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EDITED BY
Vijay Singh Meena,
CIMMYT-Borlaug Institute for South Asia (BISA), India

REVIEWED BY
Tapan Kumar Adhya,
KIIT University, India
Manish Kumar Vishwakarma,
Borlaug Institute for South Asia (BISA), India

*CORRESPONDENCE
Arne Sæbø
✉ arne.sabo@nibio.no

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N fertilization strategies for the use of P-rich organic amendments in the restoration of soil productivity—short-term responses in two soils

Arne Sæbø[1]*, Tomas Persson[1], Peter Schröder[2] and Hans Martin Hanslin[1]

[1]Norwegian Institute of Bioeconomy Research (NIBIO), Division of Food and Society, Ås, Norway, [2]Helmholz Zentrum München, German Research Center, Department Experimental Environmental Simulation, Neuherberg, Germany

To facilitate nutrient management and the use of manure as a feedstock for biogas production, manure is often separated into a solid and a liquid fraction. The former fraction is usually high in P and low in N, so when incorporated in the soil as fertilizer, it needs to be supplemented by N from, e.g., mineral fertilizers or nitrogen-fixing species. To explore strategies to manage N with solid-separated manure, we examined how the amount of digestate and the N:P ratio of pig digestate, i.e., manure that had partially undergone anaerobic digestion, affected the productivity of Westerwolds ryegrass and red clover in a pot experiment with one soil which was rich and another which was poor in plant nutrients. The soil and plant species treatments were combined with four doses of digestate, which gave plant available phosphorus (P) concentrations of 2, 4, 8, or 16 mg P100g^{-1} soil. Ammonium nitrate was dosed to obtain factorial combinations of digestate amount and N:P ratios of 1.8, 4, 8, and 16. Clover was harvested once at the beginning of flowering (15 weeks after seeding), while Westerwolds ryegrass was allowed to regrow three times after being cut at the shooting stage (in total, 4 cuts, 6, 9, 12, and 15 weeks after seeding). Ryegrass yield increased by up to 2.9 times with digestate dosage. Interactions with the N:P ratio and soil type were weak. Hence, the effect of increasing the N:P ratio was additive across digestate dosages. Red clover biomass also increased by up to 39% with digestate dosage. Residual nutrients in the soil after red clover cultivation were affected by the initial differences in soil characteristics but not by digestate treatment or biomass of harvested red clover. A targeted N management is required to benefit from the P-rich digestate in grass cultivation, while the long-term effects of red clover culture on N input need further investigation.

KEYWORDS

digestate, nitrogen, phosphorus, organic soil amendments, N:P ratio, soil productivity

1 Introduction

There is an increasing urge for agricultural production methods to decrease dependency on mineral fertilizers (Lessmann et al., 2023). Organic soil amendments can be an alternative since they contribute to increases in crop yield, long-term build-up of soil organic matter (SOM) (Chen et al., 2018), and microbial biomass (Li et al., 2024). Hence, manure application is often a long-term strategy to obtain high soil fertility (Putelat and Whitmore, 2023). In regions with high livestock density, large quantities of manure are available, and research is performed to find economically feasible and ecologically acceptable methods for treatments and use of manure (Lessmann et al., 2023). This is necessary since the composition of nutrients in manure and other soil amendments often does not meet the nutrient demand of crops. Especially the ratio between nitrogen (N) and phosphorus (P) is lower in animal manure than what is optimal for plant growth (Zhang et al., 2003). If the application of manure is repeatedly based only on N-content, excess P will reach the soils, leading to run-off and leaching, which may cause eutrophication of watersheds (Zhang et al., 2013). To increase N supply, applications of manure can be complemented with mineral nitrogen, which can easily be adjusted to plant demand and is easy to handle. However, the production of mineral fertilizers entails the use of large amounts of fossil fuel (Hasler et al., 2015). N fixation by legumes, grown either as preceding crops or grown together with N-demanding crops, is arguably a more sustainable alternative (Kebede, 2021).

The construction and operation of facilities that anaerobically digest organic materials to produce biogas to replace energy from fossil sources has increased during the last few years (Nguyen and Hermansen, 2015). In addition to biogas, a digestate, a by-product, to which most of the plant nutrients are allocated, is generated from the anaerobic digestion process. However, the nutrient levels and their plant availability in digestate and manure vary a lot, depending on the composition of parent materials and the treatment of manure and digestate in the biogas facilities (Samoraj et al., 2022). After solid/liquid phase separation, the solid fraction is rich in P and the liquid fraction has a higher N concentration (Möller and Müller, 2012) because the digestion process decreases the carbon content of the material and, as a result, its nitrogen concentration increases (Tambone et al., 2010). However, nitrogen losses from manure storage (Kupper et al., 2020; Møller et al., 2022) and treatment (Qu et al., 2022) can be as high as 50% of the excreted N.

