = 4). Cel: cellulose, Lig: lignin, Bio: no structural biomass, DM: dry matter, TOC: total organic carbon.

values, in terms of kg ha^{-1} of DM, amounted to 4620 WD, 5263 BO, 5060 RY, 3640 RD, 4030 RD + BO, and 3730 RD + RY, and they are equivalent to DM amounts obtained in the field at the sampling carried out on July 11, 2013. The other initial chemical characteristics of the residues and amounts of nutrients added to each treatment are shown in table 1.

On July 22, 2013, the litterbags were placed directly on the soil surface, between onion rows. Twenty-four bags of each treatment were placed (six bags in each plot), for a total of 144 litterbags. Bags were secured on the ground by iron bars to prevent shifting in the wind. Bags were collected at the time of deposition (time 0) and at 15 and 45 days after deposition (DAD) of the litterbags. The bags were opened in the laboratory; the residue removed after the bag remained in the experimental area contained soil particles and poultry litter, given that the experimental area contains soil particle waste. The litter bags were opened in the laboratory and the remaining plant residues were removed, washed in distilled water and then in 0.1 mol L^{-1} HCl solution and again in distilled water. Residues were dried in a drying oven with circulation at 65 °C, weighed, ground and reserved for chemical fractionation of P in the tissue.

Chemical fractionation of P in the tissue

Residue DM was subjected to P chemical fractionation according to the method proposed by Miyachi and Tamiya (1961) with adjustments proposed by Schmidt and Thannhauser (1945) and Casali et al. (2011). The following P fractions were obtained: total acid-soluble P (TASP), soluble inorganic P (Psi), soluble organic P (Pso) (by the difference between TASP and Psi), lipid P (Plip), P associated with RNA (P-RNA), P associated with DNA (P-DNA), and the residual P fraction (Pres). To obtain these fractions, 0.2 g of DM was weighed in triplicate for each replication of the treatment. After that, DM was added to 15 mL Falcon round-bottom tubes. Then 10 mL of 0.2 mol L^{-1} $HClO_4$ was added, and the tubes were shaken by hand for 5 min and centrifuged for 10 min at 6,000 *g*. The supernatant was filtered with quantitative filter with 8-micron pores. An extra 5 mL of 0.2 mol L^{-1} $HClO_4$ was added to the tissue, and the centrifugation and filtration process was repeated. Finally, supernatants were united (10 mL + 5 mL), which formed a 15 mL extract. The P content in the same extract was analyzed according to the method described by Murphy and Riley (1962), and Psi was obtained. Soluble organic P (Pso) was obtained by the difference between TASP and Psi.

The tissue sample remaining from the first extraction remained in the 15 mL Falcon tubes. This sample was added to 6 mL of ethanol + ether + chloroform solution (E + E + C) at a ratio of 2:2:1, and this remained in a water bath at 50 °C for 1 h. Then, the tissue sample with E + E + C solution was centrifuged for 10 min at 6,000 *g*, and the supernatant was reserved in a 50 mL Erlenmeyer flask. Subsequently, 4 mL of cold ether (4 °C) was added and centrifugation was repeated, and the extract of the second centrifugation was mixed with the extract of the first centrifugation. The Erlenmeyer flask extract rested for 24 h in an air extraction chamber for the ether to evaporate and for 3 h in a forced air circulation oven at 37 °C.

Following fractionation, the tissue samples remaining from the extraction with E + E + C received 6 mL of 0.5 mol L^{-1} KOH, and the Falcon tubes were closed and shaken by hand for 1 min. These samples rested for 17 h at 37 °C in a forced air circulation oven. Next, we added 1 mL of 3.0 mol L^{-1} HCl and 1 mL of 70 % $HClO_4$. Afterwards, the tubes were centrifuged for 10 min at 6,000 *g*, and the supernatant was removed and stored in 20 mL acrylic tubes. To the tissue that remained in Falcon tubes was added 5 mL of 0.5 mol L^{-1} $HClO_4$, and this was centrifuged for 10 min at 6,000 *g.* The extract of this centrifugation was mixed with the extract of the 20 mL acrylic tubes.

