Nitrogen fertilization effects on soil phosphorus dynamics under a grass-pasture system

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Abstract Nitrogen (N) and phosphorus (P) are critical to pasture productivity; however, limited information is available on how the single and combined additions of N and P affect soil P fractions and seasonal changes in microbial and biochemical processes linked to P cycling under pasture systems. A two-year field trial was conducted where N (0 or 250 kg ha^{-1} yr^{-1}) and P (0 or 50 kg ha^{-1} yr^{-1}) were applied in a full factorial design to an intensively managed grass-pasture system. Changes in plant growth and nutrient uptake, soil microbial biomass P, soil phosphatase activities, and soil inorganic and organic P fractions were assessed by regular sampling. Phosphorus addition increased Olsen P and shoot P uptake but not shoot biomass compared to the control. In contrast, N addition decreased Olsen P by 23% but increased both shoot biomass and P uptake by 1.6-fold, compared to the control. Microbial biomass P was irresponsive to N and P additions. Phosphatase enzyme activity significantly increased in summer under N addition, which was linked to labile organic P mineralization. After two growing seasons, N addition alone significantly decreased readily-available inorganic P, labile inorganic P, moderately labile inorganic P, and labile organic P by 75, 19, 7, and 28%, respectively, compared to the control. On the other hand, combined N and P addition significantly decreased readily-available inorganic P, labile inorganic P, and labile organic P by 39, 26, and 28%, respectively, but had no impact on moderately labile inorganic P compared to P addition alone. The findings of this study revealed that short-term N fertilization to N-limited grass-pastures can accelerate P cycling by mobilizing labile inorganic and organic P as well as moderately labile inorganic P pools. However, N fertilization combined with P applications exceeding plant requirements cannot mobilize moderately labile inorganic P, which accumulates under high P sorbing soils.

Keywords Soil P cycling · Nitrogen fertilization · Soil P fractions · Organic P mineralization · Phosphatase enzyme activity · Italian ryegrass (*Lolium multiflorum* Lam.)

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Introduction

Nitrogen (N) and phosphorus (P) are the most common nutrients limiting plant primary productivity (Elser et al. 2007; Vitousek et al. 2010). In general, N:P ratio in the soil, microbial biomass, and plants is 16:1, meaning that in a situation where there are > 16 mol of N to 1 mol of P, soils, microbes, or plants are P-limited, and N-limited if the ratio is < 16:1 (Redfield 1934). Such limitations may have severe impacts on productivity. Consequently, N and P fertilizers are commonly applied to agroecosystems (Tilman et al. 2002; Haygarth et al. 2013); however, excess fertilizer inputs have raised many concerns about accelerated eutrophication and build-up of soil legacy P (Bouwman et al. 2017; McDowell et al. 2020; Pavinato et al. 2020).

Nitrogen applications have been used as an effective way of phytoremediation of high P contaminated soils (Newman et al. 2009; van der Salm et al. 2009). Due to increased plant production in response to N inputs, increased offtake of soil P is observed (Perring et al. 2009; Dodd et al. 2014). Besides the increase in plant yield, N addition can affect soil chemical properties, microbial activity, and root traits, thereby influencing soil P dynamics. For example, N addition can decrease soil pH, thus enhancing the availability of more recalcitrant inorganic P forms (Sherman et al. 2006; Fan et al. 2019). Nitrogen is a principal component of phosphatase enzymes involved in organic P mineralization (Olander and Vitousek 2000; Marklein and Houlton 2012). Nitrogen supply can increase rhizodeposition with a concomitant increase in microbial activity and plant P uptake (Bicharanloo et al. 2020; He et al. 2020; Leptin et al. 2021). On the other hand, N applications can increase mycorrhizal symbiosis as well as root biomass and density, thereby expanding the soil volume explored and subjected to rhizosphere processes (Treseder 2004; Yuan and Chen 2012; Fan et al. 2019; Schleuss et al. 2020).

It is well recognised that modifying stoichiometry through fertilization can affect soil P dynamics in agroecosystems (Liu et al. 2019; Dai et al. 2020; Widdig et al. 2020; Cui et al. 2021). Using P fractionation combined with ^{18}O isotopes, Bauke et al. (2018) found that continual P applications to N deficient arable soils increased P accumulation due to lower plant production and higher P inputs compared to plant P demand. On the other hand, an adequate application of NPK increased the recycling of Ca-P pool in the subsoil, which was attributed to an increase in root growth and microbial activity under sufficient nutrient conditions in the topsoil. Under pasture systems, discrepancies have been found in soil P dynamics as affected by N fertilization. For instance, urea application to a semi-arid grassland for 11 years enhanced readily-available P and promoted the transformation of refractory to less available inorganic P fractions (Wang et al. 2021a). Labile P decreased, while alkaline phosphatase activity and organic P mineralization increased with increasing N applications (mineral + organic) to N-limited semi-arid grassland in China (Cui et al. 2021). On the other hand, N addition in the form of NH_4NO_3 decreased labile inorganic P fractions but did not stimulate organic P mineralization in a calcareous grassland in Inner Mongolia (Liu et al. 2019). These contrasting results could be ascribed to differences in soil types, sources of N applied, and plant biomass management used in these studies (Arenberg and Arai 2019, 2021; Taylor et al. 2021).

Previous studies in pasture soils have shown that N and P inputs can have a significant impact on soil P immobilization and mineralization processes as well as microbial and earthworm community composition (Sarathchandra et al. 2001; Chen et al. 2014; McLaren et al. 2020; Cui et al. 2021). Although these studies have revealed some new insights on microbial and biochemical processes linked to P cycling, they were based on soil samples taken at one single point in time and carried out under either N or P inputs. Therefore, the assessment of temporal changes and interactions between N and P inputs is recommended for a more detailed understanding of the impact of nutrient inputs on soil P dynamics in pasture systems (Dai et al. 2020; Cui et al. 2021).

Past research carried out in pasture systems mainly assessed the impact of N addition on soil P availability and changes in different inorganic P fractions (Liu et al. 2019; Wang et al. 2021a), whereas experiments combining N and P additions focused on investigating organic P mobilization and the release of phosphatase enzymes (Tian et al. 2016; Schleuss et al. 2020). Therefore, scarce data is available under pasture systems detailing transformations in inorganic and organic P pools along with changes in soil microbial biomass and activity as affected by N and P additions.

This study aimed at determining the short-term effects of N fertilization (2 years), alone or when combined with P, on plant growth, soil microbial biomass P, phosphatase enzyme activity, as well as changes in different inorganic and organic P fractions under an intensively managed biennial grass-pasture (Italian ryegrass (*Lolium multiflorum* Lam.)). We hypothesized that (1) P addition would increase plant biomass while inhibiting soil microbial activity through immobilization of P and a decrease of phosphatase activity; (2) N addition would accelerate P cycling through an increase of phosphatase activity and enhance the mobilization of recalcitrant P fractions; and (3) the combined addition of N and P would have a relative advantage in plant biomass, P uptake, and mobilization of soil P fractions compared to single P addition.

