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ORIGINAL PAPER

Impacts of long-term plant biomass management on soil phosphorus under temperate grassland

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Abstract
Aims We assessed and quantified the cumulative impact of 20 years of biomass management on the nature and bioavailability of soil phosphorus (P) accumulated from antecedent fertiliser inputs.
Methods Soil (0–2.5, 2.5–5, 5–10 cm) and plant samples were taken from replicate plots in a grassland field experiment maintained for 20 years under contrasting plant biomass regimen- biomass retained or removed after mowing. Analyses included dry matter production and P uptake, root biomass, total soil carbon (C), total nitrogen (N), total P, soil P fractionation, and ^{31}P NMR spectroscopy.
Results Contemporary plant production and P uptake were over 2-fold higher for the biomass retained compared with the biomass removed regimes. Soil C, total P, soluble and labile forms of inorganic and organic soil P were significantly higher under biomass retention than removal.
Conclusions Reserves of soluble and labile inorganic P in soil were significantly depleted in response to continued long-term removal of P in plant biomass compared to retention. However, this was only sufficient to sustain plant production at half the level observed for the biomass retention after 20 years, which was partly attributed to limited mobilisation of organic P in response to P removal.

Keywords Legacy phosphorus · Phosphorus depletion · Soil phosphorus fractionation · ^{31}P nuclear magnetic resonance spectroscopy

Introduction

Managed grassland ecosystems account for 25% of all ice-free land (FAOSTAT 2011), and therefore make a significant contribution to global food supply and ecosystem services (White et al. 2000; Wright 2005). For example, dairy, meat, and wool products from grazed pasture in New Zealand make up 35–50% of total export income (Hodgson et al. 2005; Statistics New Zealand 2015), while pastoral agriculture is a significant component of many economies in North America (e.g. USA),

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South America (e.g. Brazil) and Western Europe (e.g. Ireland).

Inputs of phosphorus (P), mainly in the form of mineral fertilizers derived from phosphate rock, are essential to enhance and maintain the productivity of agroecosystems, including grasslands (Smit et al. 2009). However, most of the P applied in fertiliser is not immediately utilised or retained by plants and grazing animals and therefore accumulates in the soil as various inorganic and organic P forms of different labilities (commonly referred to as "legacy P" or "residual P") (Haynes and Williams 1993; Nash et al. 2014; Sattari et al. 2012; Stutter et al. 2012). There are also ongoing concerns and debate around addressing the adverse environmental impacts of high P inputs and the long-term future supply and cost of finite phosphate rock resources (Cordell et al. 2009; Haygarth et al. 2013; Ulrich and Frossard 2014). These issues have highlighted the need to reassess and improve the overall utilisation efficiency of P inputs in agroecosystems, including the potential to enhance mobilisation of and use of P accumulated in soil from previous inputs (Condron et al. 2013; George et al. 2016; Haygarth et al. 2013).

A field experiment was established at Lincoln University, New Zealand in 1994 to investigate and quantify the long-term impacts of contrasting grassland management regimes on above- and below-ground properties and processes (Adair et al. 2013; Farrell et al. 2014; McDowell et al. 2016; Simpson et al. 2012). The specific objective of this study was to determine the cumulative impact of 20 years of plant biomass and P conservation and depletion on the amounts, forms and associated dynamics of topsoil P. We hypothesised that significant and relatively similar depletion of soil inorganic and organic P would have occurred in response to continued removal of P in biomass, and that changes in soil organic matter and P would be more pronounced at the soil surface (0–2.5 cm) compared with lower depths (2.5–10 cm).

Materials and methods

Long-term ecology field trial (LTET)

The field trial was established in September 1994 at Lincoln University, New Zealand (S 43°38′51, E 172°28′05) on a Wakanui silt loam soil (Mottled Immature Pallic [NZ]; Udic Ustochrept [USDA]). The trial was primarily designed to investigate and quantify the impacts of a variety of grassland management strategies (no mowing, biomass retention, biomass removal), with and without annual nitrogen (N) fertilizer inputs, on soil properties and processes (Farrell et al. 2014; McDowell et al. 2016; Simpson et al. 2012). The trial site had previously been managed under arable cropping with regular inputs of mineral fertiliser (including P) and the soil P fertility status at establishment was relatively high (Olsen P = 28 mg kg^{-1} for 0–7.5 cm soil) for lowland agricultural soils in New Zealand. The site was cultivated and sown with a mixture of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), perennial ryegrass (*Lolium perenne* L.), and cocksfoot (*Dactylis glomerata* L). Treatments were established on 5 × 5 m plots arranged in randomised blocks with four replicates each. The trial included a total of 8 treatments, although this study focused on comparison between two treatments, namely mowing with biomass retained (whereby organic matter and nutrients were conserved – hereafter referred to as "biomass retained") and mowing with biomass removed (whereby organic matter and nutrients were depleted - hereafter referred to as "biomass removed") (Fig. 1). Mowing to 4 cm was carried out when the sward reached a height of approximately 20 cm, which occurred 4–6 times per year between spring (August) and autumn (April). The trial was not grazed and the selected treatments did not receive any fertiliser or irrigation inputs.

