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Research article

Soil pH, nitrogen, phosphatase and urease activities in response to cover crop species, termination stage and termination method

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ABSTRACT

The best management options for cover cropping are largely unknown, including the growth patterns of cover crop (CC) species, optimum termination stages and termination methods. A greenhouse experiment was conducted to explore the following: (i) Effect of two termination stages (vegetative and flowering) on the chemical composition (N and C:N) of four CCs; (ii) Short-term impacts of living CCs and residues on soil pH, total N, urease and phosphatase activities at the two termination stages, and under two termination methods (slash and spray). Species tested as CCs were, vetch (*Vicia dasycarpa* L.), field pea (*Pisum sativum* L.), oats (*Avena sativa* L.), rye (*Secale cereal* L.) and a control (no CC). The experiment was set up in a randomized block design with three replications. Soil was sampled at kill and one year after CC kill. Delaying termination from vegetative till flowering stage decreased N in the tissue of *P. sativum*, *A. sativa*, *V. dasycarpa* and *S. cereal* by 59%, 65%, 44% and 56%, respectively, while their C:N ratios increased. Cover crop presence had no effect on soil pH. Living CCs had no significant effect on soil N concentration. The activities of urease and phosphatase were stimulated by all the living CC species. Unlike urease, all CC residues had a positive impact on phosphatase activity at one year. Only *P. sativum* and *V. dasycarpa* residues increased soil N concentration in the short-term. Compared to flowering, termination at vegetative stage improved soil N concentrations and phosphatase activity at both sampling times. Termination method had no effect on soil N, urease and phosphatase activity at one year. The significant interaction ($P < 0.05$) of sampling time, CC and termination stage effects on soil N concentration and phosphatase activity observed in this study indicates that these management approaches can optimize CC benefits and improve soil chemical and biological properties.

1. Introduction

Soil degradation can be averted by managing and improving soil fertility in a more sustainable way. The integration of cover crops (CCs) provides a reliable means of increasing organic matter and nitrogen (N) supply to the soil (Lawson et al., 2013). This has enabled the wide inclusion of legumes in the cover cropping systems since non-legumes may not offer significant N credit (Thilakarathna et al., 2015). Legumes biologically fix N and have been shown to contribute a considerable amount of N into the soil, resulting in increased yield of the cash crop (Thilakarathna et al., 2015). Legume residues are of superior quality with lower C:N ratio and lignocellulose content than non-legumes with higher C:N ratio. These chemical properties have been shown to be the main factor controlling the magnitude and rate of decomposition (Liang et al., 2014; Coombs et al., 2017). Nonetheless, non-legume CCs, not only increase soil C and organic matter (Fourie et al., 2007), but also trap and recycle residual available N that might have leached below the rooting zone (Fortuna et al., 2008). Cover crops improve soil physical properties, chemical properties, biological processes, weed suppression and pest control (Blanco-Canqui et al., 2015). Therefore, it is crucial to manage CCs in order to maximize their benefits for soil quality improvement. The selection of suitable CC species, termination stage and termination method may improve soil N availability, microbial activity and soil fertility in agricultural cropping systems (Wayman et al., 2015).

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A dynamic microbial community can be a reliable signal of a healthy and improved soil status (Karaca et al., 2010). This has necessitated the need to use soil biological properties to examine the impact of soil management methods and the sustainability of land-use in agricultural soils (Mganga et al., 2016). Soil microbes produce enzymes that catalyze numerous biochemical reactions which bring about the decomposition and recycling of nutrients from organic matter, N fixation, nitrification and stabilization of soil structure (Baležentienė 2012). Soil enzymes such as urease and phosphatase play a crucial role in N and P cycling, respectively (García-Ruiz et al., 2008). They are highly sensitive to environmental factors and soil management changes (García-Ruiz et al., 2008; Adetunji et al., 2020b). Additionally, phosphatase activity has been shown to respond to organic and inorganic N inputs under various cropping systems (Lemanowicz 2011; Maseko and Dakora 2013). Therefore, the activities of urease and phosphatase have been considered a more reliable index for the early evaluation of quality changes due to soil management (García-Ruiz et al., 2008; Adetunji et al., 2020b).

There are limited studies on the impacts of CC termination stages and termination methods on soil properties. In Western Washington, US, Wayman et al. (2015) evaluated the effect of *S. cereal*, barley and *V. dasycarpa* termination stage on weed control and soil N mineralization. A few other studies in the US explored how termination methods effectively killed CCs and prevented regrowth (Mirsky et al., 2009; Keene et al., 2017). The impact of these management approaches on soil microbial processes and enzyme activities is still poorly understood. Furthermore, no combined study of such processes has been reported in Africa, including the South African cropping systems. The evaluation of the management impact on urease and phosphatase activities will aid better decision in choosing appropriate CC varieties, termination methods and termination stages that best improve soil N concentrations, biological properties, fertility and sustainability of agricultural soils.

The objectives of the study were to determine the effect of: (1) termination stage on N and C:N ratio content of four CCs; (2) living CCs and residues on soil pH, total N and urease and phosphatase activities; and (3) termination stage and termination method on soil N concentration and urease and phosphatase activities. We hypothesized that: (1) the N content and C:N ratio will differ between CC species and their termination stages; (2) living CCs and residues will affect soil pH and increase total soil N, urease and phosphatase activities; though the extent of the effect may vary with species; (3) termination of CCs at vegetative stage will increase total soil N level than termination at flowering, and urease and phosphatase activities will be higher at flowering stage relative to vegetative; (4) total soil N, urease and phosphatase activities will respond more to termination stage than termination methods in the short-term.

2. Materials and methods

2.1. Site description

A greenhouse pot trial was conducted at the Agricultural Research Council (ARC) Experimental Farm, at Bien Donne, Western Cape Province, South Africa (33°50′26″S 18°58′53″E), from August 2016 to November 2017. The average minimum and maximum temperature in the greenhouse was 0.5 °C and 34 °C, respectively, and the plants were grown under natural light conditions. The soil was collected from the surface horizon (0–30 cm) of the ARC Nietvoorbij Research Farm (33°55′10″S 18°51′58″E), in Stellenbosch, Western Cape, South Africa. The soil form and series were Avalon (form: orthic, yellow-brown apedal, soft plinthic; series: sandy clay loam, mixed, superactive, mesic Typic Haplocalcids).

2.2. Experimental treatments

A randomized block design was used and each treatment was replicated three times (n = 3). Four experiments were set up which included 2 growth termination stages (vegetative and flowering) and 2 termination methods (slash and spray). Each experiment consisted of five CCs namely; field pea (*Pisum sativum* L.), grazing vetch (*Vicia dasycarpa* L.), rye (*Secale cereal* L.), oats (*Avena sativa* L.) and a control (no CC). Cover crop seeds were obtained from Barenbrug South Africa Seeds (Pty) Ltd. The test crops were selected to represent previously successful annual CC species in the Western Cape Province (Fourie et al., 2001).