Materials and methods

Site description and soil characteristics

The study site was located at Lincoln University, New Zealand (latitude: 43°38'S, longitude: 172°27'E, and altitude: 9 m). The climate is temperate, with a mean air temperature of 11.3 °C and a mean annual rainfall of 640 mm. The soil was a Wakanui silt loam (NZ classification: Mottled Immature Pallic; USDA classification: Udic Ustochrept) that had not been P fertilized for the last nine years and had been maintained under mixed cropping between 2010 and 2016 with wheat (*Triticum aestivum* L.), Italian ryegrass (*Lolium multiflorum* L.), kale (*Brassica oleracea spp. acephala*), and green globe turnips (*Brassica rapa*), respectively. In 2017, the study site was sprayed with glyphosate, ploughed, and sown with Italian ryegrass (*Lolium multiflorum* Lam. cv. Tabu) at 20 kg ha^{-1}. The soil characteristics at 0–75 mm depth at trial establishment were sand: 34.4%, silt: 54.5%, clay: 11.1%, pH: 6.3, organic matter: 4.3%, total C: 26 g kg^{-1}, total N: 2.3 g kg^{-1}, C/N: 11.3, total P: 780 mg kg^{-1}, NO_3^--N: 27 mg kg^{-1}, NH_4^+-N: 5 mg kg^{-1}, and bioavailable P (Olsen P): 10 mg kg^{-1}. The soil was considered low in total C, and total N for New Zealand soils, according to Blakemore et al. (1987). The nutrient treatments were control (no fertilization), P (application of 50 kg P ha^{-1} yr^{-1}), N (application of 250 kg N ha^{-1} yr^{-1}), and N and P (application of 50 kg P + 250 kg N ha^{-1} yr^{-1}). Each plot was 3×5 m^2 with a one-meter buffer strip to avoid nutrient transfer. The experiment was set up in a randomized block design with 5 replicates. Phosphorus fertilization (single superphosphate) was carried out in August 2018 and August 2019, whereas N (urea) was split in 5 applications (50 kg N ha^{-1} each) for optimal annual yield (Sun et al. 2008) and applied in August, October, November, March, and April each year. Phosphorus inputs aimed at increasing soil Olsen P to reach an optimum of 20 to 30 mg P kg^{-1} (Morton and Roberts 2001; Edmeades et al. 2006), while N inputs were chosen to replicate similar management practices carried out in intensive dairy pasture systems in New Zealand (Pinxterhuis and Edwards 2018; Al-Marashdeh et al. 2021). Besides P and N, the plots received 50 kg ha^{-1} of sulfur (Sulphur 90 Prill, H Sulphur Corp, South Korea, particle size of S: 18.4% < 75 μm, 52.3% < 150 μm, and 29.3% < 300 μm) in August 2018 and August 2019. All fertilizers were applied manually. The plots were irrigated with sprinklers when soil moisture in the top 0–75 mm was below 20% gravimetric water holding capacity. Following soil and plant sampling, plots were cut at 20–30 mm high with a mower, and plant biomass was removed.

Soil and plant sampling

Soil and plant samples were collected each season over two years, that is, October 2018 (Spring), January 2019 (Summer), March 2019 (Autumn), July 2019 (Winter), October 2019 (Spring), January 2020 (Summer), March 2020 (Autumn), and July 2020 (Winter). Soil samples were taken using a soil auger from the 0–75 mm horizon at 15 random locations within each plot. Shoot biomass was sampled one day later by harvesting within a 0.5 m^2 metallic frame randomly placed in each plot. Shoots were cut at 20 mm above the ground. Soil samples were sieved to pass through a 2 mm sieve and split into two portions. The first one was stored at 4 °C and used to determine soil moisture and soil biological and biochemical parameters within one week. The second portion was air-dried and used to carry out soil chemical analyses. Olsen P, microbial biomass P, acid and alkaline phosphatase activities, shoot biomass, shoot P concentration, and shoot P uptake were determined each season. Soil pH, total C and N, available N (NO_3^--N and NH_4^+-N), and soil P

fractions were measured at the end of each growing season, that is, July 2019 and July 2020.

Soil and plant analyses

Soil moisture was calculated after oven-drying 10 g of moist soil at 105 °C for 48 h. Soil pH was determined after shaking air-dried soil with deionized water (ratio 1:2.5) for one hour. Total N and C were measured using an Elementar Vario Max CN analyzer after combustion of 0.5 g of air-dried and ground soil. Available N (NH_4^+-N and NO_3^--N) was assessed by extracting field-moist soil with 2 M KCl (ratio 1:10) for one hour and then analyzing the aliquot in a total N analyzer (Blakemore et al. 1987). Olsen P was determined according to Watanabe and Olsen (1965). Microbial biomass P was determined following the fumigation-extraction method of Brookes et al. (1982) with a recovery coefficient of 40%. Potential acid and alkaline phosphatase activities were assessed according to the procedure of Tabatabai (1994) for phosphatase assays. Potential phosphatase activities were expressed as µmol p-nitrophenol produced g^{-1} fresh soil h^{-1}. Hereafter, when mentioning phosphatase activities, it means potential phosphatase activities.

Phosphorus fractionation was performed according to the Hedley method with the modifications suggested by Condron and Newman (2011), as reported elsewhere (Gatiboni et al. 2021; Touhami et al. 2021). These consisted of the addition of a salt (1 M NH_4Cl) in the first extraction to wash out excess calcium, the inclusion of a washing step using NaCl between extractions to avoid carryover of P, and the use of a second NaOH extraction after HCl to extract P firmly bound to Al and Fe oxides and P present in the microaggregates. In short, 0.5 g of air-dried soil was sequentially extracted using 10 ml of solution following the scheme outlined in Table S1. Each soil solution was shaken for 16 h, centrifuged for 15 min, and the aliquot was kept in the fridge at 4 °C for further analyses. The final soil residue was oven-dried at 50 °C to determine residual P, according to Olsen and Sommers (1982). Soil extracts were analyzed for inorganic P using two different methods to avoid organic P mineralization and overestimation of inorganic P. Inorganic P in acid extracts (NH_4Cl, HCl, and residual P) was measured using the molybdenum blue method (Murphy and Riley 1962). In comparison,

inorganic P in alkaline extracts ($NaHCO_3$, NaOH1, NaOH2) was analyzed following the procedure of Dick and Tabatabai (1977). Total P in the alkaline extracts was assessed by ICP-OES according to He and Honeycutt (2005), while organic P was calculated by deducting inorganic P from total P. Total soil P represented the sum of all nine fractions. To determine the incorporation of applied P into different P fractions, P fractionation was carried out on soil samples before starting the experiment (July 2018) and after two months of P addition (October 2018). Moreover, P fractionation was performed on soils taken after one (July 2019) and two years of plant growth (July 2020) to determine the impact of nutrient treatments on soil P dynamics.

After harvest, plant shoots were washed with deionized water, oven-dried for two days at 65 °C, and weighed. Dried plant shoots were ground in a stainless grinder, sieved less than 1 mm, and then digested in a mixture of HNO_3 and H_2O_2. Shoot P concentrations were then determined using ICP-OES, according to Wu et al. (1997). Shoot P uptake was calculated by multiplying P concentration by dry shoot biomass. Shoot biomass for each growing season was calculated as the sum of the four plant samplings from October to July, while the total shoot biomass represents the sum of the two growing seasons. Similar calculations were done for shoot P uptake. Partial P balance was calculated as the difference between P input, which is the total amount of mineral P fertilizer added over the period of the trial and P output representing the P removed in plant biomass by cutting over the same period.

Meteorological data

Monthly rainfall for the study period was retrieved from Lincoln University weather station, whereas mean soil temperature was calculated from the data provided by four sensors buried at 0–75 mm in four different locations.

Statistical analysis

Data were subjected to two-way repeated measures ANOVA with nutrient treatment as a factor and sampling dates as within-subject variables. Friedman's test was used for non-normally distributed data such as Olsen P and shoot P uptake. In the presence of

a significant effect, one-way ANOVA followed by the Tukey *post-hoc* test was performed to differentiate significant differences among treatment means and sampling dates separately at 5% probability. Soil P fractions and soil chemical properties (pH, total C, and N, available N) were analyzed using one-way ANOVA to test the effect of nutrient treatment and sampling date separately. When data did not meet homoscedasticity, the Kruskal–Wallis test was used instead, followed by the Games-Howell *post-hoc* test to separate means at 5% probability. Correlation analysis was performed and expressed as Spearman's coefficients to determine relationships between soil (Olsen P, microbial biomass P, acid and alkaline phosphatase activities, and soil moisture) and plant parameters (shoot biomass and P uptake) over all sampling dates. Spearman correlations and principal component analysis (PCA) were carried out to determine underlying relationships and explore trends between P fractions, soil (Olsen P, microbial biomass P, acid and alkaline phosphatase activity, soil pH, total C, total N, NO_3^--N, and NH_4^+-N) and plant (total shoot biomass, P concentration, and total P uptake) parameters measured after 1 and 2 years of plant growth. All data analyses were performed using SPSS 25.0 for Windows (SPSS, Chicago, IL, USA). Principal component analysis was carried out using XLSTAT Statistical Software for Excel version 2021.4. Microsoft Excel and SigmaPlot 14.0 (SYS-STAT) were used for plotting the data.