A quantity of 5 mL of 0.5 mol L^{-1} $HClO_4$ was added to the tissue remaining in the Falcon tubes. The Falcon tubes were shaken by hand for 1 min, and then kept in a water bath at 100 °C for 15 min. Afterwards, the tubes were centrifuged for 10 min at 6,000 *g*, and the

supernatant was stored in the acrylic tubes. The tissue residue remaining in the Falcon tube was transferred to 15 mL digestion tubes of 50 mL capacity obtaining Pres. A quantity of 2 mL was removed from extracts of the acrylic and Falcon tubes and digested with 2 mL concentrated H_2SO_4 and 1 mL 30 % H_2O_2 in a digester block (Tedesco et al., 1995) using 50 mL digestion tubes. As a result, we obtained Pso, Plip, P-DNA, P-RNA, and Pres. All P (λ 882 nm) fractions were determined and quantified according to Murphy and Riley (1962).

Statistical analysis

The data regarding P content obtained by chemical fractionation of the tissue samples of cover crops was subjected to analysis of variance and when the effects were significant, the means were compared by the Tukey test at 5 % probability (SAS, 2011). The proportions of P forms in the tissue of each treatment at the three sampling times were compared by multivariate analysis of principal component analysis (PCA), based on the correlation between the variables. We did not use the percentage of TASP in this analysis, to avoid redundancy in PCA, since it represents the sum of Pso and Psi.

RESULTS AND DISCUSSION

Distribution of P forms in cover crop residues

The distribution of P forms in plant tissue at time 0 (0 DAD) occurred in the following order of importance for all treatments: Psi > Pso ≈ Plip ≈ P-RNA > P-DNA ≈ Pres (Figure 2). The Psi form exhibited a content of 3068 ± 566 mg kg^{-1} in the overall average, representing approximately 73 ± 14 % of the total P content (Figures 2, 3, and 4). Accumulation of P occurs because plants typically have more than one nutrient uptake mechanism, especially in environments with high availability of Pi (Bieleski, 1973; Martinez et al., 2005). The Psi fraction represents the highest P content in the tissue because plants take up P in the form of orthophosphoric acid, and since not all of the P taken up is used metabolically, it is allocated to storage organelles, especially in the vacuole (Marschner, 2012). This is because most of the P in plant tissue is found in inorganic forms (Casali et al., 2011; Marschner, 2012) and the inorganic P in the tissue makes up the labile compartment. These results corroborate with those obtained by Fernandes et al. (2000), Pereira et al. (2008), Casali et al. (2011), and Oliveira et al. (2016), who noticed that most of the tissue P is found in the soluble inorganic form in well-nourished plants grown in soil with sufficient nutrient content.

In comparison to time 0 (0 DAD), a decrease in total P content observed in cover crop tissue was always accompanied by a decrease in Psi content at 15 and 45 DAD (Figures 2 and 3). The content of the Pso, Plip, and P-DNA fractions fluctuated over time in relation to the cover crop species. In contrast, an increase in Pres, P-RNA, and P-DNA fractions can be seen in the last sampling period in all treatments (Figure 4), and an increase in the concentration of P-RNA and Pres was consistent over time in all treatments (Figures 2 and 3). This demonstrates that in the early stages of plant residue decomposition, the Psi contained in the vacuole of the plant is released because it is soluble in water (diesters: nucleic acids, phospholipids, and phosphoproteins). However, the more recalcitrant fractions, such as Pres and P-RNA, tend to increase their proportions in the residues as they are present in organic compounds that depend on mineralization for P release (Bieleski and Ferguson, 1983; Frossard et al., 1995; Casali et al., 2011). The mineralization rate of P fractions, mainly organic fractions, is dependent on the biochemical composition of the tissue, such as the C/N ratio value and lignin and cellulose contents and their ratios (Manzoni et al., 2008; Gentile et al., 2009), as well as the form and location of the nutrient in the tissue. In general, residues with high C/N ratio, high lignin content, and reduced cellulose values, such as species of the Poaceae family, including black oat and rye (Table 1), show lower P release over time (Ferreira et al., 2014) (Figure 2).