2.3. Experimental procedures

Details of the soil type and preparation, CC seeding and fertilizer rates, irrigation method, as well as termination dates and methods have been reported by Adetunji et al. (2020a). Briefly, initial soil analysis was done by Bemlab, a commercial laboratory in the Western Cape Province, South Africa. The initial characteristics of the soil are shown in Table 1. Sixty-30 cm plastic pots were uniformly filled with 10.5 kg of the sieved soil (air - dry weight) and arranged on benches. *P. sativum* and *V. dasycarpa* seeds were inoculated by mixing them with *Rhizobium leguminosarium* biovar *viciae* at 250 g inoculant per 25 kg seed in 300 ml water (Agrimark, Stellenbosch, South Africa), prior to CC seeding on August, 2016.

2.4. Plant/soil sample collection and analysis

Prior to CC termination, from now on mentioned as kill, biomass was clipped at ground level in all pots (two legumes/four non-legume plant-stands) and was dried at 60 °C for 48 h to reach a constant weight (Coombs et al., 2017). Each dried sample was ground to pass through a 1 mm screen using a Polymix Kinematica (PX-MFC 90 D) and stored at 4 °C until chemical analysis. Soil samples were taken from the experimental pots (0–15 cm depths) just before CC kill and at one year after kill (September 21 and November 2, 2017). Soil samples were passed through 2 mm screen and then kept at 4 °C until chemical and enzyme analyses were done.

The total N concentration was measured in the plant and soil samples (0.1 g) using a TruSpec N Nitrogen Analyzer (LECO, St. Joseph, MI, USA). The total C concentration in the plant was measured by wet-combustion analysis (Dalal 1979; Shaw 2006). The C:N ratio was then determined from these two values. Soil pH was analyzed in a 1:2.5 Soil: KCl mixture (1 M KCl solution) using a glass electrode pH meter.

2.5. Soil enzyme activity assay

Urease activity (EC 3.5.1.5) was analyzed by 2 h incubation of a reaction mixture of 5.0 g of field-moist soils and 2.5 mL of 80 mM urea solution at 37 °C (Kandeler and Gerber 1988). Deionized water was added to the controls. The ammonium released was extracted with 50 mL KCl solution and product was measured at 690 nm with a digital UV–Vis spectrophotometer (Biotek ELx800) against the reagent blank. Urease activity was expressed as μg ammonium g^{-1} soil $2\ h^{-1}$. Acid phosphatase (EC 3.1.3.2) was assayed by a colorimetric method of Tabatabai and Bremner (1969) with the exception of using a homogenized reaction of 1.0 mL 25 mM *p*-nitrophenol phosphate (substrate), 4.0 mL Modified

Table 1. Initial characteristics of soil from the ARC Nietvoorbij Research Farm.

Soil characteristic	Value
Soil type	sandy clay loam
Soil Texture	61% sand, 14% silt and 25% clay
pH (KCl)	6.7
C (Walkely Black)	0.9%
P (Bray II)	11 mg kg^{-1}
K (ammonium acetate extraction)	47 mg kg^{-1}
NO_3–N (KCl)	5.31 mg kg^{-1}
NH_4–N (KCl)	10.36 mg kg^{-1}

Universal Buffer and 0.25 mL toluene, and that the product *p*-nitrophenol was extracted with 4.0 mL of 0.5 M NaOH at pH 6.5. The *p*-nitrophenol released was measured at 410 nm with a digital UV–Vis spectrophotometer (Biotek ELx800) and activity expressed as μg *p*-nitrophenol g^{-1} soil h^{-1}. One control and two replicates from each soil were used in the analyses of urease and phosphatase. Enzyme activity was expressed on a moisture-free basis. Moisture content of soil was calculated from weight lost after drying at 105 °C for 24 h.

2.6. Statistical analysis

Levene's test for homogeneity of experimental variances was verified for comparable variances (Levene 1960). Thereafter, the data were subjected to a combined analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of Statistical Analysis Software (SAS) (Version 9.4; SAS Institute Inc, Cary, USA). Observations over sampling time (kill and one year) were combined in a split-plot analysis of variance with sampling time as a sub-plot factor (Little and Hills 1972) for soil variables. The Shapiro-Wilk test was performed on the standardized residuals from the model to verify normality (Shapiro and Wilk 1965). Fisher's least significant difference (LSD) was calculated at the 5% level to compare treatment means (Ott and Longnecker 2015). A probability level of 5% was considered significant for all significance tests.

3. Results

3.1. Combined statistical analysis

It was observed from ANOVA significant sampling time, CC and termination stage interaction effects on total soil N ($P = 0.0001$) and phosphatase activity ($P = 0.0382$) (Table 2). There were significant interaction effects of sampling time and CC treatments on soil pH (0.0011) and urease activity ($P = 0.0002$) (Table 2).

3.2. Total N concentration and C:N ratio in the tissue of cover crops as affected by termination stage

The total N concentration and C:N ratio in the plant tissue of the CCs was significantly affected by termination stage. The total N concentration significantly decreased from vegetative to flowering stage across all CC tissues with *V. dasycarpa* (3.9–2.2%) being the highest followed by *P. sativum* (3.2–1.3%), *S. cereal* (2.5–1.1%) and *A. sativa* (2.3–0.8%) (Table 3). On the contrary, C:N ratio considerably increased from vegetative to flowering stage across all CCs with the highest concentration observed under *A. sativa* (22:1–66:1) followed by *S. cereal* (21:1–55:1), *P. sativum* (16:1–45:1) and *V. dasycarpa* (13:1–25:1) (Table 3). The C:N ratios of *V. dasycarpa* and *P. sativum* were lower than that of *S. cereal* and *A. sativa* at vegetative and flowering stages.

3.3. Soil pH as affected by cover crop species and sampling time

At kill, there was no significant difference in soil pH among living *P. sativum* (7.19), *S. cereal* (7.17), *V. dasycarpa* (7.17) and control (7.17) except *A. sativa* (7.06) which had significantly lower soil pH (Table 4). However, soil pH significantly increased across all treatments at one year with control (7.31) and *A. sativa* (7.30) being the highest followed by *S. cereal* (7.27) and the lowest being *P. sativum* (7.22) and *V. dasycarpa* (7.20) compared to the initial measured pH of 6.7.

3.4. Total soil nitrogen

3.4.1. Total soil nitrogen concentration as affected by cover crop species and sampling time

At kill, there was no significant difference in soil N concentration between living CC species and the control soils (Table 4). There was a marginal increase in N concentration in *S. cereal* (0.27%) and *V. dasycarpa* (0.27%) soils than the control (0.26%) soils. At one year, *P. sativum* and *V. dasycarpa* residues significantly increased soil N by about 16% and about 7%, respectively, whereas soil N decreased under *S. cereal* and control (Table 4). *A. sativa* soils maintained the same soil N concentration.

3.4.2. Total soil nitrogen concentration as affected by cover crop species and termination stage at different sampling times

Soil N concentration at kill was significantly higher at the vegetative stage than flowering under *V. dasycarpa*, whereas no significant differences were observed between the termination stages for *P. sativum*, *S. cereal*, *A. sativa* and control soils (Table 5). When averaged over all CCs, termination stage had no significant effect on soil N concentration at kill (Table 6). At one year, vegetative stage had higher soil N concentration

Table 2. Analysis of variance (Pr > F) for total soil nitrogen, soil pH, urease activity and phosphatase activity in combined sampling times as affected by cover crop species, termination stage, and termination method.