Results

Environmental conditions

The study site received a total of 926 mm of water (731 mm rainfall, 195 mm irrigation) between August 2018 and July 2019 in comparison to 850 mm (645 mm rainfall, 205 mm irrigation) in the second growing season from August 2019 to July 2020 (Fig. 1a). Maximum temperatures were recorded in January each year, whereas minimums were noted in June 2019 and July 2020 (Fig. 1a). Soil moisture varied from 16 to 29%, with lower values observed in summer and higher values in winter (Fig. 1b). Albeit N and N and P treatments showed lower soil moisture throughout the study period, no significant differences were detected in soil moisture between nutrient treatments (Fig. 1b, Table S2).

Soil properties

Soil pH showed no significant changes across nutrient treatments at the end of the first growing season (July 2019). However, a significant decrease in soil pH was observed at the end of the second growing season (July 2020) under N addition treatments (Table S3), though changes in soil pH were small (0.2 units). Total C and N were not affected by nutrient treatment and sampling date in this study (Table S3). In comparison to the control and P treatments, NO_3^--N concentrations were significantly higher under treatments with N addition in July 2019 and July 2020. However, NH_4^+-N concentrations were similar regardless of nutrient treatment (Table S3).

Olsen P concentrations significantly increased after P addition and reached an average of 22 mg kg^{-1} under P and N and P treatments compared to an initial Olsen P of 10 mg kg^{-1} (Fig. 2a). Throughout the growing season, Olsen P concentrations declined irrespective of nutrient treatment but remained significantly higher under P and N and P treatments compared to treatments without P addition (Fig. 2a, Table S2). Olsen P concentrations decreased by 32% under N treatment compared to the control, while this decrease was 14% under N and P treatment compared to P alone. Microbial biomass P did not respond to N and P additions but was significantly different between seasons regardless of nutrient treatment (Fig. 2b, Table S2). In general, higher microbial biomass P was observed in summer, especially under N and N and P treatments, though differences between nutrient treatments were not significant. Acid and alkaline phosphatase activities significantly decreased following P addition (P and N and P treatments) in October 2019 and October 2020. Significantly higher phosphatase activities were observed in summer (January 2019 and January 2020) under N and N and P treatments, especially for alkaline phosphatases, compared to the control and P treatments (Fig. 3, Table S2). Acid and alkaline phosphatase activities followed similar seasonal trends, with maximum values measured in summer and minimum values in winter (Fig. 3).
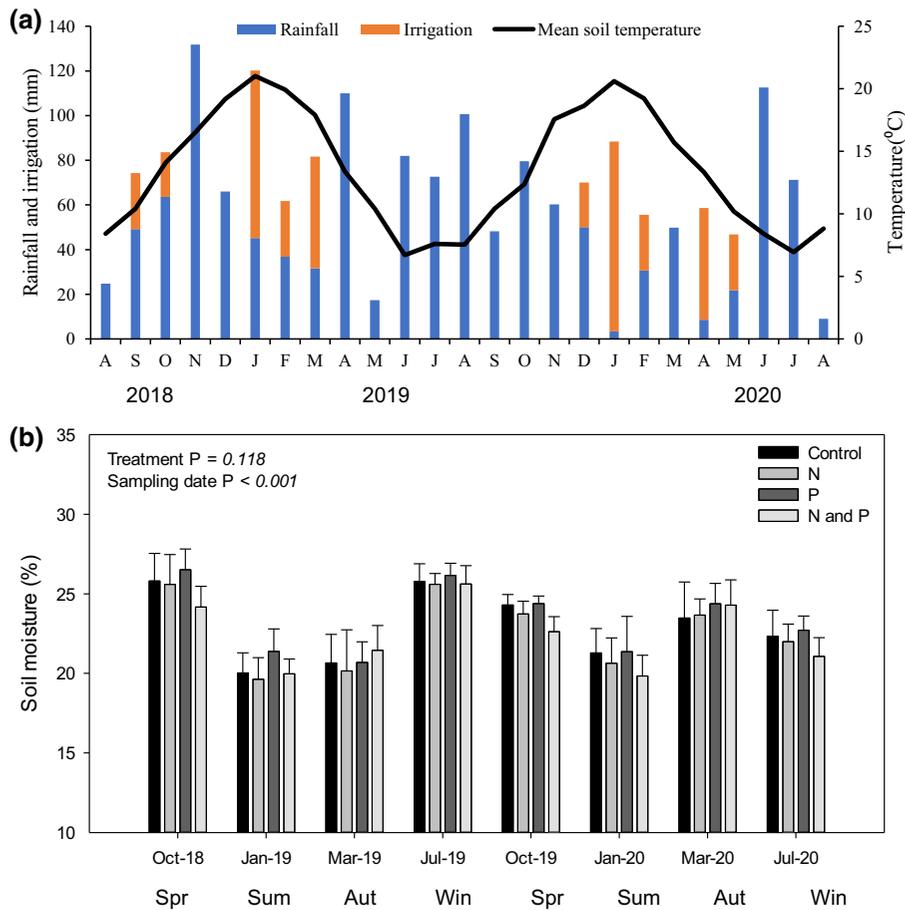
Fig. 1 Climatic conditions (**a**) (monthly mean soil temperature, rainfall, and irrigation) at the trial site during the growing seasons of 2018–2019 and 2019–2020, and temporal changes in soil moisture (**b**) under the control, N (250 kg N ha^{-1} yr^{-1}), P (50 kg P ha^{-1} yr^{-1}), and N and P (50 kg P + 250 kg N ha^{-1} yr^{-1}) treatments. Soil samples were taken from 0–75 mm horizon under Italian ryegrass (*Lolium multiflorum* Lam.) between October 2018 and July 2020. Values are means of 5 replicates, and bars represent standard deviations

Soil P fractions and partial P balance

Comparison between P fractionations of the original soil (July 2018) and two months after P addition (P and N and P treatments in October 2018) revealed that mineral P fertilizer was incorporated into labile inorganic P (NaHCO$_3$-Pi) (16 mg kg^{-1}) but also moderately labile inorganic P (NaOH1-Pi) (14 mg kg^{-1}) (Tables S4, 1).

Changes in soil P fractions illustrated in Table 1 showed that at the end of the second growing season (July 2020), readily-available, labile, and moderately labile inorganic P (NaOH1-Pi) were significantly depleted under the control and N treatments, while labile organic P (NaHCO$_3$-Po) decreased significantly

under N and N and P treatments. After two consecutive years of plant growth, total P significantly decreased under N treatment and was unchanged under P and N and P treatments. Other soil P fractions (HCl-Pi, NaOH1-Po, NaOH2-Pi, NaOH2-Po, residual-Pi) were similar regardless of nutrient treatment and sampling date (Table 1).