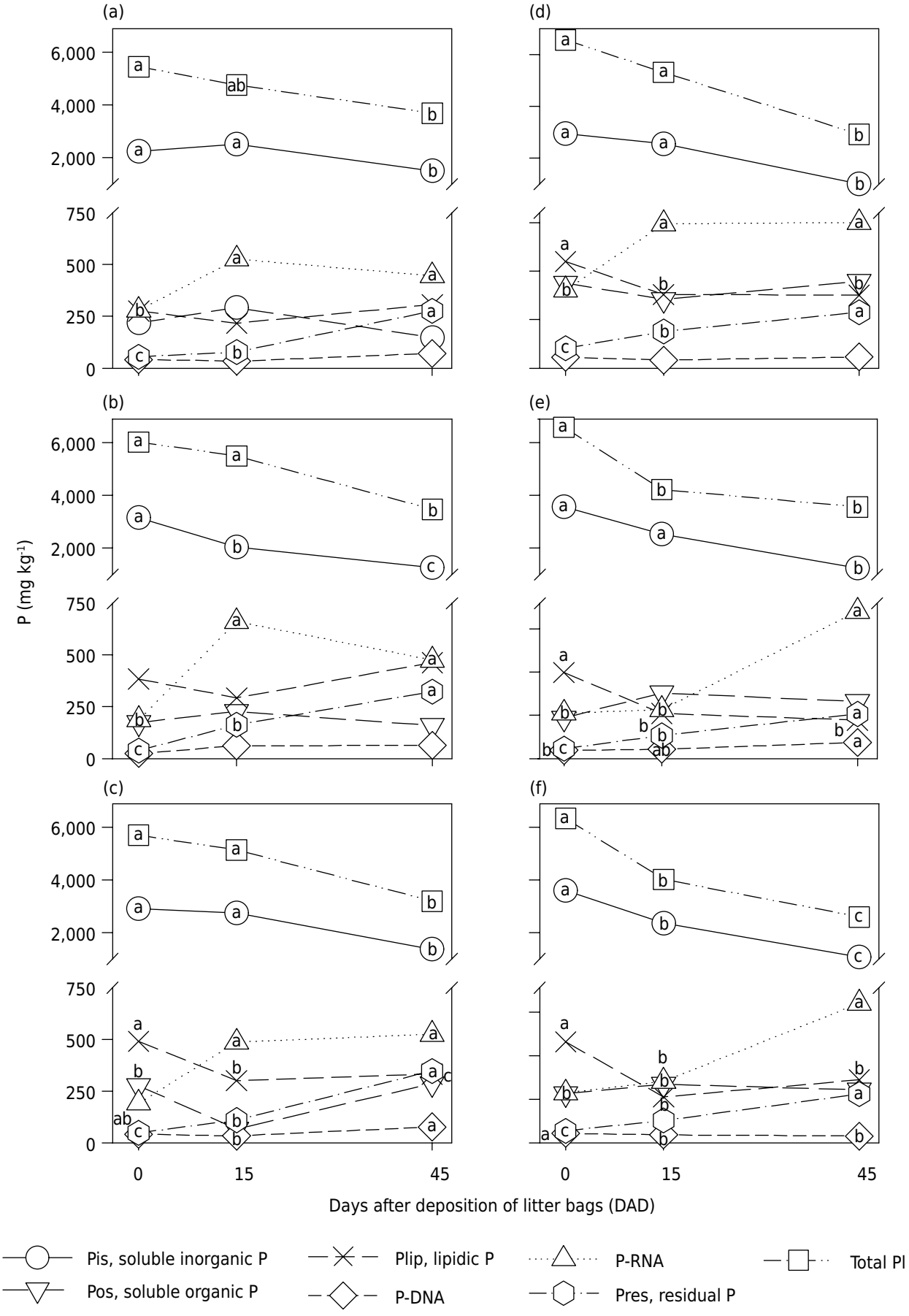

Figure 2. Changes in phosphorus forms (a) in residues of weeds (WD); (b) black oat (BO); (c) rye (RY); (d) oilseed radish (RD); (e) oilseed radish + black oat (RD + BO); and (f) oilseed radish + rye (RD + RY) at 0, 15, and 45 days after deposition (DAD) of the litterbags on the soil surface in agroecological no-till onion. Means followed by the same letter in DAD do not differ by the Tukey test ($p<0.05$).