Our hypothesis was that plant responses to digestate, used as soil amendments and fertilizers, will depend on the soil type and on the N:P ratio of both the soil and the amendments. The aim of this study was to determine how the N:P ratio of separated solid phase digestate affects responses to P dosage supplied by the same digestate phase in plants with high (Westerwolds ryegrass) or low (red clover) N demand.

2 Materials and methods

We established a pot experiment under controlled greenhouse conditions at NIBIO Særheim Research Station (Latitude 58.76053, Longitude 5.65078) to test the combined effect of soil N:P ratios and digestate on Westerwolds ryegrass and red clover productivity. Treatments included combinations of three levels of applied P, 4 N:P ratios, in addition to unfertilized and unamended controls, and 2 soils in the Westerwolds ryegrass part of the experiment. The treatments of the red clover plants included three levels of applied P, 2 N:P ratios, in addition to 8 units without digestate or fertilizers, and 2 soils. All treatments were replicated 4 times. In total, there were 104 Westerwolds ryegrass units and 56 red clover units (see below for further details).

The first soil was a fine sand classified as an Arenosol (IUSS Working Group WRB, 2015), which was dug up from the tilled layer (0–25 cm) of an agricultural field originating from fluvial and alluvial processes at the seashore of southwestern Norway. The second soil, a coarse sand of moraine origin with no history of cultivation, classified as an Anthropic Regosol (IUSS Working Group WRB, 2015), was extracted from a sand quarry in the same region. Soil characteristics are given in Table 1. The fine sandy soil had about 10 times more plant available P, 11 times more total N, and almost double the amount of soil organic matter than that of the coarse sand (Table 1).

Digestate was obtained from an anaerobic digestion biogas system. In this system, pig manure was only partially subjected to the digestion process since only the washed-out energy-rich substances from a percolation process were cycled through the anaerobic digestion tank. The fluid from the digestion tank was continuously returned and used as percolation fluid, washing through the manure several times. Even though the manure was only partially digested, we, for simplicity, refer to it as digestate. After approximately 1 month of percolation, the masses were composted for 60 days, where temperatures reached a range between 60 and 70°C and subsequently started to decrease during the termination phase of the composting. The digestate had a pH of 6.4, a total content of 5.4 g N, 3.0 g P, 1.5 g K, and 4.6 g Mg per kg, and a C/N ratio of 23.0. The temperature in the greenhouse was set to 18°C under the natural day length (long day) from the start of the experiment (28 May) to its termination in September of the same year.

Soil mixtures with 3 levels of digestate were established by mixing components in a concrete blender for 2 min. These mixtures were added to 2 L pots, placed on individual trays, and randomly allocated to treatments and positions on a greenhouse table. Digestate levels were chosen to give plant available P (ammonium lactate–acetate extractable P, P-AL, Egnér et al., 1960) of 2, 4, or 6 mg/100 g soil. These pots were seeded with the tetraploid Westerwolds ryegrass (*Lolium multiflorum* var. *westerwoldicum* Wittm) cv. Bartigra (5 plants per pot) or *Trifolium pratense* L. cv. Lea (5 plants per pot). Ammonium nitrate solutions were injected 4 cm below the soil surface with a syringe 1 week after germination to give N:P ratios of 1.8, 4, 8, or 16 for digestate and ammonium nitrate combined. Red clover only received the N:P ratios of 1.8 and 4. All pots with red clover, including pots unamended with chemical fertilizers, were inoculated with a field-collected red clover rhizosphere soil slurry (1:1 soil to water: 10 mL) after germination. The inoculation of Rhizobia bacteria was confirmed by investigating roots in a limited number of supplemental pots at

TABLE 1 Chemical characteristics of the fine and coarse sandy soils used in the experiment.

	Unit	Anthropic Regosol	Arenosol
pH		6.0	6.4
P-AL	mg/100 g	1.3	12
K-AL	mg/100 g	3.6	0.69
Mg-AL	mg/100 g	5.1	2
Ca-AL	mg/100 g	39	93
Kjeldahl-N	mg/100 g	11	125
Loss on ignition	%TS	1.2	2.2

P, K, Mg, and Ca were quantified as ammonium lactate–acetate extractable nutrients.

harvest. Inoculation was effective, with many relatively small nodules distributed over large parts of the root system.