Compared to the control, two consecutive years of N addition alone significantly decreased readily-available inorganic P, labile inorganic P, labile organic P, and moderately labile inorganic P by 75, 19, 28, and 7%, respectively. On the other hand, the combined addition of N and P significantly decreased readily-available inorganic P, labile inorganic P, and labile organic P by 39, 26, and 28%, respectively, compared
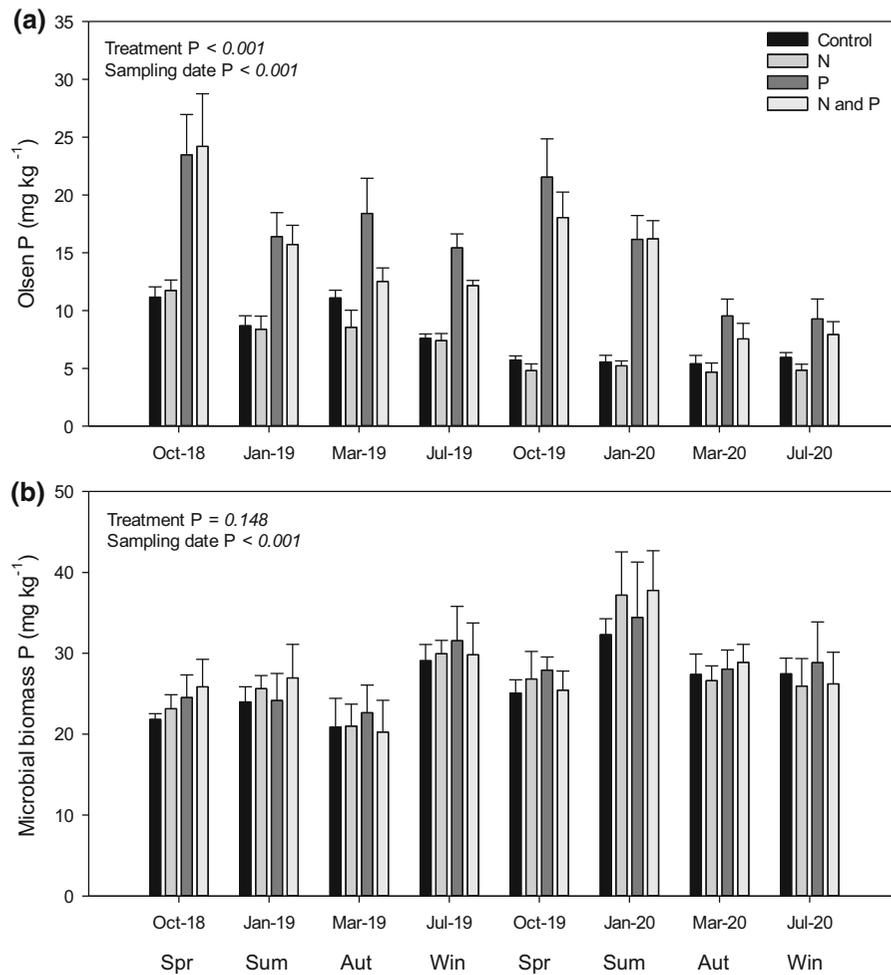
Fig. 2 Temporal changes in Olsen P (**a**), and microbial biomass P (**b**) under the control, N (250 kg N ha^{-1} yr^{-1}), P (50 kg P ha^{-1} yr^{-1}), and N and P (50 kg P + 250 kg N ha^{-1} yr^{-1}) treatments. Soil samples were taken from 0–75 mm horizon under Italian ryegrass (*Lolium multiflorum* Lam.) between October 2018 and July 2020. Values are means of 5 replicates, and bars represent standard deviations

to the application of P alone, whereas moderately labile inorganic P was not affected (Table 1). Additionally, as compared to the initial soil before starting the experiment (Table S4), N addition alone decreased labile inorganic P (from 33 to 15 mg kg^{-1}), moderately labile inorganic P (from 127 to 105 mg kg^{-1}), and labile organic P (from 41 to 21 mg kg^{-1}) (Table 1). Phosphorus addition alone increased soil labile inorganic P (from 33 to 42 mg kg^{-1}), increased moderately labile inorganic P (from 127 to 142 mg kg^{-1}) and depleted labile organic P (41 to 32 mg kg^{-1}) (Table 1), compared to the initial soil (Table S4). On the other hand, the combined addition of N and P had similar soil labile inorganic P (from 33

to 31 mg kg^{-1}), increased moderately labile inorganic P (from 127 to 140 mg kg^{-1}) and depleted labile organic P (41 to 22 mg kg^{-1}) (Table 1), compared to the initial soil (Table S4).

The control and N treatments had a negative partial P balance, but N treatment decreased the partial P balance by 51% compared to the control (Table 2). The partial P balance under P and N and P treatments was positive, but N and P treatment decreased the partial P balance by 28% compared to P treatment (Table 2). Results also showed that 56 and 40% of added P accumulated in the topsoil (0–75 mm) under P and N and P treatments, respectively (Table 2).
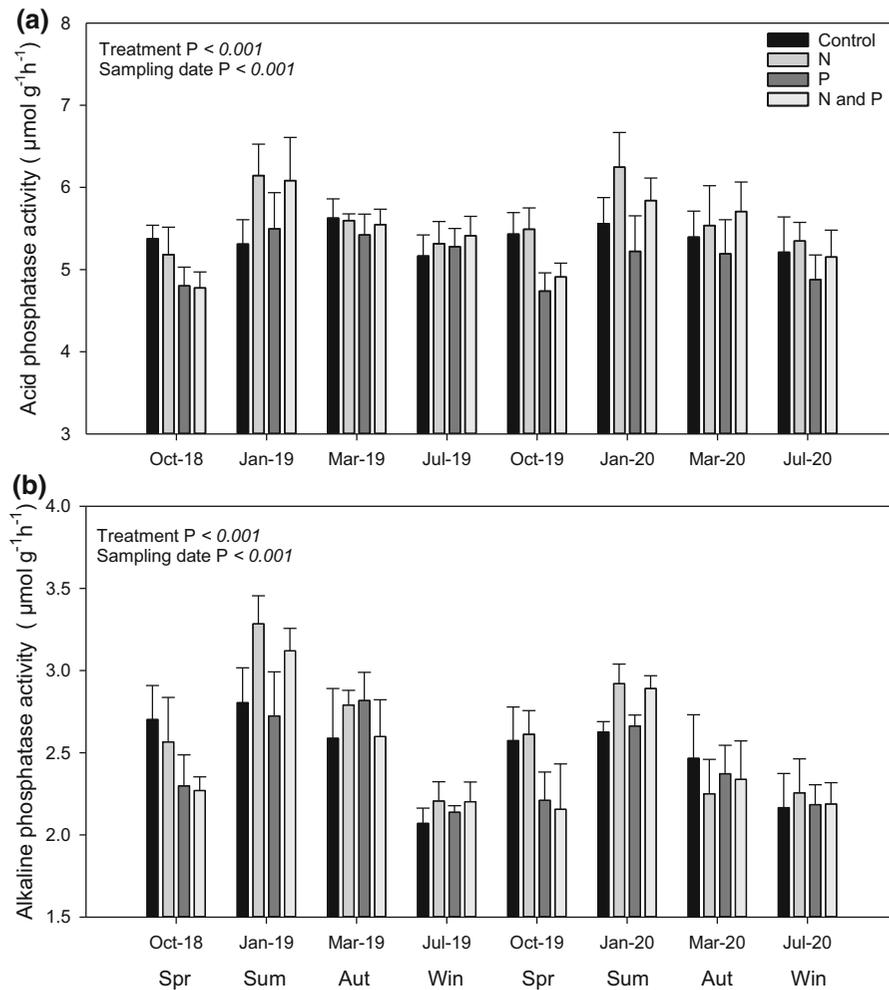
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Fig. 3 Temporal changes in acid (**a**) and alkaline (**b**) phosphatase activities under the control, N (250 kg N ha^{-1} yr^{-1}), P (50 kg P ha^{-1} yr^{-1}), and N and P (50 kg P + 250 kg N ha^{-1} yr^{-1}) treatments. Soil samples were taken from 0–75 mm horizon under Italian ryegrass (*Lolium multiflorum* Lam.) between October 2018 and July 2020. Values are means of 5 replicates, and bars represent standard deviations

Shoot biomass and shoot nutrient uptake

Total shoot biomass of Italian ryegrass over two growing seasons did not significantly respond to P addition alone (P treatment) compared to the control. However, a significant increase of 65 and 63% was observed in the total shoot biomass under N and N and P treatments, respectively, in comparison to the control (Table 3). The total shoot P uptake under P, N, and N and P treatments was significantly higher by 1.3-, 1.5-, and 1.7-fold compared to the control, respectively (Tables 3, S2). Shoot biomass and shoot P uptake were higher in season 1 than season 2, irrespective of nutrient treatments and showed

significant differences between sampling dates (Tables 3, S2, Fig. S1). Peaks of shoot biomass were recorded in January 2019 and January 2020 (summer), especially under N and N and P treatments. In contrast, shoot P uptake was maximum in October 2018 and January 2020 (Fig. S1).