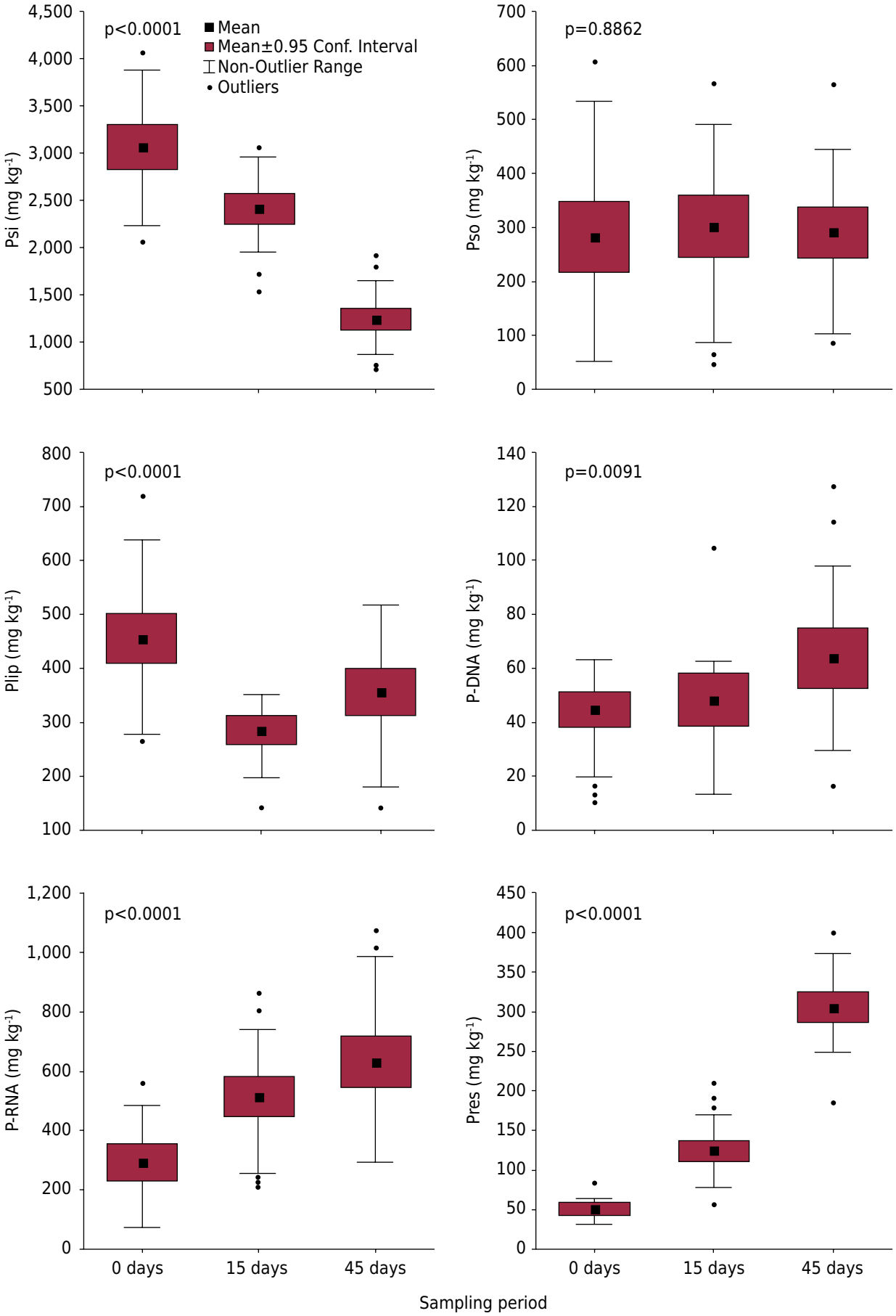

Figure 3. Content of P forms in the plant tissue of all treatments evaluated in terms of sampling time (time 0 and at 15 and 45 days after the deposition of decomposition bags). Psi: soluble inorganic P, Pso: soluble organic P, Plip: lipidic P, P-DNA: P associated with DNA; P-RNA: P associated with RNA; and Pres: residual P fraction.

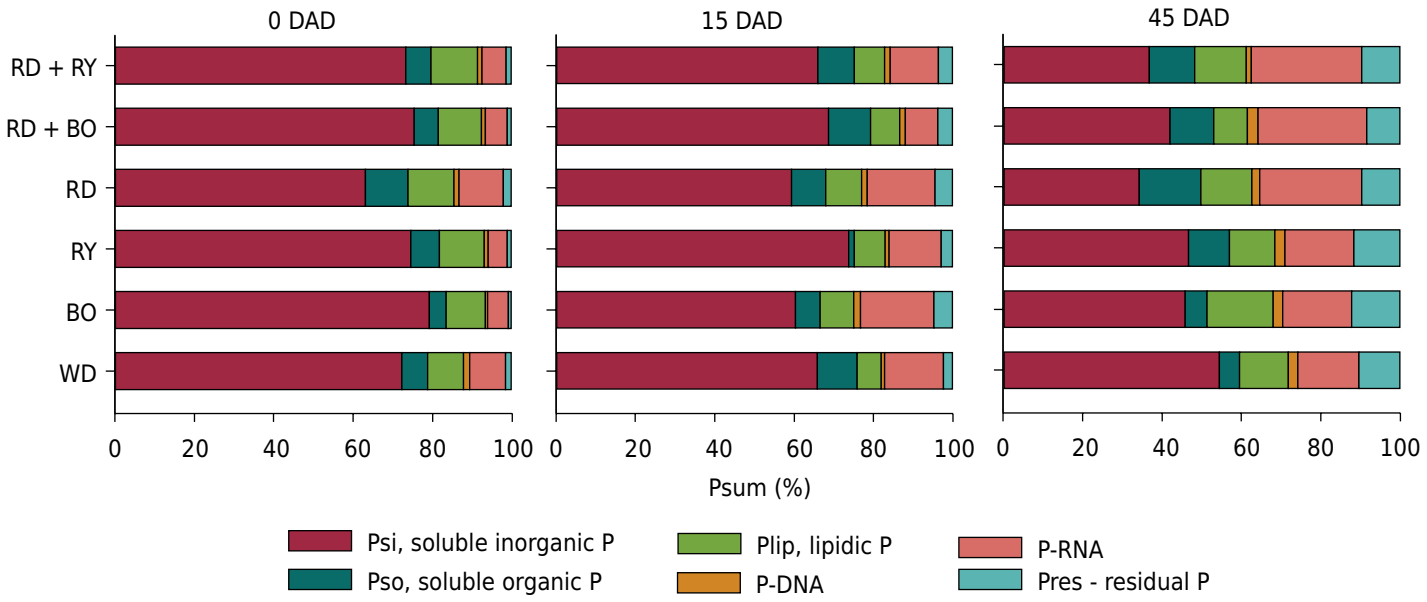

Figure 4. Relative percentage of P fractions in the plant tissue of the residues of weed (WD), black oat (BO), rye (RY), oilseed radish (RD), oilseed radish + black oat (RD + BO), and oilseed radish + rye (RD + RY) extracted by chemical fractionation proposed by Miyachi and Tamiya (1961) with adjustments proposed by Schmidt and Thannhauser (1945) and Casali et al. (2011) in cover crop residues at the time of deposition of cover crop residues on the soil (time 0) and at 15 and 45 days after the deposition (DAD) of litterbags on the soil surface in agroecological no-till onion.