Plant sampling and analysis

Plant production and nutrient uptake were not routinely determined on the field trial. Accordingly, assessment of the impact of 20 years of contrasting biomass management on plant growth and P uptake were carried out for the spring-early summer part of the 2013–2014 growing season (August–November). This involved harvesting vegetation at ground-level using randomly placed, 50 × 50 cm quadrats in each replicate plot of the two treatments (8 plots) on three consecutive occasions (mid-August, mid-September, early November). Biomass from the three harvests from each plot were dried at 65 °C, bulked and weighed prior to digestion and P analysis (Miller 1998). Aboveground plant biomass dry matter and P uptake were expressed in units of Mg ha^{-1} and kg ha^{-1}, respectively. Root biomass assessment was carried out from soil core samples in November 2014. Duplicate 5.4 cm diameter cores were collected in each

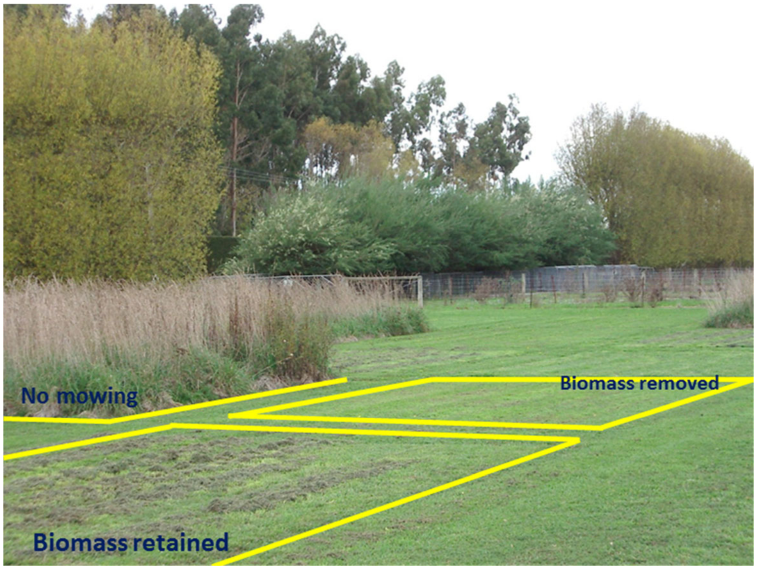

Fig. 1 The long-term ecology field trial (Lincoln University, New Zealand) showing the three main treatments, namely no mowing, mowing with biomass removed (upper right), and mowing with biomass retained (lower left)

replicate plot to 10 cm depth. The cores were divided into 0–2.5, 2.5–5, and 5–10 cm depth intervals. Roots (>1 mm) were separated from soil by washing, dried at 65 °C, and weighted. Root biomass dry matter to 10 cm was expressed in units of Mg ha^{-1}, while root biomass for each soil depth increment was expressed in units of g dm^{-3} to allow comparison between depths.

Soil sampling and analyses

Soil samples were taken from the biomass retained and removed treatments in November 2014 after 20 years of contrasting plant biomass management. Soil samples were randomly taken from the four replicate plots of each treatment by using a spade to collect a 3 cm thick × 20 cm wide slice of soil. These were then partitioned into three different depth increments of 0–2.5, 2.5–5, and 5–10 cm. Duplicate sub-samples from each plot and at each depth were bulked, dried at 65 °C, and ground to <2 mm prior to soil analyses.

Soil pH was determined in water (ratio 1:2.5; v/v), and total organic carbon (C; also referred to as total C) and total nitrogen (N) were analysed by an elemental analyser (LECO 2000 CNS Analyser, LECO, Brisbane, Australia). Soil bulk density was determined for each soil depth in order to correct the soil attributes and concentrations to units of mass per hectare. This involved collecting a known volume of soil in metal rings (5.4 cm diameter) at the respective depth, which was then dried to constant mass at 105 °C, and weighed. The quantities of total C and N in the top 10 cm were expressed in units of Mg ha^{-1} and kg ha^{-1}, respectively, while for each depth increment total C and N were expressed in units of Mg ha^{-1} cm^{-1} and kg ha^{-1} cm^{-1}, respectively, to allow comparison between depths.

Soil P fractionation involved sequential extraction of replicate samples with ammonium chloride (1 M NH_4Cl), sodium bicarbonate (0.5 M $NaHCO_3$ pH 8.5), sodium hydroxide (0.1 M NaOH), hydrochloric acid (1 M HCl), a second extraction with 0.1 M NaOH (concentrations of inorganic and organic P in this fraction were respectively summed to the first extraction with NaOH), and finally digestion with concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) (Condron et al. 1996; Olsen and Sommers 1982). Total organic P was determined as the sum of organic P extracted with $NaHCO_3$ and NaOH, and total soil P was calculated as the sum of total P in all fractions. These multiple soil P fractions were designated as "soil solution P" (NH_4Cl), "labile inorganic and organic P" ($NaHCO_3$), "moderately labile inorganic and organic P" (NaOH), "calcium-bound inorganic P" (HCl), and "highly stable P" (conc. H_2SO_4 + H_2O_2) (Cross and Schlesinger 1995). The quantities of total P, total inorganic P, total organic P and inorganic and organic P fractions in the top 10 cm were expressed in units of kg ha^{-1}, while for each depth increment these P parameters were expressed in units of kg ha^{-1} cm^{-1} to allow comparison between depths.