Relationships between soil and plant parameters and P fractions

Results of correlation analysis on all sampling dates showed that alkaline phosphatase activity was positively correlated with microbial biomass P while Olsen P was negatively correlated with acid

Table 1 Distribution of different inorganic P (Pi) and organic P (Po) fractions (mg kg^{-1}) under the control, N (250 kg N ha^{-1} yr^{-1}), P (50 kg P ha^{-1} yr^{-1}), and N and P (50 kg P + 250 kg N ha^{-1} yr^{-1}) treatments. Soil samples were taken from the 0–75 mm horizon under Italian ryegrass (*Lolium multiflorum* Lam.) in October 2018, July 2019, and July 2020

	Control	N	P	N P
October 2018				
NH$_4$Cl	0.8 ± 0.1[a] bA	1.0 ± 0.1 bA	1.8 ± 0.5 aA	1.7 ± 0.2 aA
NaHCO$_3$-Pi	29 ± 1 bA	31 ± 3 bA	49 ± 6 a	48 ± 5 aA
NaHCO$_3$-Po	43 ± 4 A	43 ± 3 A	42 ± 6 A	44 ± 5 A
NaOH1-Pi	126 ± 1 bA	128 ± 2 bA	139 ± 9 ab	144 ± 12 a
NaOH1-Po	248 ± 16	255 ± 10	252 ± 4	255 ± 9
HCl-Pi	131 ± 19	116 ± 10	119 ± 13	118 ± 13
NaOH2-Pi	26 ± 1	28 ± 2	26 ± 1	26 ± 1
NaOH2-Po	26 ± 1	26 ± 2	28 ± 3	28 ± 2
Residual-Pi	139 ± 10	141 ± 14	140 ± 13	145 ± 12
Total P [b]	770 ± 19	769 ± 24 A	797 ± 24	809 ± 18
July 2019				
NH$_4$Cl	0.8 ± 0.1 cA	0.8 ± 0.1 cA	1.2 ± 0.1 aB	1.0 ± 0.1 bB
NaHCO$_3$-Pi	26 ± 1 cB	24 ± 2 cB	41 ± 3 a	33 ± 2 bB
NaHCO$_3$-Po	30 ± 2 B	31 ± 2 B	34 ± 2 AB	29 ± 2 B
NaOH1-Pi	132 ± 2 bA	129 ± 4 bA	153 ± 7 a	147 ± 8 a
NaOH1-Po	257 ± 9	253 ± 10	252 ± 10	255 ± 11
HCl-Pi	117 ± 8	125 ± 21	129 ± 16	127 ± 14
NaOH2-Pi	27 ± 3	26 ± 1	27 ± 2	26 ± 1
NaOH2-Po	27 ± 2	28 ± 2	28 ± 2	27 ± 2
Residual-Pi	144 ± 9	140 ± 13	142 ± 10	140 ± 10
Total P [b]	760 ± 19 b	756 ± 14 bA	796 ± 23.9 a	785 ± 13 ab
July 2020				
NH$_4$Cl	0.4 ± 0.1 cB	0.1 ± 0.1 dB	1.2 ± 0.2 aB	0.8 ± 0.3 bB
NaHCO$_3$-Pi	19 ± 1 cC	15 ± 1 dC	42 ± 4 a	31 ± 2 bB
NaHCO$_3$-Po	29 ± 2 aB	21 ± 1 bC	31 ± 3 aB	22 ± 2 bC
NaOH1-Pi	114 ± 2 bB	105 ± 4 bB	142 ± 7 a	140 ± 4 a
NaOH1-Po	252 ± 8	261 ± 7	261 ± 7	259 ± 12
HCl-Pi	120 ± 14	124 ± 18	123 ± 16	125 ± 19
NaOH2-Pi	27 ± 2	28 ± 1	28 ± 2	27 ± 2
NaOH2-Po	29 ± 2	28 ± 2	30 ± 2	27 ± 2
Residual-Pi	144 ± 15	140 ± 14	141 ± 12	142 ± 13
Total P [b]	735 ± 23 b	726 ± 21 bB	801 ± 10 a	781 ± 19 a

[a]Values represent means of five replicates ± standard deviations (n = 5)

[b]Calculated as the sum of different inorganic and organic P fractions

Within rows, different lowercase letters indicate significant differences ($p < 0.05$) among nutrient treatments, while within columns, different uppercase letters indicate significant differences ($p < 0.05$) among sampling dates

phosphatase activity (Table 4). Shoot biomass was significantly correlated with acid and alkaline phosphatase activities, whereas P uptake was significantly correlated with Olsen P and acid and alkaline phosphatase activities. Shoot biomass, shoot P uptake, and phosphatase activities were negatively correlated with soil moisture (Table 4). Principal component analysis results revealed that inorganic P fractions

Table 2 Partial P balance (difference between P inputs and outputs) in the 0–75 mm soil depth for the period of the trial (2 years) under the control, N (250 kg N ha^{-1}y^{-1}), P (50 kg P ha^{-1}y^{-1}), and NP (50 kg P + 250 kg N ha^{-1}y^{-1}) treatments

Treatment	P input (kg P ha^{-1})	P output (kg P ha^{-1})	Partial P balance (kg P ha^{-1})
Control	0	35	− 35
N	0	53	− 53
P	100	44	56
N P	100	60	40

Table 3 Shoot biomass, shoot P uptake, and shoot N uptake in season 1 (July 2018-July 2019), season 2 (October 2019 – July 2020), and the whole period of the study (total) under the control, N (250 kg N ha^{-1} yr^{-1}), P (50 kg P ha^{-1} yr^{-1}), and N and P (50 kg P + 250 kg N ha^{-1} yr^{-1}) treatments

	Control	N	P	N and P
	Shoot biomass (T ha^{-1})			
Season 1	5.7 ± 0.3[a]b	8.8 ± 0.3a	5.9 ± 0.3b	9.1 ± 0.4a
Season 2	5.9 ± 0.5b	10.3 ± 0.9a	6.6 ± 0.3b	9.8 ± 0.4a
Total	11.5 ± 0.7b	19.1 ± 1.0a	12.5 ± 0.4b	18.9 ± 0.6a
	Shoot P uptake (kg ha^{-1})			
Season 1	14.3 ± 0.8c	19.5 ± 1.1b	18.5 ± 0.7b	24.6 ± 1.2a
Season 2	20.3 ± 2.7c	33.1 ± 4.1a	25.6 ± 1.9b	35.4 ± 2.5a
Total	34.7 ± 3.3d	52.6 ± 3.0b	44.1 ± 2.1c	60.0 ± 2.4a
	Shoot N uptake (kg ha^{-1})			
Season 1	109.2 ± 5.7b	210.4 ± 7.1a	112.7 ± 5.6b	206.2 ± 9.1a
Season 2	128.0 ± 13.6b	232.3 ± 30.7a	146.7 ± 6.7b	224.2 ± 13.9a
Total	237.2 ± 16.5c	442.7 ± 31.8a	259.4 ± 3.5b	430.5 ± 19.7a

[a]Values represent means ± standard deviations (n = 5)

Within rows, different letters indicate significant differences ($p < 0.05$) among nutrient treatments

*Significant difference at $p < 0.05$; **Significant difference at $p < 0.01$; MBP: microbial biomass P; Acid P: acid phosphatase activity; Alk P: alkaline phosphatase activity

depleted in this study, namely readily-available, labile, and moderately labile inorganic P had positive loadings with the first component, which explained 26.6% of the data variability (Fig. 4). These P fractions were also positively correlated with Olsen P and microbial biomass P while negatively correlated with total shoot biomass and P uptake (Table 5). The second component explained another 16.5% of data variability and was mainly composed of labile organic P (loading = 0.654), total shoot biomass (loading = − 0.581), and total P uptake (loading = − 0.515) (Fig. 4). Spearman correlations further showed that NO_3^--N concentration was significantly positively correlated with total shoot biomass. Labile organic P was significantly negatively

correlated with total shoot biomass and P uptake (Table 5).