On the other hand, species of the Brasicaceae family, such as oilseed radish, have lower C/N ratio values, lower lignin content, and higher cellulose values, which stimulate the release of P (Doneda et al., 2012).

Phosphorus-RNA is considered a fraction of high recalcitrance because it is the P portion associated with the plant RNA structures and will only be solubilized in the event of decomposition of the plant tissue. The increase in P-RNA content over time may have occurred due to P immobilization by microbial biomass, as well as the formation of byproducts derived from microbial decomposition. Among these byproducts are organic P forms such as teichoic acid, which is an acidic polysaccharide found in the bacterial cell wall (Turner et al., 2005) that is obtained with the cultivation of cover crop species such as legumes (Guggenberger et al., 1996); in addition, P-RNA is present in microbial biomass, which decomposes the residue and makes these levels increase over time.

The P-RNA contents in the mixed cover crops showed no increase from time 0 to 15 DAD. However, the P-RNA contents increased for all single treatments and WD increased from 0 to 15 DAD (Figures 1e, 1f, and 4). These differences may be a consequence of new conditions established in the mixed crops, which seem to have reduced mineralization of plant residues by microorganisms compared to the single treatments (Aita and Giacomini, 2003; Doneda et al., 2012).

The increase in Pres over time in the tissue (Figures 2, 3, and 4) typically happens because it is associated with P-DNA and plant protein structures that will be solubilized with the complete decomposition of plant tissue (Hogue et al., 1970). The release of more recalcitrant P forms to the soil solution is controlled by the mineralization rate of organic matter and depends on microbial activity, which uses carbon skeletons as energy sources, hydrolyzing phosphate esters (Tarafdar and Claassen, 2005).

In order to facilitate interpretation of the results of temporal variation of P forms in the tissue, we carried out a principal component analysis (PCA) using the proportions of P forms in the plant tissue (Figure 5). PCA indicated that the first two principal components (PC1 and PC2) explained 83.56 % of the existing total variation in the samples. The variables that contributed most to PC1 were Psi, P-DNA, P-RNA, and Pres, while the ones

that contributed most to PC2 were Pso and Plip (Table 2). The projection of P forms in the cover crop tissue at different sampling times clearly demonstrates that over time the proportion of Psi in plant residue decreases and the contribution of P-RNA, P-DNA, and Pres fractions increases (Figure 5).

As the P fractions were ranked in terms of times and treatments by PCA (Figure 5), we observed a predominance of the P-RNA and Pso fractions in the RD, RD + RY, and RD + BO treatments, which is in accordance with the higher proportion of those fractions in those treatments at 45 DAD (Figure 4, Table 3). The values of the Pres and P-DNA fractions are close and separated from the other fractions (Figure 5). This confirms that the increase of Pres over time in plant tissue is associated with P-DNA, especially in the single RY and BO treatments, which are closer to the values of the Pres and P-DNA fractions and reach their highest proportions at 45 DAD (Figure 4, Table 3). As for the