Equivalent data for total C, total N, total P, total organic P, and labile organic P were used to calculate the following mass ratios: C:N, C:total P, C:total organic P, N:total organic P, C: labile organic P.

In order to assess whether long-term biomass management affected the chemical nature of soil organic P, soils from each depth (0–2.5, 2.5–5, 5–10 cm) were extracted with 0.25 M NaOH +0.05 M EDTA and P species analysed using ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR) (Cade-Menun and Liu 2014). It was not possible to analyse all four replicate soil samples from each depth due to time and cost constraints associated with NMR analysis. Therefore, soil extracts from each replicate were combined using equal volumes of solution. The inorganic phosphorus present was determined in the combined extracts using molybdate blue colorimetry (Dick and Tabatabai 1977), and the total phosphorus content was measured by ICP-OES (Pierzynski 2000). Combined NaOH-EDTA extracts were prepared and analysed by ^{31}P NMR according to the procedures and protocols described in McDowell et al. (2016). The quantities of P species determined by ^{31}P NMR were expressed in units of kg ha^{-1} cm^{-1} to allow comparison between depths.

Statistical analysis

Statistical analyses of the data were carried out using GenStat 16 (GenStat 2013). One-way ANOVA was carried out to test differences in plant and soil properties between treatments (2 treatments, 3 depths and 4 replicates; total $n = 24$). For soil data (including mass ratios) where significant differences between treatments were observed, an additional two-way ANOVA was carried out to test treatment × depth interactions. If the treatment × depth interaction was not significant, then one-way ANOVA was used to test the significance of differences between treatments within each depth.

Results

After 20 years dry matter production was markedly and significantly higher under biomass retention (12.5 Mg ha^{-1}) compared with biomass removal (5.4 Mg ha^{-1}) which was also reflected in the corresponding data for above-ground P uptake (Table 1). Similarly, below-ground root biomass was significantly higher in the topmost soil layer (0–2.5 cm) under biomass retention (27.2 g dm^{-3}) compared with removal (15.7 g dm^{-3}), and while a similar trend was evident in the lower soil depths (2.5–10 cm) the differences were not significant.

Data for selected soil properties and ratios for the different treatments and depths are shown in Fig.2 and Table 2. Total C to 10 cm was significantly higher under biomass retention than removal, while corresponding differences in total N were not significant (Fig. 2). The C:N and C:total organic P ratios were significantly higher as a consequence of biomass retention compared with removal, while the opposite trend was observed for C:labile organic P. Differences in the other mass ratios between treatments were not significant. Data presented in Table 2 revealed that soil bulk density and pH were not affected by long-term biomass management. However, total C was consistently and significantly higher under biomass retention than removal in all soil depths, although there was no significant interaction between treatment and depth. The magnitude of these differences was similar for each depth, whereby total C was 17–26% higher as a result of biomass retention. Similarly, for the 0–2.5 cm soil all mass ratios except C:labile organic P were significantly higher under the biomass retention regime, while similar differences were found for C:N and C:organic P in the 5–10 cm soil depth. On the other hand, C:labile organic P was significantly lower under biomass retention in the 2.5–5 cm soil.

Soil P data are presented in Figs. 3 and 4. Figure 3 shows the quantities of total and extracted soil P fractions determined for the 0–10 cm soils. Total P and total inorganic P were significantly higher under biomass retention compared with removal, while there was no difference in total organic P. For the P fractions, quantities of soil solution, labile and moderately labile inorganic P were consistently and significant higher as a consequence of biomass retention compared with removal, while labile organic P was also significantly higher under biomass retention. Within the inorganic P fractions, the relative magnitude of the difference between biomass retention and removal was greater for soil solution inorganic P (2.7-fold) compared with labile inorganic P (2-fold), and moderately labile inorganic P (1.4-fold). There were no significant differences between biomass treatments for moderately labile organic P, calcium-bound inorganic P, and highly stable P. Figure 4 shows data for the different depths for total P, total inorganic P, total organic P, soil solution inorganic P, labile inorganic and organic P and moderately labile inorganic and organic P. These data confirmed that

Table 1 Mean data for above-ground dry matter production and P uptake determined between August and November 2014 for the biomass retained and biomass removed treatments, together with below-ground biomass data for 0–10 cm soil determined in November 2014

Above-ground	Dry matter (Mg ha^{-1})		P uptake (kg P ha^{-1})	
Biomass retained	12.5		5.9	
Biomass removed	5.4		2.2	
	*		*	
Below-ground	Dry Matter (g dm^{-3})			0-10 cm (Mg ha^{-1})
	0-2.5 cm	2.5-5 cm	5-10 cm	
Biomass retained	27.2	3.4	1.5	8.4
Biomass removed	15.7	2.5	1.3	5.2
	*	*ns*	*ns*	*

One-way ANOVA summary: *n* = 4; LSD = least significant difference set at 5% probability; Asterisk indicate significant statistical differences associated to treatments in each depth (columns), degrees of freedom (treatments within depth) total = 7; *ns* = not significant

quantities of total P and total inorganic P were significantly higher under biomass retention, except for total P in the 0–2.5 cm soil. Soil solution inorganic P, labile inorganic and organic P, and moderately labile inorganic P were significantly higher under biomass retention at all soil depths. Significant interactions were determined