Source	DF	Nitrogen	pH	Urease	Phosphatase
Method (M)	1	0.7084	0.6667	0.5566	0.4364
Stage (S)	1	<.0001	0.0037	0.4793	<.0001
M x S	1	0.6594	0.7622	0.338	0.1606
Block (Method*Stage)	8	0.2793	0.5868	0.1066	0.8503
Cover crop (CC)	4	<.0001	0.2588	<.0001	0.0552
CC x M	4	0.2097	0.6709	0.5832	0.0785
CC x S	4	0.0652	0.0093	0.0107	0.1463
CC x M x S	4	0.4951	0.0099	0.2035	0.2403
Block (Method*Stage*Cover Crop)	32	-	-	-	-
Sampling Time (ST)	1	0.1452	<.0001	<.0001	<.0001
ST x M	1	0.4509	0.8337	0.6105	0.0203
ST x S	1	<.0001	0.0043	0.1578	<.0001
ST x M x S	1	0.6381	0.4313	0.0029	0.9004
ST x CC	4	0.0003	0.0011	0.0002	0.4262
ST x CC x M	4	0.1857	0.3003	0.5813	0.5265
ST x CC x S	4	0.0001	0.118	0.0645	0.0382
ST x CC x M x S	4	0.7346	0.3461	0.2615	0.7217
Error	40	-	-	-	-
Corrected Total	119	-	-	-	-

Table 3. Interaction effects of cover crop species (*A. sativa*, *P. sativum*, *S. cereal* and *V. dasycarpa*) and termination stage (vegetative and flowering) at kill on total N and C:N ratio in the plant tissue.

Cover crop	Termination stage	Total N (%)	C:N
A. sativa	Vegetative	2.3 d	22.3 de
P. sativum		3.2 b	16.4 fg
S. cereal		2.5 c	20.5 ef
V. dasycarpa		3.9 a	13.1 g
A. sativa	Flowering	0.8 h	66.3 a
P. sativum		1.3 f	44.7 c
S. cereal		1.1 g	54.9 b
V. dasycarpa		2.2 e	25.2 d

Mean values (n = 3) within a column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

Table 4. Cover crop species and sampling time effects on soil pH and nitrogen.

Sampling time	Cover crop	Soil pH	Soil nitrogen (%)
Kill	*A. sativa*	7.06 (±0.06) d	0.22 (±0.01) d
	P. sativum	7.19 (±0.03) c	0.25 (±0.02) bcd
	S. cereal	7.17 (±0.03) c	0.27 (±0.01) abc
	V. dasycarpa	7.17 (±6.09) c	0.27 (±0.02) abc
	Control	7.17 (±0.03) c	0.26 (±0.01) abcd
One year	*A. sativa*	7.30 (±0.02) a	0.22 (±0.03) d
	P. sativum	7.22 (±0.01) bc	0.29 (±0.02) a
	S. cereal	7.27 (±0.02) ab	0.23 (±0.03) cd
	V. dasycarpa	7.20 (±0.05) bc	0.29 (±0.01) ab
	Control	7.31 (±0.02) a	0.17 (±0.02) e

Standard error values are shown in parenthesis. Mean values (n = 3) within a column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

than flowering for all the CC residues except *V. dasycarpa* which was similar at both termination stages (Table 5). Overall, there were significant interaction effects of sampling time, CC and termination stage on soil N concentration (Table 2; $P = 0.0001$).

3.4.3. Total soil nitrogen concentration as affected by termination method

At one year, slash and spray treatments had no significant effect on soil N concentrations when averaged over all CC residues (Figure 1).

Table 5. Cover crop species and termination stage effects on total soil nitrogen at different sampling times (kill and one year).

Sampling time	Cover crop	Termination stage	Soil nitrogen (%)
Kill	*A. sativa*	Vegetative	0.21 (±0.02) g
	P. sativum		0.26 (±0.03) defg
	S. cereal		0.27 (±0.02) bcdef
	V. dasycarpa		0.30 (±0.02) abcd
	Control		0.26 (±0.01) cdefg
	A. sativa	Flowering	0.23 (±0.01) fg
	P. sativum		0.24 (±0.03) efg
	S. cereal		0.27 (±0.02) bcdefg
	V. dasycarpa		0.24 (±0.02) efg
	Control		0.25 (±0.01) defg
One year	*A. sativa*	Vegetative	0.31 (±0.01) abc
	P. sativum		0.32 (±0.04) ab
	S. cereal		0.33 (±0.00) a
	V. dasycarpa		0.28 (±0.01) abcde
	Control		0.24 (±0.01) efg
	A. sativa	Flowering	0.13 (±0.02) h
	P. sativum		0.25 (±0.02) defg
	S. cereal		0.13 (±0.01) h
	V. dasycarpa		0.29 (±0.01) abcde
	Control		0.10 (±0.02) h

Standard error values are shown in parenthesis. Mean values (n = 3) within a column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

3.5. Soil enzyme activity

3.5.1. Soil urease and phosphatase activities as affected by cover crop species and sampling time

At kill, urease activity was significantly higher in living CC soils than in control with *S. cereal* (48.71 μg NH_4^+ g^{-1} soil 2 h^{-1}) and *A. sativa* (43.75 μg NH_4^+ g^{-1} soil 2 h^{-1}) showing higher activity than *V. dasycarpa* (34.70 μg NH_4^+ g^{-1} soil 2 h^{-1}) and *P. sativum* (34.60 μg NH_4^+ g^{-1} soil 2 h^{-1}) (Table 7). There was also significantly higher phosphatase activity in living *P. sativum*, *S. cereal* and *V. dasycarpa* soils than the control soils, at kill. Phosphatase activity in living *A. sativa* soil was marginally higher than the control soil (Table 7).

Urease activity decreased over time with no significant differences between CC residue soils and the control, at one year (Table 7). The phosphatase activity also decreased across all CC residue soils, with *A. sativa* (80.04 μg PNP g^{-1} soil h^{-1}) and *P. sativum* residues (79.90 μg PNP g^{-1} soil h^{-1}) being the highest and significantly higher than the control (61.84 μg PNP g^{-1} soil h^{-1}) (Table 7). However, there were no significant differences in phosphatase activity under *V. dasycarpa* (66.37 μg PNP g^{-1} soil h^{-1}), *S. cereal* (67.10 μg PNP g^{-1} soil h^{-1}) and the control (61.84 μg PNP g^{-1} soil h^{-1}) soils (Table 7).

3.5.2. Soil urease and phosphatase activities as affected by cover crop species and termination stage

At kill, the flowering treatment showed significantly higher urease activity than vegetative treatment under *S. cereal* and *A. sativa* while no significant differences were observed in termination stage among the other CCs and the control (Table 8). Also, the activity of phosphatase was higher at the vegetative stage than the flowering stage irrespective of CC species (Table 8). However, there was no significant difference in urease activity between the vegetative and flowering stages across all CC residues at one year (Table 8).

Generally, the activity of urease was not significantly affected by termination stages at both sampling times (Table 9 and Table 2; $P = 0.0645$). Higher phosphatase activity was observed at the vegetative compared to the flowering stage regardless of the CC residue species (Table 8), at one year. Overall, the termination stage greatly affected phosphatase activity regardless of the sampling time, with the vegetative stage being about 168% and about 29% higher than flowering, at kill and one year, respectively (Table 9 and Table 2; $P = 0.0382$).