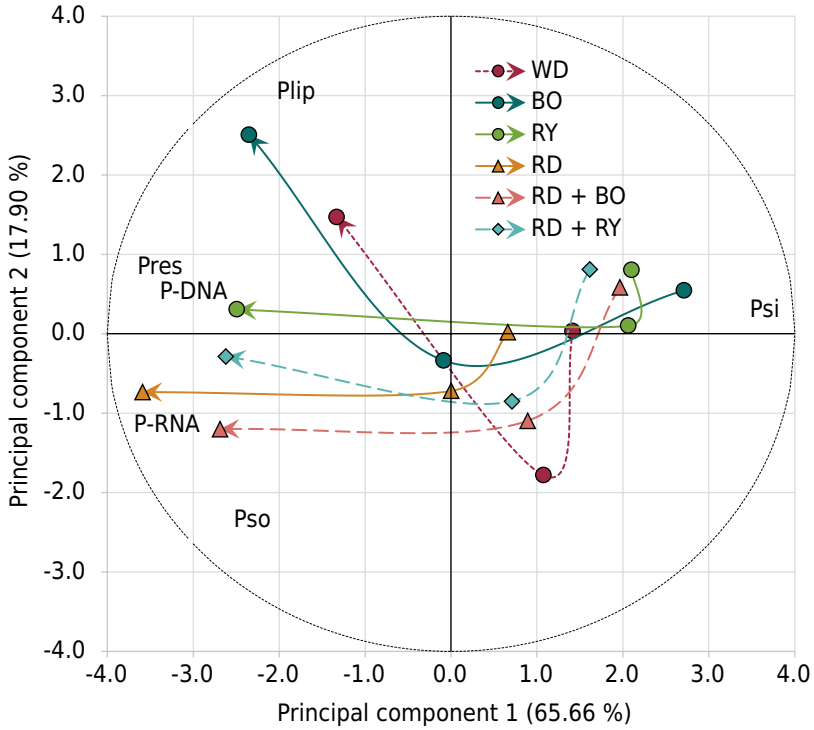

Figure 5. Projection of P forms in the plant residues of weed (WD), black oat (BO), rye (RY), oilseed radish (RD), oilseed radish + black oat (RD + BO), and oilseed radish + rye (RD + RY) at different sampling times. Numbers in parentheses indicate the percentage of variance explained by each axis. The direction of the arrows indicates the sampling times, the first at 0 and then at 15 and 45 days after deposition (DAD) of litterbags.

Table 2. Contribution of the variables for the first two principal components

Phosphorus form	PC1	PC2
	%	
Soluble inorganic P (Psi)	**24.8**	0.5
Soluble organic P (Pso)	9.8	**32.7**
Lipid P (Plip)	6.1	**53.5**
P associated with DNA (P-DNA)	**17.6**	1.9
P associated with RNA (P-RNA)	**19.4**	7.4
Residual P fraction (Pres)	**22.3**	4.0
Total	100.0	100.0

Values in bold indicate variables contributing more than 60 % of the variable with the highest contribution for each principal component.

Plip fraction, we observed that it separated from the other fractions, and it is related to the BO and WD treatments, which showed the two highest proportions of Plip at 45 DAD (Figure 4, Table 3).

Mineralization of cover crop P forms

The forms of total P, Psi, Plip, Pso, and Pres decreased from time 0 (0 DAD) to 45 DAD during decomposition of WD, BO, RY, RD, RD + BO, and RD + RY (Figure 2). In contrast, we observed increased contents of P-DNA and P-RNA during decomposition of the residues of all the treatments in the same period. This is due to the difference in accumulation of P forms in the tissue. It indicates that the P release rate depends not only on total content in the tissues (Oliveira et al., 2016), but also on how P is stored, in addition to other characteristics of the residue, such as C/N ratio (Tables 1 and 4) and leaching of water-soluble compounds degraded by the microbial population of plant residues (Ferreira et al., 2014). According to Aita and Giacomini (2003), this is because most microorganisms, especially fungi and bacteria, which colonize plant residues and part of total soil organic C, are used during decomposition as a source of ATP and are in part released into the atmosphere in the form of CO_2 (Manzoni et al., 2008).

Table 3. Difference in the relative proportion of phosphorus forms between the sampling times of weed (WD), black oat (BO), rye (RY), oilseed radish (RD), oilseed radish + black oat (RD + BO), and oilseed radish + rye (RD + RY) residues

Treatment	Psi	Pso	Plip	P-DNA	P-RNA	Pres
			Δ% in the first 15 days (initial % - % 15 DAD)			
WD	-6.3	3.4	-3.0	-0.5	5.8	0.8
BO	-19.9	2.3	-1.0	1.0	13.7	3.9
RY	-0.9	-5.3	-3.7	0.0	8.2	1.8
RD	-4.4	-0.8	-2.7	0.2	6.0	1.7
RD + BO	-6.5	4.3	-3.5	0.4	2.5	2.8
RD + RY	-7.2	2.8	-4.1	0.2	6.1	2.2
			Δ% in the first 45 days (initial % - % 45 DAD)			
WD	-17.8	-1.4	3.2	0.9	6.5	8.5
BO	-32.9	1.5	6.7	1.7	11.9	11.1
RY	-27.9	3.2	0.0	1.7	12.5	10.5
RD	-31.8	5.5	0.9	0.7	14.0	10.7
RD + BO	-33.4	4.9	-2.2	1.6	21.9	7.3
RD + RY	-36.4	5.0	1.2	0.2	21.7	8.3