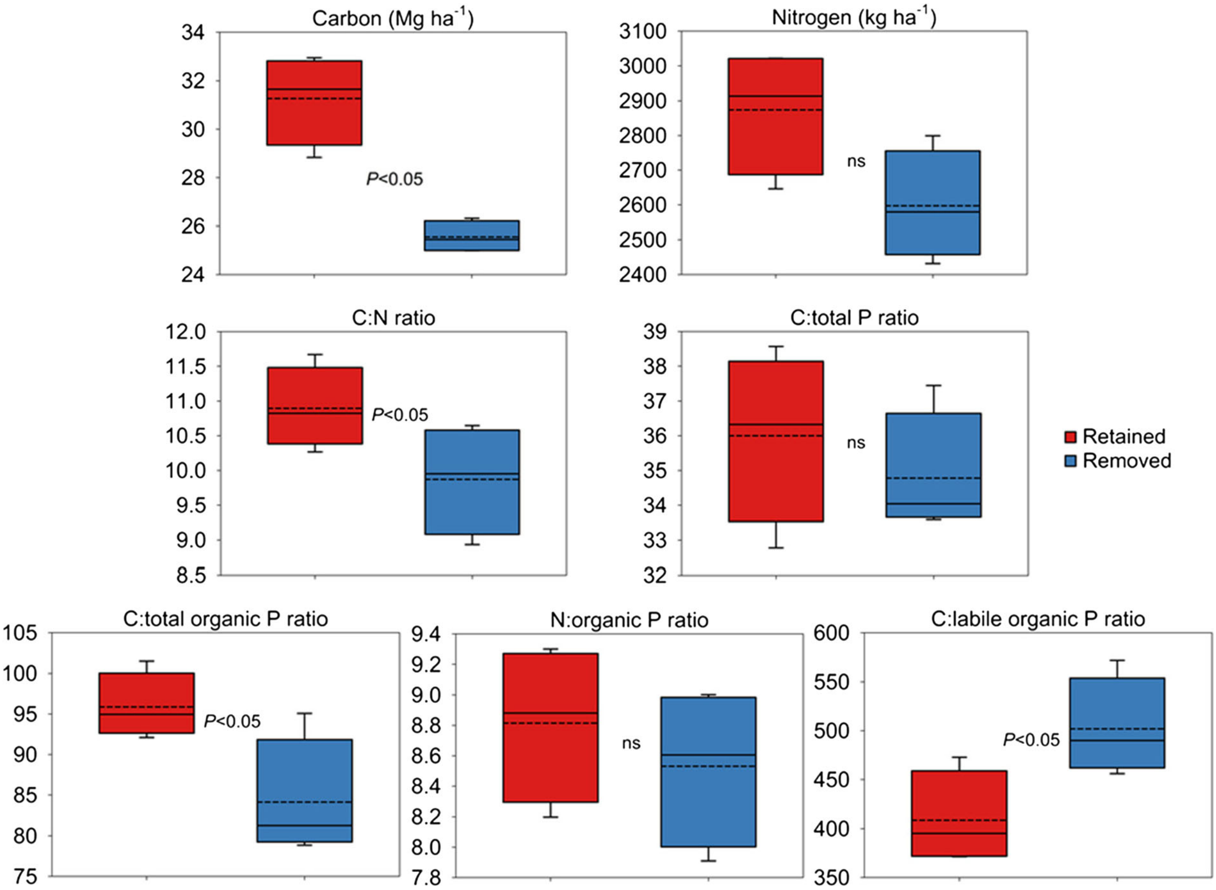

Fig. 2 Amounts of carbon, nitrogen and various component mass ratios determined for the 0–10 cm soil layer after 20 years of aboveground biomass retention compared to removal, in a long-term field experiment under temperate grassland at Lincoln, New Zealand. Continuous line within the box is the median (50th percentile), whereas the dashed line is the mean. Note that the scales are different. $P < 0.05$ indicates significant difference at 5% level, while ns = not significant

Table 2 Mean data ($n = 4$) for bulk density, pH, total C, total N, and various component mass ratios determined for soils taken after 20 years of biomass retention and removal

Soil attribute	Plant biomass management	Depth (cm)			Treat. × Depth[§]
		0–2.5	2.5–5.0	5.0–10	
Soil bulk density (g cm^{-3})	Retained	0.7	0.8	1.1	-
	Removed	0.8	0.9	1.1	
		ns	*ns*	*ns*	
pH	Retained	5.6	5.4	5.5	-
	Removed	5.7	5.4	5.6	
		ns	*ns*	*ns*	
Carbon (Mg ha^{-1} cm^{-1})	Retained	3.4	3.5	2.8	*ns*
	Removed	2.7	3.0	2.3	
		*	*	*	
Nitrogen (kg ha^{-1} cm^{-1})	Retained	299	324	264	-
	Removed	276	295	234	
		ns	*ns*	*ns*	
C:N	Retained	11.4	10.8	10.7	-
	Removed	9.9	10.1	9.7	
		*	*ns*	*	
C:total P	Retained	49.0	40.3	29.3	-
	Removed	40.8	37.6	30.6	
		*	*ns*	*ns*	
C:total organic P	Retained	127	103	80.7	-
	Removed	101	95.4	71.6	
		*	*ns*	*	
N:organic P	Retained	11.2	9.5	7.6	-
	Removed	10.2	9.5	7.4	
		*	*ns*	*ns*	
C:labile organic P	Retained	746	436	315	-
	Removed	714	539	413	
		ns	*	*ns*	