3.5.3. Soil urease and phosphatase activities as affected by termination method

At one year, termination methods did not have a significant effect on the activities of urease and phosphatase when averaged across all CCs (Figure 2).

4. Discussion

4.1. Cover crop tissue

Results from this study revealed that termination stage considerably affected CC chemical composition. This is consistent with other studies which indicated that late termination increased CC C:N ratio while tissue

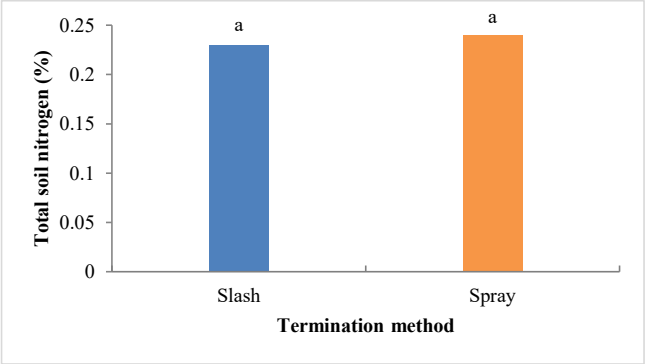

Figure 1. The overall effect of termination method on total soil nitrogen at one year. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$).

N concentration decreased from early termination to late termination (Alonso-Ayuso et al., 2014; Lawson et al., 2015; Coombs et al., 2017). Results showed that termination of *V. dasycarpa*, *P. sativum*, *S. cereal* and *A. sativa* at the vegetative stage can serve as a management approach to optimize biomass N accumulation and quality, as well as soil N input. According to Whittinghill et al. (2012), CC C:N ratio and lignocellulose index are biochemical indicators that may serve as quantitative parameters for modeling soil and microbial activities. They may also serve as qualitative variables when examining residue effects on soil microbial properties and processes (Liang et al., 2014). For example, residues with low C:N ratios (less than 35) are generally of better quality than residues with high C:N ratios (greater than 35) and they have, therefore, been used as pointer to rapid and optimum microbial decomposition and N mineralization (Coombs et al., 2017). Thus, at vegetative stage, the termination of *P. sativum*, *V. dasycarpa* and most importantly *A. sativa* and *S. cereal*, will enhance soil microbial and enzymatic activity resulting in rapid decomposition, N cycling and nutrient release for the subsequent cash crop. Alonso-Ayuso et al. (2014) reported that postponement of termination date led to CC biomass increase and more residues with higher fiber content and C:N ratio that, however do not easily decay and, thus, are more suitable to protect the soil and enhance the slow release of the N pool. The choice of CC termination stage can be targeted towards reducing N immobilization, improving N input and microbial processes.

4.2. Soil pH

Living CCs and their residues do not appear to have significant impact on soil pH as the sampling time did; as shown by this study. The observed increase in soil pH across all CC residue treatments including control at one year is in agreement with a study by Balota and Chaves (2011), which showed an increase in soil pH from about 4 to 6 after 10 years under seven summer legumes (*Mucuna pruriens, Vigna unguiculata, Leucaena leucocephala, Arachis hypogaea, Crotalaria breviflora, Mucuna deeringiana, Crotalaria spectabilis* and control). However, Mukherjee and Lal (2015) indicated that in one season *Brassica rapa* and *P. sativum* residues significantly decreased soil pH from about 6.7 to 5.7 compared to the

Table 6. The overall effect of termination stage (vegetative and flowering) on total soil nitrogen at different sampling times (kill and one year).

Sampling time	Termination stage	Soil nitrogen (%)
Kill	Vegetative	0.26 (±0.01) b
	Flowering	0.24 (±0.01) b
One year	Vegetative	0.30 (±0.01) a
	Flowering	0.18 (±0.02) c

Standard error values are shown in parenthesis. Averaged values within a column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

Table 7. The activities of urease and phosphatase as affected by cover crop species and sampling time.

Sampling time	Cover crop	Urease (μg NH_4^+ g^{-1} soil 2 h^{-1})	Phosphatase (μg PNP g^{-1} soil h^{-1})
Kill	*A. sativa*	43.75 (±2.60) a	104.21 (±13.23) ab
	P. sativum	34.60 (±1.56) b	108.27 (±19.37) a
	S. cereal	48.71 (±4.29) a	106.68 (±13.33) a
	V. dasycarpa	34.70 (±3.18) b	106.21 (±15.40) a
	Control	25.93 (±1.77) c	91.75 (±15.93) bc
One year	*A. sativa*	10.36 (±1.35) d	80.04 (±9.90) cd
	P. sativum	9.73 (±1.33) d	79.90 (±6.23) cd
	S. cereal	11.25 (±1.38) d	67.10 (±3.08) de
	V. dasycarpa	9.55 (±0.80) d	66.37 (±4.65) de
	Control	5.87 (±1.07) d	61.84 (±4.22) e

Standard error values are shown in parenthesis. Mean values (n = 3) within a column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

Table 8. Cover crop species and termination stage effects on urease and phosphatase activities at different sampling times (kill and one year).

Sampling time	Cover crop	Termination stage	Urease (μg NH_4^+ g^{-1} soil 2 h^{-1})	Phosphatase (μg PNP g^{-1} soil h^{-1})
Kill	*A. sativa*	Vegetative	38.28 (±1.42) cd	146.62 (±6.09) b
	P. sativum		36.13 (±1.28) cde	167.78 (±13.58) a
	S. cereal		41.67 (±1.85) bc	144.70 (±8.75) b
	V. dasycarpa		35.48 (±5.80) cde	155.56 (±5.88) ab
	Control		29.63 (±2.25) ef	138.77 (±12.21)b
	A. sativa	Flowering	49.22 (±3.97) ab	61.79 (±3.61) efg
	P. sativum		33.08 (±2.85) de	48.75 (±7.08) fg
	S. cereal		55.75 (±7.60) a	68.66 (±11.28) ef
	V. dasycarpa		33.92 (±3.27) de	56.86 (±5.85) efg
	Control		22.24 (±1.78) f	44.72 (±9.08) g
One year	*A. sativa*	Vegetative	10.42 (±2.05) gh	101.88 (±12.71) c
	P. sativum		9.68 (±2.11) gh	90.64 (±8.30) cd
	S. cereal		11.18 (±2.56) g	73.01 (±3.05) de
	V. dasycarpa		9.15 (±0.95) gh	74.37 (±5.96) de
	Control		8.45 (±1.21) gh	61.10 (±5.73) efg
	A. sativa	Flowering	10.29 (±1.94) gh	58.20 (±3.32) efg
	P. sativum		9.78 (±1.82) gh	69.17 (±7.46) e
	S. cereal		11.32 (±1.36) g	61.19 (±4.30) efg
	V. dasycarpa		9.95 (±1.36) gh	58.38 (±5.85) efg
	Control		3.30 (±0.96) h	62.58 (±6.72) efg

Standard error values are shown in parenthesis. Mean values (n = 3) within a column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

control. Similarly, *Vicia villosa* (hairy vetch) and *Trifolium incarnatum* decreased soil pH by 11% and it was attributed to greater exchangeable ions (Al and Mn) under CCs compared to those under fallow (Mcvay et al., 1989). In the present study, despite the general increase in soil pH, *P. sativum* and *V. dasycarpa* were the least, and this is consistent with previous studies that showed lower soil pH under legumes compared to non-legumes (Nuruzzaman et al., 2006; Li et al., 2007; Maltais-Landry 2015). The observed lower soil pH under legumes has been indicated to probably be a result of the effect of soil N fixation (Nuruzzaman et al., 2006; Maltais-Landry 2015).