Discussion

Nutrient addition impacts on plant biomass and P uptake

Single or combined fertilizer inputs can modify the soil nutrient balance, thereby affecting plant primary productivity and, in turn, plant nutrient uptake (Deng et al. 2016; Schleuss et al. 2020; Cui et al. 2021). In contrast to our first hypothesis, our results showed that P addition alone did not increase the aboveground

Table 4 Values of Spearman correlation coefficients between soil and plant parameters, assessed from October 2018 to July 2020 (n = 160)

	MBP	Olsen-P	Acid P	Alk P	Shoot biomass	Shoot P uptake	Soil moisture
MBP	1	− 0.047	0.141	0.379**	0.095	− 0.127	− 0.029
Olsen-P	− 0.047	1	− 0.349**	− 0.090	0.063	0.405**	0.085
Acid P	0.141	− 0.349**	1	0.382**	0.359**	0.201*	− 0.434**
Alk P	0.379**	− 0.090	0.382**	1	0.292**	0.216**	− 0.145
Shoot biomass	0.095	0.063	0.359**	0.292**	1	0.760**	− 0.356**
P uptake	− 0.127	0.405**	0.201*	0.216**	0.760**	1	− 0.366**
Soil moisture	− 0.029	0.085	− 0.434**	− 0.145	− 0.356**	− 0.366**	1

plant biomass of Italian ryegrass. Ikoyi et al. (2018) found that the single P application to a P-deficient soil cultivated with ryegrass (*Lolium perenne* L.) had negative feedback on soil microbial activity and diversity, thus cancelling out the positive effect of P application on plant biomass. Shi et al. (2020) highlighted that low P requirements of timothy grass (*Phleum pratense* L.) were responsible for the non-response of plant biomass to increased P applications. Edmeades et al. (2006) estimated that Olsen P of 30 mg kg^{-1} was the critical value below which pasture production will be less than 97% of optimal production under sedimentary soils in New Zealand. However, this value was established for perennial grass-legume pastures but not for biennial Italian ryegrass (*Lolium multiflorum* Lam.). It is acknowledged that the P requirements of legumes are higher than grasses due to the key role of P in N_2 fixation (Peoples et al. 1998; Sprent 1999; Lam et al. 2012). Therefore, it is possible that soil P availability was not a limiting factor for the growth of Italian ryegrass in the current study. Nevertheless, studies determining the critical values of available soil P for Italian ryegrass under sedimentary soils in New Zealand are needed to confirm this result. Another possibility would be that Italian ryegrass could have taken up some P from deeper soil layers (> 75 mm), especially knowing that our study site was previously under a cropping system, where P might have been evenly distributed in the soil profile. Future studies under Italian ryegrass need to investigate changes in available soil P with depth and its relationship to plant growth.

Sprengel-Liebig Law of the Minimum states that growth is limited not by the total resources available but by the most limiting nutrient (de Baar 1994; van der Ploeg et al. 1999). In contrast to P addition, N addition alone or combined with P significantly increased shoot biomass by 1.6-fold, emphasizing that plant primary productivity in this study was limited by N availability rather than P. Indeed, PCA and correlation analysis revealed that shoot biomass was positively correlated with NO_3^--N concentration ($P < 0.01$). Our findings partly agreed with the results reported by Schleuss et al. (2020) but stressed again that plant productivity in our soil was mainly N-limited since there were no significant differences between shoot biomass under N addition alone and the combined addition of N and P. In fact, our soil was previously under an arable cropping system characterized by high losses of C and N due to increased removal of soil nutrients, depletion of soil organic matter, and degradation of soil structure (Haynes et al. 1991; Nguyen et al. 1995; Haynes 2000).

Phosphorus addition alone significantly increased total shoot P uptake compared to the control treatment in both years, which was not seen for shoot biomass. This was indicative of a luxury uptake of P by Italian ryegrass. It is well established that P uptake is a function of N availability in soils (Ford et al. 2016; Gao et al. 2016; Wang et al. 2021a, b). In fact, our results indicated that N addition, alone and in combination with P, significantly increased total shoot P uptake by 19 and 37%, respectively, compared to the single addition of P. These results confirmed our second hypothesis and partly our third hypothesis.

Nutrient addition impacts on soil properties linked to P cycling

Throughout the growing season, Olsen P concentrations decreased regardless of nutrient treatment,
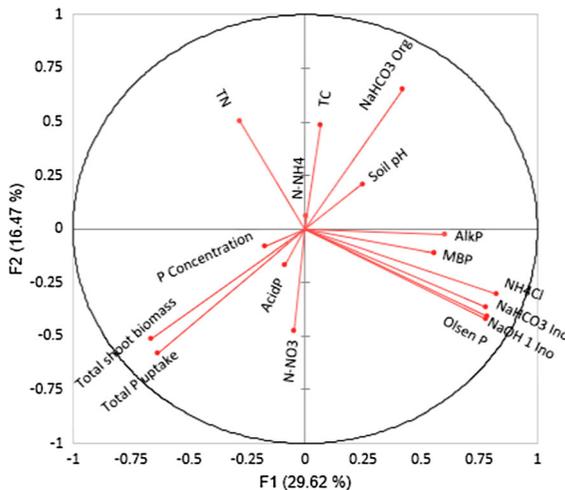
Fig. 4 Loading plot from the results of principal component analysis (PCA) showing the relationships between soil and plant parameters and P fractions measured in July 2019 and July 2020 under Italian ryegrass (*Lolium multiflorum* Lam.). MBP: microbial biomass P; Acid P: acid phosphatase activity; Alk P: alkaline phosphatase activity; TN: total nitrogen; TC: total carbon; Ino: inorganic P; Org: organic P

mainly due to plant P uptake and P removal in plant biomass. Under N addition treatments, Olsen P concentrations decreased by an average of 23% compared to the control and P treatments. In N-limited soils, N addition increases net primary productivity, thereby accelerating soil P depletion (Deng et al. 2017; Cui et al. 2021). In fact, our results showed that shoot biomass under N addition treatments was 1.6-fold higher than the control and P treatments. Schleuss et al. (2021) found that available inorganic P was not significantly different under grassland soils receiving either P or N and P inputs. Their result could be explained by the similar aboveground biomass found across treatments along with the recycling of P through plant returns. McDowell et al. (2016) tested the application of 50 kg N ha^{-1} yr^{-1} to a P-enriched soil and found that this quantity was not enough to speed up the depletion of bioavailable P under a cut and carry system. Similarly, Dodd et al. (2014) noted that the application of 150 or 300 kg N ha^{-1} yr^{-1} increased plant P uptake but had no significant impact on Olsen P compared to the unamended control. The higher bioavailable P stocks present in the soils investigated by McDowell et al. (2016) (Olsen P $\approx$ 40 mg kg^{-1}) and Dodd et al. (2014) (Olsen P $\approx$ 35 mg kg^{-1}) as compared to our soil (Olsen P:

10 mg kg^{-1}) could explain the lack of bioavailable P depletion in their studies. Another explanation could be the mobilization of other inorganic P fractions to replenish the available P pool (Liu et al. 2019).

Nutrient stoichiometry plays a critical role in regulating soil microbial growth and activity (Sinsabaugh et al. 2008; Maaroufi and De Long 2020). Microbial biomass P did not respond to N and P additions in this study. In their meta-analysis, Deng et al. (2017) pointed out that N addition had no effect on microbial biomass P, while Gong et al. (2020) described a decrease in microbial biomass P in response to long-term N inputs to a temperate meadow. Randall et al. (2019) found that soil microbial biomass C, N, and P were unchanged in a grassland soil under cut and carry system regardless of P applications (0, 15, 30 kg P ha^{-1} yr^{-1}). They suggested that this result was attributed to low C and N availabilities and P adsorption to clay particles. In an intensively managed grassland system, Massey et al. (2016) explained the non-response of microbial biomass P to long-term inorganic P applications (0, 15, 30, 45 kg P ha^{-1} yr^{-1}) by the similar availability of C (rhizodeposition) across P treatments due to the long-term plant biomass removal. Heterotrophic soil microbes are generally C-limited, implying that their growth and activity are chiefly regulated by C availability (energy) (Griffiths et al. 2012; Heuck et al. 2015; Spohn and Schleuss 2019). Our soil was previously under an arable cropping system characterized by low labile organic C (Haynes et al. 1991; Nguyen et al. 1995; Haynes 2000). Hence, it is possible that the low availability of C has cancelled out the effect of N and P addition on microbial biomass P due to microbial C-limitation (Xu et al. 2020). In fact, Soong et al. (2020) highlighted that microbial C-limitation is often overshadowed by plant nutrient limitation, yet it can feedback on soil nutrient cycling.