One-way ANOVA was carried out to test differences on the overall treatment effect (2 treatments, 3 depths, 4 replicates; total n = 24). [§] In case of presence of significant differences between treatment means, an additional two-way ANOVA was carried out to test the treatment × depth interaction. Significance was set to 5% probability, where $P < 0.05 = P$-values are shown; otherwise, *ns* = not significant is shown. In the absence of treatment × depth interaction, the statistical analysis was complemented by one-way ANOVA testing differences between treatments within depths separately, where asterisks indicate $P < 0.05$

between treatment and depth for total P, total inorganic P, soil solution inorganic P, labile organic P and moderately labile inorganic P (Table S1 and Table S2).

Extractable P forms determined by ^{31}P NMR analysis of soils to 10 cm is shown in Table 3. As expected, orthophosphate inorganic P and monoester organic P were the predominant P forms present, with trace quantities of diester organic P and pyrophosphate P detected. Amounts of orthophosphate and monoester P were higher under biomass retention compared with biomass removal, which is consistent with the soil P fractionation results described above. However, in the 0–2.5 cm soil the relative proportion of extracted P present as orthophosphate was higher for biomass removed (78%) than retained (69%), while the opposite trend was evident for

Fig. 3 Amounts of total P, total inorganic P (Pi), total organic P (Po) and various P pools (kg P ha^{-1}) quantified for the topsoil (0-10 cm depth) after over 20 years of aboveground biomass retention compared to removal, in a long-term field experiment under temperate grassland at Lincoln, New Zealand. Continuous line within the box is the median (50th percentile), whereas the dashed line is the mean. Note that the scales are different. $P<0.05$ indicates significant difference at 5% level, while *ns* = not significant ▶

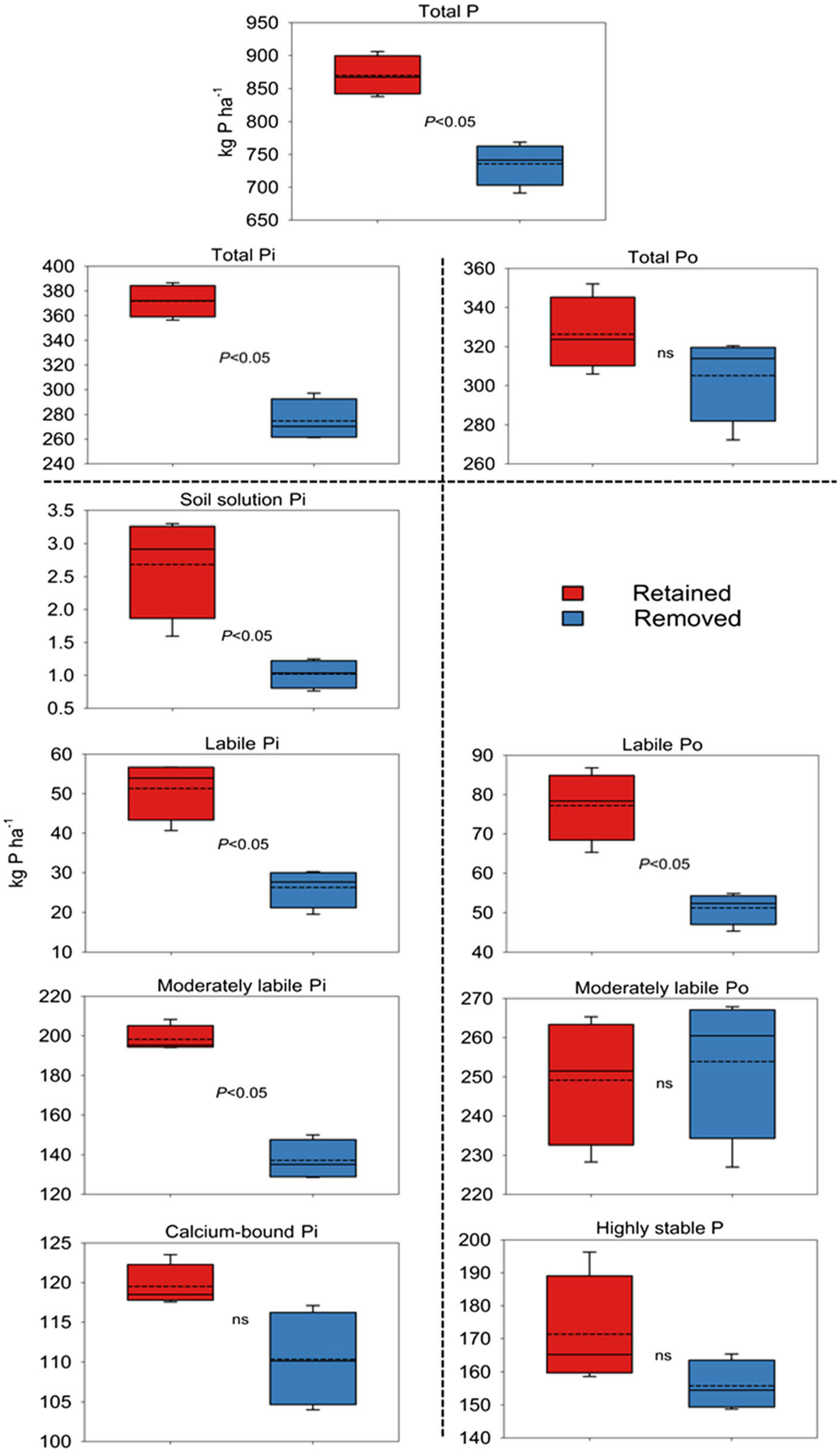
Total P
kg P ha^{-1}
950
900
850
800
750
700
650
P<0.05
Total Pi
400
380
360
340
320
300
280
260
240
P<0.05
Total Po
360
340
320
300
280
260
ns
Soil solution Pi
3.5
3.0
2.5
2.0
1.5
1.0
0.5
P<0.05
Retained
Removed
Labile Pi
kg P ha^{-1}
60
50
40
30
20
10
P<0.05
Labile Po
90
80
70
60
50
40
P<0.05
Moderately labile Pi
220
200
180
160
140
120
P<0.05
Moderately labile Po
270
260
250
240
230
220
ns
Calcium-bound Pi
125
120
115
110
105
100
ns
Highly stable P
200
190
180
170
160
150
140
ns