4.3. Total soil nitrogen

Most research has been on examining CC residues in relation to soil ecosystem and fertility improvement, the outcome which is usually controlled by complex interactions between CCs and other management

Table 9. The overall effect of termination stage (vegetative and flowering) on the activities of soil urease and phosphatase at different sampling times (kill and one year).

Sampling time	Termination stage	Urease (μg NH_4^+ g^{-1} soil 2 h^{-1})	Phosphatase (μg PNP g^{-1} soil h^{-1})
Kill	Vegetative	36.24 (±1.45) a	150.69 (±4.49) a
	Flowering	38.84 (±2.88) a	56.16 (±3.63) c
One year	Vegetative	9.78 (±0.80) b	80.20 (±4.21) b
	Flowering	8.93 (±0.83) b	61.90 (±2.49) c

Standard error values are shown in parenthesis. Averaged values within a column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's LSD.

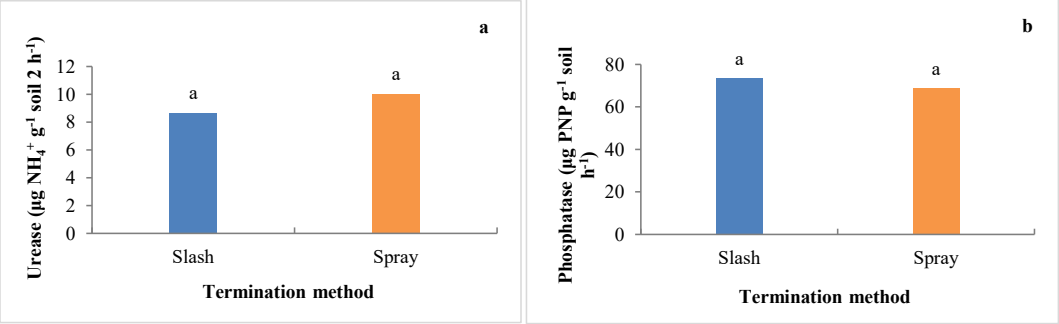

Figure 2. The overall effect of termination method on the activities of (a) urease and (b) phosphatase at one year. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$).

factors (Lienhard et al., 2014). In this sense, there is not much information documenting the impact of living CC stands on soil nutrient availability, microbial processes and soil enzyme activities (Qian et al., 2015).

Findings from this study indicate that living CC stands of *V. dasycarpa* and *S. cereal* slightly increased total soil N concentrations. Other authors have reported similar superior total soil N indices under *T. incarnatum* and *Securigera varia* (crown vetch) (Xu et al., 2013; Qian et al., 2015). Living CCs may affect soil nutrient concentrations and availability by enhancing biological activity thereby improving mineralization and nutrient levels (Yao et al., 2005), root exudation, which provide substrates and labile carbon leading to optimum nutrient release (Hoagland et al., 2008), and sequestering of nutrients thereby reducing availability (Qian et al., 2015). The observed slight increase in soil N level under living *V. dasycarpa* may be as a result of biological N fixation processes in legume roots which add extra N (Maseko and Dakora 2013). The marginally higher N concentrations under living *S. cereal* soils may be explained by root exudates effects which provide substrate and boost soil soluble compounds for soil microbial activity and nutrient release (Mukumbareza et al., 2015). However, Qian et al. (2015) reported that soil mineral N was lower in non-legume living *Lolium perenne* compared to no CC treatments in apple orchard soils. This may be due to scavenging ability and N use competition between *L. perenne* and the apple trees (Qian et al., 2015). At one year, residues of *P. sativum* and *V. dasycarpa* increased soil N concentration compared to the control and *S. cereal*, which is consistent with reports from previous studies (Balkcom et al., 2015; Blanco-Canqui et al., 2015; Coombs et al., 2017). The increased soil N concentration observed under *P. sativum* and *V. dasycarpa* soils in contrast to the other treatments could be due to higher tissue N input detected in this study and the biological N fixation associated with the legume crops. Furthermore, lower C:N ratios of *P. sativum* and *V. dasycarpa* residues compared to *S. cereal* and *A. sativa* might have contributed to enhanced microbial decomposition and enzyme activity that resulted in optimum N cycling and mineralization. This is consistent with the findings of Balkcom et al. (2015) and Coombs et al. (2017). Several studies have shown that legumes, including *Medicago sativa*, *V. dasycarpa*, *T. incarnatum*, *Crotalaria juncea*, *Glycine max*, *P. sativum* considerably increased soil N through biological N fixation processes compared to grasses and no CC under different management methods (Blanco-Canqui et al., 2011; Jani et al., 2016). Nonetheless, there are reports of little or no N increase in *V. dasycarpa* treated soils (Benincasa et al., 2010; Alonso-Ayuso et al., 2014). Non-legumes including *S. cereal* and *A. sativa* do not fix N but rather sequester and scavenge N (Balkcom et al., 2015; Thilakarathna et al., 2015) and this explains why no increase in soil N concentration was observed under their respective soils in this study at one year. It was also observed that *P. sativum* and *V. dasycarpa* residues markedly enhanced soil N concentration in the short-term (one year). This showed that *P. sativum* and *V. dasycarpa* can rapidly improve soil fertility, reduce N fertilizer requirement for subsequent cash crop and mitigate potential field loss.

Generally, legumes through symbiosis with N fixing bacteria add more N to the soil compared to grasses, while early termination is associated with quality residues, rapid decomposition and optimum N release (Alonso-Ayuso et al., 2014; Lawson et al., 2015). The observed significantly higher soil N concentration at vegetative stage relative to flowering under *V. dasycarpa*, at kill, is in agreement with other studies (Alonso-Ayuso et al., 2014; Coombs et al., 2017; Boyrahmadi and Raiesi 2018). Other authors found that postponing *V. dasycarpa* termination time for eight days (Cline and Silvernail 2001), fourteen days (Clark et al., 1995) or till flowering (Drinkwater et al., 2000) augmented *V. dasycarpa* biomass rather than increasing N contribution from N fixation. As stated by Sullivan and Andrews (2012), plant available N of legumes rises as growth advances, but peaks at bud growth stage and gradually decreases as the flowering growth stage continues. The current results showed that termination at vegetative stage compared to flowering not only significantly increased soil N under *P. sativum* soils, but also considerably elevated N concentrations under non-legume residues of *S. cereal* and *A. sativa* soils at one year. Termination at vegetative stage maintains lower C:N ratio and reduces N uptake by CCs especially grasses and allows more time for N release from CC residues (Alonso-Ayuso et al., 2014). In a study reported by Thorup-Kristensen and Dresbøll (2010), it was noted that there was a reduction in net N mineralization, and availability of nutrient to the following cash crop when *S. cereal* termination was delayed. This further supports the current findings that CC termination stage is an important soil management strategy for manipulating C:N ratio for reduced N immobilization, optimum decomposition and N cycling, as well as increased soil N availability for the succeeding cash crop.