Phosphatase enzymes are released by plants and soil microbes to cleave phospho-monoester compounds into inorganic orthophosphates easily available for plant uptake (Tarafdar and Claassen 1988; Nannipieri et al. 2011). In this study, acid and alkaline phosphatase activities significantly decreased following the application of P fertilizers (October 2019, 2020). This indicated that soil phosphatase enzymes were sensitive to P availability (Marklein and Houlton 2012), which is consistent with the negative correlations ($P < 0.01$) found between acid and alkaline

Table 5 Values of Spearman correlation coefficients between soil and plant parameters, soil P fractions assessed in July 2019 and 2020 ($n = 40$)

	MBP	Olsen P	Acid P	Alk P	Soil pH	NH_4^+-N	NO_3^--N	TN	TC	NH_4Cl	$NaHCO_3$ Ino	$NaHCO_3$ Org	NaOH 1 Ino	Shoot biomass	P uptake
MBP	1	0.372*	0.115	0.255	0.316*	− 0.083	0.053	− 0.337*	− 0.155	0.324*	0.355*	0.163	0.356*	− 0.260	− 0.398*
Olsen P	0.372*	1	0.111	0.443*	0.230	− 0.025	0.111	− 0.234	0.041	0.763**	0.809**	− 0.047	0.810**	− 0.282	− 0.272
Acid P	0.115	0.111	1	0.179	− 0.128	− 0.195	0.258	0.082	− 0.111	− 0.176	− 0.210	− 0.119	− 0.056	0.211	− 0.042
Alk P	0.255	0.443*	0.179	1	0.484**	0.027	0.326*	− 0.167	0.014	0.371*	0.249	0.095	0.361*	− 0.306	− 0.516**
Soil pH	0.316*	0.230	− 0.128	0.484**	1	0.082	− 0.156	− 0.050	0.143	0.269	0.263	0.592**	0.237	− 0.664**	− 0.643**
NH_4^+-N	− 0.083	− 0.025	− 0.195	0.027	0.082	1	− 0.032	0.057	0.231	0.044	0.070	− 0.089	0.022	− 0.032	0.104
NO_3^--N	0.053	0.111	0.258	0.326*	− 0.156	− 0.032	1	− 0.238	− 0.215	0.015	− 0.120	− 0.203	0.010	0.483**	0.064
TN	− 0.337*	− 0.234	0.082	− 0.167	− 0.050	0.057	− 0.238	1	0.583**	− 0.245	− 0.212	0.213	− 0.256	− 0.006	0.115
TC	− 0.155	0.041	− 0.111	0.014	0.143	0.231	− 0.215	0.583**	1	0.056	0.050	0.279	− 0.017	− 0.171	− 0.090
NH_4Cl	0.324*	0.763**	− 0.176	0.371*	0.269	0.044	0.015	− 0.245	0.056	1	0.896**	0.236	0.824**	− 0.310	− 0.275
$NaHCO_3$ Ino	0.355*	0.809**	− 0.210	0.249	0.263	0.070	− 0.120	− 0.212	0.050	0.896**	1	0.113	0.873**	− 0.297	− 0.098
$NaHCO_3$ Org	0.163	− 0.047	− 0.119	0.095	0.592**	− 0.089	− 0.203	0.213	0.279	0.236	0.113	1	0.139	− 0.455*	− 0.571**
NaOH 1 Ino	0.356*	0.810**	− 0.056	0.361*	0.237	0.022	0.010	− 0.256	− 0.017	0.824**	0.873**	0.139	1	− 0.208	− 0.175
Shoot biomass	− 0.260	− 0.282	0.211	− 0.306	− 0.664**	− 0.032	0.483**	− 0.006	− 0.171	− 0.310	− 0.297	− 0.455*	− 0.208	1	0.787**
P uptake	− 0.398*	− 0.272	− 0.042	− 0.516**	− 0.643**	0.104	0.064	0.115	− 0.090	− 0.275	− 0.098	− 0.571**	− 0.175	0.787**	1

*Significant difference at $p < 0.05$; ** Significant difference at $p < 0.01$; MBP: microbial biomass P; Acid P: acid phosphatase activity; Alk P: alkaline phosphatase activity; TN: total nitrogen; TC: total carbon; Ino: inorganic P; Org: organic P

phosphatase activities and Olsen P concentration, confirming our first hypothesis. However, the suppression of phosphatase activities under P and N and P treatments was short-lived and was not observed throughout the growing season. This suggested that inorganic P addition inhibited newly synthesized phosphatase enzymes rather than stabilized enzymes (Spiers and McGill 1979).

Nitrogen is a dominant component of phosphatase enzymes (Olander and Vitousek 2000); thus, plant and soil microbes invest more in phosphatase enzyme production when N availability is high (Marklein and Houlton 2012). However, our results showed that acid and alkaline phosphatase activities were only significantly higher in summer under N and N and P treatments, which were coincident with peaks of plant production. Higher shoot biomass indicates higher C inputs and, in turn, higher C release into the belowground compartment (Lu et al. 2011; Schleuss et al. 2021). Based on 35 studies using C isotope tracers, Huang et al. (2020) found that N addition increased newly synthesized C derived from plant roots. A recent study showed that the addition of 550 kg N ha^{-1} (as urea) to 5 grassland plant species, including perennial ryegrass (Lolium perenne L.), increased the concentration of water-extractable C released in the soil compared to the unplanted control (Leptin et al. 2021). Hence, the higher N:P ratio and rate of rhizodeposition under N and N and P treatments could stimulate the mobilization of organic P by soil microbes via alkaline phosphatase enzymes to maintain microbial stoichiometry (Heuck et al. 2015; Spohn et al. 2015; Tian et al. 2016; Li et al. 2016). On the other hand, increased plant P demand in summer can trigger the release of acid phosphatase enzymes derived from plant roots to mineralize organic P (Tate et al. 1991; Scott and Condron 2003; Wasaki et al. 2018). This was further confirmed by the positive correlation found between acid ($P < 0.05$) and alkaline phosphatase activities ($P < 0.01$) and shoot biomass. Therefore, our study showed that short-term N addition caused an indirect increase in phosphatase activities through the stimulation of plant P demand and the release of rhizodeposits rather than directly affecting the production of phosphatase enzymes per se. This result highlights the importance of assessing seasonal changes of phosphatase activity for a better understanding of the effects of nutrient inputs on soil P dynamics.

Nutrient addition impacts on soil P fractions

Soil P fractionation data revealed that almost half of total P was in the form of moderately labile P, indicating the presence of high amounts of Al and Fe oxides in the soil of the study site. This explains why the addition of mineral P fertilizer caused an increase in both labile and moderately labile inorganic P fractions, which was in line with previous studies under P fertilized soils in New Zealand (Condron and Goh 1989; McDowell and Condron 2000; Touhami et al. 2020). Readily-available, labile, and moderately labile inorganic P as well as labile organic P were the main P fractions depleted by Italian ryegrass in this study. Readily-available inorganic P is considered a rapidly mobilizable inorganic P fraction (Rose et al. 2010), while most plant species acquire their P from labile inorganic P (Hedley et al. 1982; Gatiboni et al. 2021). Moderately labile P is abundant in New Zealand soils and is considered as the P adsorbed to Al and Fe oxides (Maher and Thorrold 1989; Perrott and Mansell 1989; McDowell and Condron 2012). In pasture systems, several studies have indicated that labile and moderately labile P could account for the majority of plant P uptake (Condron and Goh 1989; Chen et al. 2002, 2003). Indeed, in the current study, results from correlation analysis and PCA indicated that readily-available, labile, and moderately labile inorganic P fractions were negatively correlated with total shoot biomass and P uptake.