Fig. 4 Amounts of total P, total inorganic P (Pi), total organic P (Po) and various P pools (normalised to constant depth increment, kg P ha^{-1} cm^{-1}) quantified for different depths (0–2.5, 2.5–5 and 5–10 cm) in soils under contrasting long-term aboveground biomass management (retention versus removal) at Lincoln, New Zealand. Note that the scales are different. Line within the box is the median (50th percentile). Dots indicate data points that fall outside the 1.5 × the interquartile range

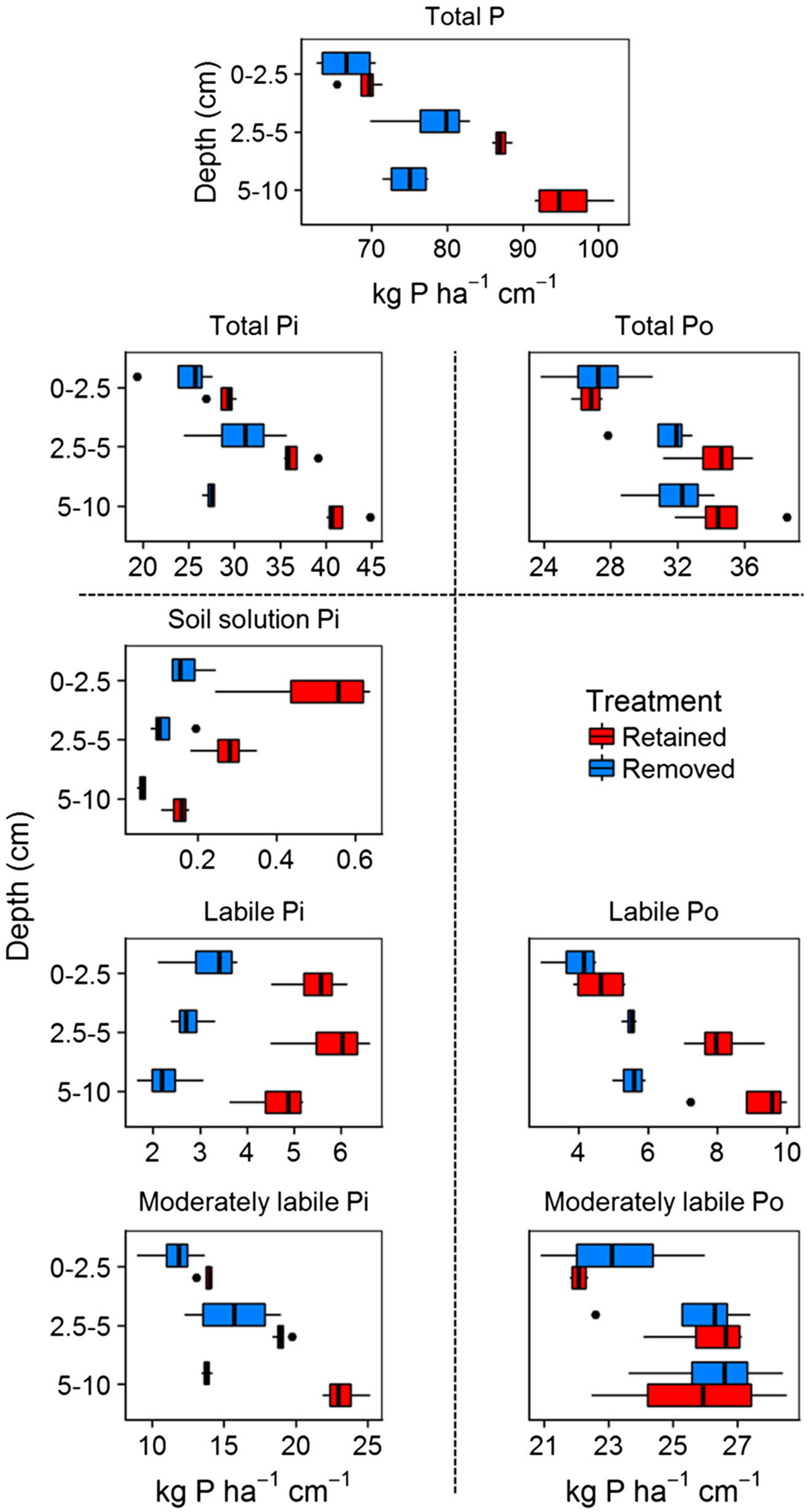

Table 3 Extractable soil P forms (kg ha^{-1} cm^{-1}) identified by ^{31}P NMR spectroscopy analysis for combined soil replicates at different depths (0–2.5, 2.5–5 and 5–10 cm) sampled after 20 years of biomass retention and removal

Plant biomass management	NaOH-EDTA-P	Orthophosphate [6.5][b]	Monoesters [3 to 6]	Diesters [2 to −1]	Pyro-phosphate [−3 to −6]
0–2.5 cm					
Retained	46.8 (44)[a]	34.2 (69)[c]	13.7 (29)	0.1 (0.1)	0.7 (2)
Removed	44.0 (34)	32.4 (78)	8.8 (20)	–	0.7 (1)
2.5–5 cm					
Retained	61.6 (45)	37.8 (61)	24.2 (39)	–	0.4 (1)
Removed	51.8 (32)	30.9 (60)	20.0 (39)	–	0.9 (2)
5–10 cm					
Retained	57.7 (45)	35.6 (62)	22.1 (38)	–	0.7 (1)
Removed	43.6 (29)	26.4 (61)	17.2 (39)	–	–

[a] Percentage of the NaOH-EDTA-P extract present as inorganic P determined by colorimetry

[b] Chemical shift (δ ppm)

[c] Proportion of the NaOH-EDTA-P detected as P species by ^{31}P NMR

monoester P (20% and 29% for the removed and retained treatments, respectively). The corresponding relative proportions of orthophosphate and monoester P were similar between treatments in the 2.5–5 cm and 5–10 cm soils.

Discussion

The above- and below-ground plant data clearly demonstrated that current dry matter production and root biomass had been markedly reduced by 38–57% as a consequence of continued biomass removal over 20 years, while seasonal plant P uptake had been reduced by 63%. The latter in particular indicated that the plant availability of soil P had been dramatically lowered by