Generally, termination method had no impact on soil N concentration when averaged over all the tested CCs. Termination methods are driven towards the same goal, but the main difference between slashing, disking, roller-crimping and spraying a CC stand is the point of placement of residues after termination and the extent of alteration on soil ecological properties (Liang et al., 2014). Previous research found that disking of CCs led to rapid shoot decay and higher mineral N availability in contrast to herbicide use (Jani et al., 2016). It has also been reported that mowing of the *S. cereal* and *V. dasycarpa* mixture (Snapp et al., 2005) and roller crimping of *V. dasycarpa* (Parr et al., 2011) resulted in enhanced N mineralization and increased soil N concentrations. Haney et al. (2002) reported that CC termination with glyphosate was also indicated to enhance N mineralization under different soil types.

4.4. Soil enzymes

In agricultural soils, urease and phosphatase play crucial roles in N and P cycling respectively. Their activities are sensitive to environmental factors and rapidly respond to soil management changes, making them a more reliable biological soil quality indicator (Adetunji et al., 2020b).

However, there are still limited studies on the use of soil enzyme assays to monitor soil management changes in cover cropping systems.

At kill, the observed higher urease and phosphatase activities under living CCs relative to control may be as a result of the stimulant effect of plant root activity (Boyrahmadi and Raiesi 2018). Cover crops produce enzymes such as phosphatase and urease from living roots (Follmer 2008), and root exudates from the plants serve as C substrate and energy source for microbial activity (Sanaullah et al., 2011). Furthermore, living CCs enhance soil physical and chemical properties which may result in higher microbial population and activity that improves enzyme activity (Diouf et al., 2010). Previous studies have reported significant increase in urease activity under *Trifolium repens*, *S. varia* and *M. sativa* living mulches compared to perennial *L. perenne* in vineyard soils (Xi et al., 2011; Qian et al., 2015). Adamavičienė et al. (2012) similarly reported that only living legume mulches increased urease activity in a cornfield, indicating that higher urease activity can be traced back to the N fixing ability of legumes (Qian et al., 2015). A number of studies have reported that legumes namely *Aspalathus caledonensis*, *Vigna unguiculata*, *Cicer arietinum*, and *Cyclopia genistoides* release more phosphatase enzymes than non-legumes (Makoi et al., 2010; Maseko and Dakora 2013). This is because legumes make use of more P in the symbiotic N fixation process than grasses (Makoi and Ndakidemi 2008). Phosphatase activity has been linked to organic matter and N availability through inorganic and organic N amendments in various studies (Lemanowicz 2011; Kalembasa and Symanowicz 2012). Consistent with other studies (Liang et al., 2014; Weerasekara et al., 2017), urease and phosphatase activities decreased over time (at one year); and this may be as a result of a reduction by decomposition of CC residue materials and substrates (Weerasekara et al., 2017). However, while phosphatase maintained higher activity under CC residues than the control, urease activity did not respond to CC residues at one year. The observed higher phosphatase activity under CC residue soils is consistent with previous findings which indicated that CC residues do not only add labile C, organic matter and substrate that stimulate phosphatase synthesis, but also moderate soil moisture and temperature conditions that enhance microbial processes (Balota and Chaves 2010; Jat et al., 2013).

The CC termination stage is a crucial soil management technique that can affect N availability for subsequent cash crops and fallow land (Balkcom et al., 2015). Despite the importance, there is limited or no report of the effect of CC termination timing on urease and phosphatase activities in South African soils. In the present study, the higher urease activity observed under *S. cereal* and *A. sativa* at flowering relative to vegetative stage may be a result of higher organic matter and over synthesis of this enzyme by microbes due to delay in termination. Nevertheless, when averaged over all the CCs, termination stage generally had no significant impact on urease activity across the two sampling times (Kill and one year). However, there was significant interaction of CCs, termination stage and sampling time effects on phosphatase activity, with vegetative stage showing a positive effect in contrast to flowering. This is an indication that phosphatase activity responds more to CC residue and substrate quality than quantity. Termination at vegetative stage favors lower C:N ratio which enhances microbial decomposition and N mineralization in agricultural soils (Alonso-Ayuso et al., 2014). The present results, therefore, suggest that CCs should be terminated at vegetative stage for improved soil phosphatase activity, microbial decomposition, N and P cycling.

It was observed that termination by spray and slashing had no considerable effect on phosphatase and urease activities in the short term. However, there was a decrease in microbial biomass C, urease and phosphatase activities under *A. sativa* soils terminated with glyphosate in a study by García-Orenes et al. (2010). It has been reported that herbicides such as glyphosate could accumulate in the soil and be toxic to soil microbes, consequently affecting biogeochemical reactions, nutrient cycling and soil fertility (Abbas et al., 2014). Furthermore, glyphosate application adversely affected soil bacterial diversity and rhizobacterial community structure under maize (Barriuso and Mellado 2012) and grass (Druille et al., 2016). In contrast to spraying, CC termination by slashing promoted shoot-soil contact as well as surface mulch cover, which enhanced soil moisture, temperature regulation and biological decomposition (Liang et al., 2014). However, there have been conflicting reports of the impact of glyphosate use on soil ecology with other findings indicating no substantial effect on diversity and activity of soil beneficial microbes (Weaver et al., 2007; Lane et al., 2012). According to Haney et al. (2002), glyphosate application stimulated microbial processes and inorganic N release under different soils. The present study indicated that termination methods did not have a significant impact on urease and phosphatase activity when averaged over all the CCs. Therefore, there is a need for further research on the impact of CC termination methods on soil enzyme activities and N availability under different soils in the short and long term.

5. Conclusion

This study indicates that early CC termination at the vegetative stage maintains higher tissue N (*V. dasycarpa* > *P. sativum* > *S. cereal* > *A. sativa*) and lower C:N ratio concentration (*V. dasycarpa* < *P. sativum* < *S. cereal* < *A. sativa*), irrespective of CC species. Living CCs and their residues had no effect on soil pH but sampling time did. *P. sativum* and *V. dasycarpa* followed by *S. cereal* are better CC options to farmers in increasing soil N concentrations. Living CCs stimulated urease (*S. cereal* > *A. sativa* > *V. dasycarpa* > *P. sativum*) and phosphatase activities (*P. sativum* > *S. cereal* > *V. dasycarpa* > *A. sativa*), whereas their dead residues considerably increased only phosphatase activity at one year. Termination methods had no significant effect on soil N concentrations and the activities of urease and phosphatase, at one year. Compared to flowering, termination at vegetative stage raised soil N levels and phosphatase activity irrespective of sampling time. This study, therefore, suggests that CC chemical composition/quality and termination stage should be considered when selecting management methods that improve soil N concentration and microbial activity. Further research with longer time spans should be done at field level including examining the N input of the CCs to the subsequent cash crop. We also suggest future sampling and more research to better understand the impact of living CCs and their residues on pH since soil acidity or alkalinity affects organic acids and enzymatic activity to some extent.