Previous investigations have shown that changes in labile P fractions as affected by N addition were partly related to plant biomass management and plant residue returns. For instance, N addition depleted recalcitrant and occluded P fractions but increased soil P availability under forest ecosystems (Sherman et al. 2006; Block et al. 2013; Fan et al. 2019). In fact, forest litterfall plays a pivotal role in recycling inorganic P, which continually replenishes the soil available P pool. On the other hand, increased plant P demand in response to N inputs and the removal of P in plant products under intensive cropping systems decreases soil available P and enhances the depletion of sparingly available P fractions (Wang et al. 2021b). Our results showed that N addition alone significantly decreased readily-available inorganic and labile inorganic P by 75 and 19%, respectively, compared to the control treatment. This was mainly attributed to the higher shoot P uptake together with the continual removal of

P in plant biomass (Perring et al. 2009; Boitt et al. 2018). Our results corroborated the findings of Wang et al. (2021b), where a significant decrease in resin and labile inorganic P fractions was observed in a crop rotation subjected to long-term N inputs in Germany. Similarly, Cui et al. (2021) described that the depletion of labile P was proportional to the level of N addition and accumulation of plant biomass in a semi-arid grassland.

Changes in moderately labile inorganic P in response to N addition have shown discrepancies between studies and seems to be related to soil chemical properties, including pH. Fan et al. (2019) found that long-term N addition to an acidic soil under forest ecosystem was able to mobilize moderately labile inorganic P by increasing the desorption of P from Al and Fe oxides due to soil acidification. Conversely, Wang et al. (2021a) noted that 11 years of urea addition to a calcareous grassland soil increased moderately labile inorganic P, which was linked to the complexation of free inorganic P by Al and Fe released from soil minerals. The soil used in this study had an acidic pH; thus depletion of moderately labile inorganic P in response to N addition is expected. Indeed, our results revealed that N treatment showed significantly higher depletion of moderately labile inorganic P compared to the control. There is increasing evidence that organic anions can contribute to the mobilization of moderately labile inorganic P via chelation, complexation, and ligan exchange processes (Wang et al. 2015, 2016; Wang and Lambers 2020). A recent study showed that N addition increased rhizodeposition, available P, and plant P uptake when wheat was grown in an acidic unfertilized grassland soil (Bicharanloo et al. 2020). He et al. (2020) revealed that under P deficiency and high N supply, alfalfa (*Medicago sativa* L.) released high amounts of carboxylates, especially tartrate. Therefore, it is suggested that the decrease in moderately labile inorganic P observed in the present study could be attributed to organic anion release rather than soil acidification because differences in soil pH between the control and N treatment were small. Nevertheless, future research needs to quantify organic anions, and their relationship to soil P fractions under N fertilized pasture systems.

Because of the low P use efficiency of P fertilizers and the need to increase and maintain plant productivity, P applications often exceed plant P requirements leading to soil P accumulation (Perrott et al. 1992; Condron 2003). Nitrogen addition has been suggested as one of the effective strategies to increase plant production and reduce soil legacy P (Perring et al. 2009; Dodd et al. 2014). Investigating a calcareous soil under meadow steep, Liu et al. (2019) found a decrease in labile and moderately labile inorganic P fractions under the combined N and P additions compared to P addition alone. Liu et al. (2019) ascribed the mobilization of these P fractions to increased plant biomass as a result of N fertilization. In the present study, the combined addition of N and P significantly decreased readily available and labile inorganic P by 39 and 26%, respectively, compared to P addition alone. Additionally, although plant biomass increased by 1.6-fold and partial P balance decreased by 28% under N and P treatment compared to P treatment, sparingly available inorganic P was similar under both treatments. The non-response of Italian ryegrass to P addition and the presence of high amounts of free Al and Fe oxides in this soil not only promoted the accumulation of P in the moderately labile inorganic P fraction but also prevented its mobilization through N fertilization in the topsoil (0–75 mm). Under four grassland soils differing in soil properties, Perring et al. (2009) showed that N applications combined with P for 5 years had no impact on the depletion of moderately labile inorganic P. They attributed this result to the high amount of P sorbed to the soil solid phase in the soils investigated. Therefore, it is suggested that when P applications exceed plant P requirements, especially under soils with high free Al and Fe oxides, short-term N fertilization can mobilize labile P pools (readily available and labile inorganic P and labile organic P) but not sparingly P fractions (moderately labile inorganic P) even under an intensively managed grass-pasture system. Therefore, N and P inputs to pasture soils needs to be tailored according to plant P requirements and the availability of free cations (free P-binding sites). Due to the short-time scale of this study and the non-response of Italian ryegrass to P addition, longer-term studies under N and P co-limited grassland soils are warranted to quantify if more recalcitrant P pools would be depleted.

It is well established that N fertilization enhances organic P mineralization in a range of ecosystems (Heuck et al. 2018; Cui et al. 2021; Wang et al. 2021b); however, there is a lack of data on different organic P

pools depleted under N fertilized pasture systems. Past research on the topic indicates that long-term N addition inhibits phosphatase enzyme activity and biological P cycling (Tian et al. 2016), while short-term N supply promotes organic P mineralization in pasture soils (Cui et al. 2021). In this short-term study, P fractionation data revealed that labile organic P was depleted by Italian ryegrass regardless of nutrient treatment in this soil. However, the extent of this depletion was 28% higher under treatments with N addition. Plants tend to mobilize organic P to meet increased P demand (Ford et al. 2016; Schleuss et al. 2020). Furthermore, external N inputs can modify the soil N:P ratio, thereby promoting higher organic P mineralisation by soil microbes to maintain stoichiometric homeostasis (Li et al. 2016; Heuck et al. 2018). Results from correlation analysis and PCA showed that NO_3^--N concentration correlated positively with total shoot biomass, while labile organic P correlated negatively with total shoot biomass and P uptake. As previously shown, shoot biomass and shoot P uptake were significantly higher by an average of 1.6-fold under N addition treatments compared to the control. These treatments also exhibited significantly higher acid and alkaline phosphatase activities in summer. Therefore, N addition in this study increased plant growth, which in turn accelerated biological P cycling via the mobilization of labile organic P through phosphatase enzymes (Marklein and Houlton 2012; Heuck et al. 2018; Schleuss et al. 2020).

Conclusions

A better understanding of N and P fertilization effects on soil P dynamics under pastoral systems is crucial to maintain pasture productivity and sustain the use of dwindling P reserves. The results of this field study showed, for the first time, that N addition alone to a biennial grass-pasture system accelerated soil P cycling by mobilizing labile and moderately labile inorganic P and mineralizing labile organic P, whereas combined addition of N and P caused an accumulation of moderately labile inorganic P. In soils where P is adsorbed to Al and Fe oxides and present in organic form, N fertilization could be a good strategy to mobilize these P pools. Nevertheless, N and P inputs need to be tailored according to plant requirements and the availability of free cations to avoid soil P build-up

under pastoral systems. Nitrogen addition indirectly increased phosphatase enzyme activity in summer by stimulating plant P demand and the release of rhizodeposits. This increase was linked to labile organic P mineralization. These findings emphasize the importance of seasonally assessing phosphatase enzyme activity to unravel the effect of nutrient inputs on soil P cycling. Due to the short time scale of this study and the non-response of Italian ryegrass to P addition, longer-term studies under N and P co-limited pasture soils need to be undertaken to quantify if more recalcitrant P pools can be depleted under N and P additions. Investigation of changes in soil P fractions with depth under pastoral systems is also warranted.

Author' contributions DT, LMC, and RWM contributed to the study conception and design. DT carried out the experiment. DT and MB collected soil and plant samples and carried out laboratory analyses. DT analyzed the data, wrote the first draft of the manuscript, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final version of the manuscript.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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