cumulative removal of P in biomass over 20 years. This was confirmed by the corresponding relative differences determined for various soil inorganic P fractions under the contrasting biomass management regimes. Thus quantities of solution, labile, and moderately labile inorganic P were reduced by 63, 49, and 31%, respectively, as a consequence of long-term biomass P removal (Fig. 5). These data are consistent with changes in soil inorganic P fractions determined over time in archived 0–7.5 cm soils taken from the biomass removed treatment (McDowell et al. 2016). The declining proportional decreases with reducing relative solubility (i.e. solution > labile > moderately labile), together with the absence of any concomitant differences in the calcium bound and highly stable P

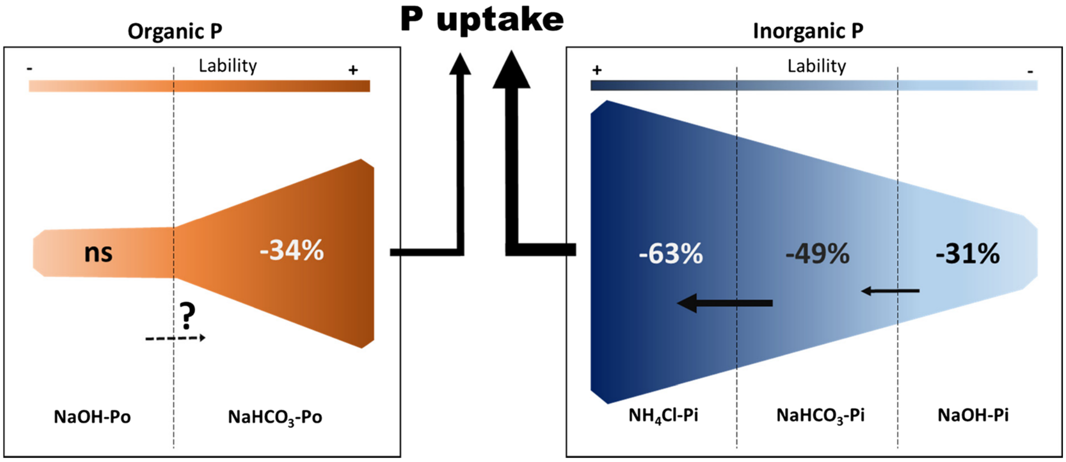

Fig. 5 Relative difference in selected soil inorganic and organic P fractions (0–10 cm) determined after 20 years of aboveground biomass/P removal versus biomass/P retention

pools, provides empirical confirmation of the long-term relative bioavailability of these soil P pools, which in turn validates the use of sequential soil P fractionation in biogeochemical studies (Chen et al. 2003; Condron and Newman 2011; Cross and Schlesinger 1995; Negassa and Leinweber 2009; Yang and Post 2011). These findings clearly demonstrate that soluble and readily available inorganic P reserves accumulated from previous fertiliser inputs were at least partly utilised by plants in response to cessation of P inputs and continued removal of P in biomass over 20 years. However, this mobilisation of soil inorganic P was only sufficient to maintain plant production and P uptake at 37–45% compared with biomass retention, while there was no significant depletion of more stable forms of inorganic P. This in turn may reflect the relative capacity of the plant species present and their associated flora and fauna to mobilise stable/recalcitrant forms of soil inorganic and organic P (Clarholm et al. 2015; Condron and Newman 2011).