Declarations

Author contribution statement

Adewole Tomiwa Adetunji: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Bongani Ncube: Conceived and designed the experiments.

Andre Harold Meyer, Olatunde Stephen Olatunji: Contributed reagents, materials, analysis tools or data.

Reckson Mulidzi, Francis Bayo Lewu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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References

Abbas, Z., Akmal, M., Khan, K.S., 2014. Effect of buctril super (Bromoxynil) herbicide on soil microbial biomass and bacterial population. Braz. Arch. Biol. Technol. 57, 9–14.
Adamavičienė, A., Romaneckas, K., Pilipavičius, V., Avižienytė, D., Šarauskis, E., Sakalauskas, A., 2012. Interaction of maize and living mulch: soil chemical properties and bioactivity. J. Food Agric. Environ. 10, 1219–1223.
Adetunji, A.T., Ncube, B., Meyer, A.H., Mulidzi, R., Lewu, F.B., 2020a. Soil β-glucosidase activity, organic carbon and nutrients in plant tissue in response to cover crop species and management practices. S. Afr. J. Plant Soil 1–9.
Adetunji, A.T., Ncube, B., Mulidzi, R., Lewu, F.B., 2020b. Potential use of soil enzymes as soil quality indicators in agriculture. Front. Soil Environ. Microbiol. 57–64.
Alonso-Ayuso, M., Gabriel, J.L., Quemada, M., 2014. The kill date as a management tool for cover cropping success. PloS One 9, e109587.
Baležentienė, L., 2012. Hydrolases related to C and N cycles and soil fertility amendment: responses to different management styles of agro-ecosystems. Pol. J. Environ. Stud. 21, 1153–1159.
Balkcom, K.S., Duzy, L.M., Kornecki, T.S., Price, A.J., 2015. Timing of cover crop termination: management considerations for the Southeast. Crop Forage Turfgrass Manage. 1.
Balota, E.L., Chaves, J.C.D., 2010. Enzymatic activity and mineralization of carbon and nitrogen in soil cultivated with coffee and green manures. Rev. Bras Cienc. Solo. 34, 1573–1583.
Balota, E.L., Chaves, J.C.D., 2011. Microbial activity in soil cultivated with different summer legumes in coffee crop. Braz. Arch. Biol. Technol. 54, 35–44.
Barriuso, J., Mellado, R.P., 2012. Relative effect of glyphosate on glyphosate-tolerant maize rhizobacterial communities is not altered by soil properties. J. Microbiol. Biotechnol. 22, 159–165.
Benincasa, P., Tosti, G., Tei, F., Guiducci, M., 2010. Actual N availability from winter catch crops used for green manuring in maize cultivation. J. Sustain. Agric. 34, 705–723.
Blanco-Canqui, H., Mikha, M.M., Presley, D.R., Claassen, M.M., 2011. Addition of cover crops enhances no-till potential for improving soil physical properties. Soil Sci. Soc. Am. J. 75, 1471–1482.
Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: insights from studies in temperate soils. Agron. J. 107, 2449–2474.
Boyrahmadi, M., Raiesi, F., 2018. Plant roots and species moderate the salinity effect on microbial respiration, biomass, and enzyme activities in a sandy clay soil. Biol. Fertil. Soils 54, 509–521.
Clark, A.J., Decker, A.M., Meisinger, J.J., Mulford, F.R., Mcintosh, M.S., 1995. Hairy vetch kill date effects on soil water and corn production. Agron. J. 87, 579–585.
Cline, G.R., Silvernail, A.F., 2001. Residual nitrogen and kill date effects on winter cover crop growth and nitrogen content in a vegetable production system. HortTechnology 11, 219–225.
Coombs, C., Lauzon, J.D., Deen, B., Van Eerd, L.L., 2017. Legume cover crop management on nitrogen dynamics and yield in grain corn systems. Field Crop. Res. 201, 75–85.
Dalal, R., 1979. Simple procedure for the determination of total carbon and its radioactivity in soils and plant materials. Analyst 104, 151–154.
Diouf, M., Baudoin, E., Dieng, L., Assigbetsé, K., Brauman, A., 2010. Legume and gramineous crop residues stimulate distinct soil bacterial populations during early decomposition stages. Can. J. Soil Sci. 90, 289–293.
Drinkwater, L., Janke, R., Rossoni-Longnecker, L., 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume-based grain systems. Plant Soil 227, 99–113.
Druille, M., García-Parisi, P.A., Golluscio, R., Cavagnaro, F.P., Omacini, M., 2016. Repeated annual glyphosate applications may impair beneficial soil microorganisms in temperate grassland. Agric. Ecosyst. Environ. 230, 184–190.
Follmer, C., 2008. Insights into the role and structure of plant ureases. Phytochemistry 69, 18–28.
Fortuna, A., Blevins, R., Frye, W., Grove, J., Cornelius, P., 2008. Sustaining soil quality with legumes in no-tillage systems. Commun. Soil Sci. Plant Anal. 39, 1680–1699.
Fourie, J., Agenbag, G., Louw, P., 2007. Cover crop management in a Chardonnay/99 Richter vineyard in the coastal region, South Africa. 3. Effect of different cover crops and cover crop management practices on organic matter and macro-nutrient content of a medium-textured soil. S. Afr. J. Enol. Vitic. 28, 61.
Fourie, J., Louw, P., Agenbag, G., 2001. Effect of seeding date on the performance of grasses and broadleaf species evaluated for cover crop management in two wine grape regions of South Africa. S. Afr. J. Plant Soil 18, 118–127.
García-Orenes, F., Guerrero, C., Roldán, A., Mataix-Solera, J., Cerdà, A., Campoy, M., Zornoza, R., Bárcenas, G., Caravaca, F., 2010. Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. Soil Tillage Res. 109, 110–115.
García-Ruiz, R., Ochoa, V., Hinojosa, M.B., Carreira, J.A., 2008. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol. Biochem. 40, 2137–2145.
Haney, R., Senseman, S., Hons, F., 2002. Effect of Roundup Ultra on microbial activity and biomass from selected soils. J. Environ. Qual. 31, 730–735.
Hoagland, L., Carpenter-Boggs, L., Granatstein, D., Mazzola, M., Smith, J., Peryea, F., Reganold, J.P., 2008. Orchard floor management effects on nitrogen fertility and soil biological activity in a newly established organic apple orchard. Biol. Fertil. Soils 45, 11.
Jani, A.D., Grossman, J., Smyth, T.J., Hu, S., 2016. Winter legume cover-crop root decomposition and N release dynamics under disking and roller-crimping termination approaches. Renew. Agric. Food Syst. 31, 214–229.
Jat, M., Gathala, M., Saharawat, Y., Tetarwal, J., Gupta, R., 2013. Double no-till and permanent raised beds in maize–wheat rotation of north-western Indo-Gangetic plains of India: effects on crop yields, water productivity, profitability and soil physical properties. Field Crop. Res. 149, 291–299.
Kalembasa, S., Symanowicz, B., 2012. Enzymatic activity of soil after applying various waste organic materials, ash, and mineral fertilizers. Pol. J. Environ. Stud. 21, 1635–1641.
Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fertil. Soils 6, 68–72.
Karaca, A., Cetin, S.C., Turgay, O.C., Kizilkaya, R., 2010. Soil Enzymes as Indication of Soil Quality. Soil Enzymology. Springer, Berlin, Heidelberg.
Keene, C., Curran, W., Wallace, J., Ryan, M., Mirsky, S., Vangessel, M., Barbercheck, M., 2017. Cover crop termination timing is critical in organic rotational no-till systems. Agron. J. 109, 272–282.
Lane, M., Lorenz, N., Saxena, J., Ramsier, C., Dick, R.P., 2012. Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. Pedobiologia 55, 153–159.
Lawson, A., Cogger, C., Bary, A., M Fortuna, A.-, 2015. Influence of seeding ratio, planting date, and termination date on rye-hairy vetch cover crop mixture performance under organic management. PloS One 10, e0129597.
Lawson, A., Fortuna, A.M., Cogger, C., Bary, A., Stubbs, T., 2013. Nitrogen contribution of rye-hairy vetch cover crop mixtures to organically grown sweet corn. Renew. Agric. Food Syst. 28, 59–69.
Lemanowicz, J., 2011. Phosphatases activity and plant available phosphorus in soil under winter wheat (Triticum aestivum L.) fertilized minerally. Pol. J. Agron. 4, 12–15.
Levene, H., 1960. Robust tests for equality of variances. In: Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling Stanford University Press, Palo Alto, CA, pp. 278–292.
Li, L., Li, S.-M., Sun, J.-H., Zhou, L.-L., Bao, X.-G., Zhang, H.-G., Zhang, F.-S., 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc. Natl. Acad. Sci. Unit. States Am. 104, 11192–11196.
Liang, S., Grossman, J., Shi, W., 2014. Soil microbial responses to winter legume cover crop management during organic transition. Eur. J. Soil Biol. 65, 15–22.
Lienhard, P., Terrat, S., Prévost-Bouré, N.C., Nowak, V., Régnier, T., Sayphoummie, S., Panyasiri, K., Tivet, F., Mathieu, O., Levèque, J., 2014. Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. Agron. Sustain. Dev. 34, 525–533.
Little, T.M., Hills, F., 1972. Statistical Methods in Agricultural Research UCD Book Store. University of California Davis.
Makoi, J.H., Chimphango, S.B., Dakora, F.D., 2010. Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere of cowpea (Vigna unguiculata L. Walp.) genotypes grown in mixed culture and at different densities with sorghum (Sorghum bicolor L.). Crop Pasture Sci. 61, 279–286.
Makoi, J.H., Ndakidemi, P.A., 2008. Selected soil enzymes: examples of their potential roles in the ecosystem. Afr. J. Biotechnol. 7, 181–191.
Maltais-Landry, G., 2015. Legumes have a greater effect on rhizosphere properties (pH, organic acids and enzyme activity) but a smaller impact on soil P compared to other cover crops. Plant Soil 394, 139–154.
Maseko, S., Dakora, F., 2013. Rhizosphere acid and alkaline phosphatase activity as a marker of P nutrition in nodulated Cyclopia and Aspalathus species in the Cape fynbos of South Africa. South Afr. J. Bot. 89, 289–295.
Mcvay, K., Radcliffe, D., Hargrove, W., 1989. Winter legume effects on soil properties and nitrogen fertilizer requirements. Soil Sci. Soc. Am. J. 53, 1856–1862.
Mganga, K.Z., Razavi, B.S., Kuzyakov, Y., 2016. Land use affects soil biochemical properties in Mt. Kilimanjaro region. Catena 141, 22–29.
Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R., Shumway, D.L., 2009. Control of cereal rye with a roller/crimper as influenced by cover crop phenology. Agron. J. 101, 1589–1596.
Mukherjee, A., Lal, R., 2015. Short-term effects of cover cropping on the quality of a Typic Argiaquolls in Central Ohio. Catena 131, 125–129.
Mukumbareza, C., Muchaonyerwa, P., Chiduza, C., 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. S. Afr. J. Plant Soil 32, 189–197.
Nuruzzaman, M., Lambers, H., Bolland, M.D., Veneklaas, E.J., 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant Soil 281, 109–120.
Ott, R.L., Longnecker, M.T., 2015. An Introduction to Statistical Methods and Data Analysis. Nelson Education.