Psi: soluble inorganic P; Pso: soluble organic P; Plip: lipid P; P-DNA: P associated with DNA; P-RNA: P associated with RNA; and Pres: residual P fraction.

Table 4. Carbon/nitrogen ratio of remaining dry matter from the residues of weed (WD), black oat (BO), rye (RY), oilseed radish (RD), oilseed radish + black oat (RD + BO), and oilseed radish + rye (RD + RY) deposited on the onion row

Treatment	Days after deposition of litterbags (DAD)		
	0	15	45
WD	16 b	17 b	15 b
BO	19 ab	27 ab	21 a
RY	23 a	28 a	20 a
RD	17 b	25 ab	24 ab
RD + BO	17 b	29 a	23 a
RD + RY	16 b	31 a	27 a
CV (%)	10.5	18.3	17.4

Means followed by the same letter, in each column, do not differ by the Tukey test at 5 % probability. CV: coefficient of variation.

The variation over time in the relative distribution of P forms was similar in every treatment (Figure 4), except for the BO and WD treatments. After 15 DAD, the BO treatment showed a more marked decrease in the proportion of Psi (20 %) compared to the other treatments (5.1 ± 2.5 %), while P-RNA showed the highest increase (13.7 %) among treatments (5.7 ± 2.1 %) (Table 3). This shows that in the first weeks, BO had the highest capacity for release of P stored in the vacuolar inorganic form. This is similar to results observed by Casali et al. (2011), who compared the release of P forms in black oat, vetch, and forage turnip residues in an experiment conducted in an Oxisol under no-tillage and conventional tillage. Furthermore, the predominance of P-RNA after 15 DAD in AV residues depends on its mineralization and release of P, with consequent uptake by the onion root system (Oliveira et al., 2016).

After 45 DAD, the release of Psi from cover crops (BO, RY, and RD) and mixed cover crops (RD + BO and RD + RY) was similar (32.5 ± 3.1 %), and it was approximately two times higher than the release of Psi by WD (17.8 %) (Table 3). However, WD had the lowest increase in the proportion of P-RNA (6.5 %) after 45 DAD, and this was approximately two times lower than in the other treatments (16.4 ± 5.0 %) (Table 3). This is due to lignin content and the C/N ratio (Tables 1 and 4), because WD residues with proportionally higher N content and a C/N ratio lower than 30 (16 in WD) are more readily colonized by the microbial population, compared to those with a C/N ratio higher than 30. This is because there is more N (26.2 g kg^{-1}) available for the formation of its tissue, which increases mineralization of residue components and is consequently reflected in less residual P (Ferreira et al., 2014). Therefore, the lower amount of P at 45 DAD in WD is partly due to the higher contents of N (26.2 g kg^{-1}) and the N added by dry matter (120.9 kg ha^{-1}) and lower values for lignin and the C/N ratio, which were lower due to the mixture of several species, including grasses and legumes (Table 1).

CONCLUSIONS

In addition to total P concentration, the release of P from cover crop tissue also depends on the form of P accumulated in the tissue and the quality of the residue.

The highest accumulation of P in cover crops occurred in the soluble inorganic P fraction, which is the fraction of fastest release in the tissue of all the plants.

Black oat had the highest initial release rate of soluble inorganic P, but the rate became equal to the release rate of other cover crop residues at 45 days after deposition. However, weeds released only half the amount of soluble inorganic P in the same period, despite accumulating a considerable amount of P in their biomass.

The oilseed radish + rye and oilseed radish + black oat mixtures showed a higher release of P-RNA at 45 days after deposition compared to the single treatments.

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