In addition to differences in soil inorganic P, the fact that labile soil organic P was 34% lower under biomass removal compared with retention (Fig. 5) could be attributed to either enhanced immobilisation as a consequence of continued biomass retention or enhanced mineralisation of organic P in this pool in response to depletion of soil P by long-term P removal in biomass.

With regard to the possibility of increased P immobilisation under biomass retention, the regular and sustained cutting and return of grassland biomass C to the soil could have resulted in enhanced net conversion of inorganic to organic P in the soil (Hopkins and Dungait 2010; Magid et al. 1996). This is consistent with the fact that after 20 years total organic C was 17–26% higher in all three soil layers to 10 cm under biomass retention compared with removal. However, the C:total organic P ratios were higher under biomass retention in all 3 depths (significant for the 0–2.5 cm and 5–10 cm soils). This is not consistent with enhanced immobilisation of inorganic P, as the C:total organic P ratios would have been similar if this was the case. This reflects the fact that relative differences in soil C between the biomass treatments were markedly greater than the corresponding non-significant differences in total organic P (−2 to +10%). The absence of any significant differences in the predominant moderately labile soil organic P pool, which accounts for 76–83% of total soil organic P compared with 17–24% for the labile pool, does not indicate that enhanced immobilisation of P occurred in response to continued biomass retention over 20 years. Furthermore, C:N ratios determined in the different soil depths were similarly higher under biomass retention (10.7–11.4) than removal (9.7–10.1) compared with total C. These various differences indicate that there was a shift towards more nutrient depleted soil organic matter in response to increased organic matter inputs under biomass retention (Condron et al. 2010; Horwath 2017; McGill and Cole 1981; Tipping et al. 2016), especially in the top 2.5 cm soil which reflects the continued mowing and return of plant biomass to the soil surface.

With regard to the possibility of increased organic P mineralisation under biomass removal, with particular reference to the significant differences in labile organic P observed between the biomass management treatments. While the C:labile organic P ratio in the 0–2.5 cm soil was lower under biomass removal than retention, the opposite trend was evident in the lower soil depths (significant for the 2.5–5 cm soil), while the corresponding ratio for the combined 0–10 cm soil was significantly higher under biomass removal (502) compared with retention (409). These trends may be attributed to differences in the relative magnitude of changes in total C and labile organic P between treatments at different depths. Thus differences in total C and labile organic P in the 0–2.5 cm soil were similar (26 and 17%, respectively), whereas differences in labile organic P were substantially higher than total C in the 2.5–5 cm (17 and 47%, respectively) and 5–10 cm (22 and 65%, respectively) soils. This means that there was less labile organic P relative to organic carbon in the soils maintained under biomass removal after 20 years, which in turn could be attributed to enhanced mineralisation of labile organic P in response to continued depletion of soil inorganic P. Other studies conducted in temperate grasslands have demonstrated that labile soil organic P can make a significant contribution to short-term plant P requirements (Perrott et al. 1990; Scott and Condron 2003; Tate et al. 1991). On the other hand, the absence of any significant change in moderately labile organic P in response to soil inorganic P depletion was not consistent with results from P depletion studies related to land-use changes in plant species and associated flora and fauna (Chen et al. 2000; Condron et al. 1996; Hedley et al. 1982; Richter et al. 2006).

The ^{31}P NMR data showed that almost all of the extractable organic P in the LTET soils was present as monoesters with little or no diesters detected. This is consistent with results reported by McDowell et al. (2016) for archived 0–7.5 cm soils taken from the LTET, but is not consistent with data from temperate grazed pasture soils (Condron et al. 1985; Magid et al. 1996).

Conclusions

The findings of this study revealed that plant production and P uptake were 45 and 63% lower, respectively, as a result of 20 years of biomass and P removal in the absence of P inputs. Consequently quantities of soluble and labile inorganic P were 31–63% lower in soil maintained under biomass removal than retention. Corresponding differences in organic C and organic P in the different soil depths to 10 cm indicated that significant mineralisation of labile organic P had occurred in response to continued P removal, while there was no change in the main moderately labile organic P pool. Changes in soil inorganic and organic P that occurred in response to long-term P removal highlight limitations in the mobilisation and reutilisation of legacy or residual P accumulated in soil from previous fertiliser P inputs, especially for organic P and recalcitrant inorganic P. This in turn suggests that enhanced utilisation of legacy P may require changes in plant species/varieties and/or management, including the use of green manure/cover crops and microbial innoculants (Calabi-Floody et al. 2017; Haygarth et al. 2013; Richardson et al. 2011; Simpson et al. 2014).

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