Parr, M., Grossman, J., Reberg-Horton, S., Brinton, C., Crozier, C., 2011. Nitrogen delivery from legume cover crops in no-till organic corn production. Agron. J. 103, 1578–1590.
Qian, X., Gu, J., Pan, H.-J., Zhang, K.-Y., Sun, W., Wang, X.-J., Gao, H., 2015. Effects of living mulches on the soil nutrient contents, enzyme activities, and bacterial community diversities of apple orchard soils. Eur. J. Soil Biol. 70, 23–30.
Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. Appl. Soil Ecol. 48, 38–44.
Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 591–611.
Shaw, K., 2006. Determination of organic carbon in soil and plant material. Eur. J. Soil Sci. 10, 316–326.
Snapp, S., Swinton, S., Labarta, R., Mutch, D., Black, J., Leep, R., Nyiraneza, J., O'neil, K., 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. Agron. J. 97, 322–332.
Sullivan, D.M., Andrews, N., 2012. Estimating Plant-Available Nitrogen Release from Cover Crops. Oregon State University Extension Service.
Tabatabai, M., Bremner, J., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.
Thilakarathna, M.S., Serran, S., Lauzon, J., Janovicek, K., Deen, B., 2015. Management of manure nitrogen using cover crops. Agron. J. 107, 1595–10607.
Thorup-Kristensen, K., Dresbøll, D.B., 2010. Incorporation time of nitrogen catch crops influences the N effect for the succeeding crop. Soil Use Manag. 26, 27–35.
Wayman, S., Cogger, C., Benedict, C., Burke, I., Collins, D., Bary, A., 2015. The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems. Renew. Agric. Food Syst. 30, 450–460.
Weaver, M.A., Krutz, L.J., Zablotowicz, R.M., Reddy, K.N., 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. Pest Manag. Sci. 63, 388–393.
Weerasekara, C.S., Udawatta, R.P., Gantzer, C.J., Kremer, R.J., Jose, S., Veum, K.S., 2017. Effects of cover crops on soil quality: selected chemical and biological parameters. Commun. Soil Sci. Plant Anal. 48, 2074–2082.
Whittinghill, K.A., Currie, W.S., Zak, D.R., Burton, A.J., Pregitzer, K.S., 2012. Anthropogenic N deposition increases soil C storage by decreasing the extent of litter decay: analysis of field observations with an ecosystem model. Ecosystems 15, 450–461.
Xi, Z.-M., Tao, Y.-S., Zhang, L., Li, H., 2011. Impact of cover crops in vineyard on the aroma compounds of Vitis vinifera L. cv Cabernet Sauvignon wine. Food Chem. 127, 516–522.
Xu, L.-F., Peng, Z., Han, Q.-F., Li, Z.-H., Yang, B.-P., Nie, J.-F., 2013. Spatial distribution of soil organic matter and nutrients in the pear orchard under clean and sod cultivation models. J. Integr. Agric. 12, 344–351.
Yao, S., Merwin, I.A., Bird, G.W., Abawi, G.S., Thies, J.E., 2005. Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. Plant Soil 271, 377–389.