The Westerwolds ryegrass was cut at the shooting stage with a cutting height of 3 cm at 4 consecutive times in total over the experimental period, 6, 9, 12, and 15 weeks after seeding. The cut material was dried at 60°C for at least 48 h and weighed. The red clover plants were harvested once, at the beginning of flowering (15 weeks after sowing), dried, and weighed as above. Moreover, to test for residual nutrients in the soil after harvest of the red-clover culture, pots with soil were frozen for 1 week at −20°C after harvest and thawed to promote the release of nutrients from the roots. Westerwolds ryegrass was seeded at the same rate as above and cultivated until harvest at the beginning of the first shooting. The harvested material was dried and weighed as described above.

2.1 Statistical analysis

Effects of treatments on accumulated biomass production were analyzed with a full factorial three-way ANOVA model using Minitab 20.2 (Minitab Ltd., Coventry, United Kingdom) for the Westerwolds ryegrass and red clover datasets separately. Model diagnostics were performed with normal probability plots and plots of residuals vs. fitted values. To further investigate the temporal patterns, accumulated biomass was also modeled in a mixed effect model with soil, digestate dosage, and N:P ratio as fixed factors and time and pot as random. Both intercept and slope were allowed to differ between pots. These effects were modeled using the lmer function of the lme4 package in R 3.4.2 (R Core Team, 2017). Even though pots did not contribute to much of the explained variation, this mixed effect model gave a better fit to the data than a simple linear model AIC (1,694 vs. 1,497), BIC (1773 vs. 1,589), and log-likelihood (−830 vs. -728).

3 Results

Digestate dosage and N:P ratio had strong effects on the cumulative biomass yield of Westerwolds ryegrass (Table 2; Figures 1, 2). Averaged across N:P ratio treatments, accumulated biomass was 1.6–1.9 and 2.4–2.9 times higher for the 13 and 20 g/kg than for the 7 g/kg digestate levels for the two soils. Comparing the effects of N:P ratios, accumulated biomass increased by a factor of 1.3, 1.6, and 1.8 across soils when going from the original N:P ratio of 1.8 to a ratio of 4, 8, and 16. Although accumulated yield increased slightly more with the N:P ratio at higher dosages for both soils (dose x N:P interaction, Table 2; Figure 1) and the effects of both dose and N:P ratio depended on soil type (Table 2; Figure 1), these interactions were weak giving a close to a linear increase in accumulated biomass from the four harvests with increasing amounts of digestate. The main effect of soil type was low, where the nutrient-rich fine sandy soil, on average, gave only 11.2% more yield than the coarse and nutrient-poor sandy soil.

Treatment effects varied throughout the experiment (Figure 2). The initial differences between soils disappeared after the first harvest. The interaction plot of P dose by N:P ratio over the harvests clearly indicated a dynamic of the available nutrients, going from a surplus and partly saturation at the first harvest to a deficit and stronger effect of dose and N:P ratio at the final harvest (Figure 3; Table 3).

Red clover biomass increased with digestate dosage and was 1.2 times higher in fine sandy soil than in coarse sandy soil across treatments but was not affected by the N:P ratio (Table 2; Figure 4). The response to dosage showed a threshold where the increase in red clover biomass increased by 39% in coarse sand and 19% in fine sand when increasing from digestate dose 1 to 2 and leveled off at intermediate dosage of digestate (doses 13 and 20 g/kg, both with significantly higher effect than 7 g/kg). The initial effects of soil type (without digestate) were considerably larger in red clover than in Westerwolds ryegrass (Figure 1 vs. Figure 4), pointing to an N limitation in the grass.

The residual nutrients after the red clover culture gave a higher biomass of Westerwolds ryegrass in Arenosol soil than in the Anthropic Regosol soil, with a difference of 59% between the soils (Figure 5). A linear model including red clover biomass as a covariate showed that only the original soil had an effect ($p < 0.001$), while dose, N:P ratio, and red clover biomass all were non-significant ($p > 0.4$). Hence, the residual effects of the digestate were driven by initial differences in soil characteristics and were not affected by pre-cultivation with red clover (Table 4).

4 Discussion

We found that the yield of Westerwolds ryegrass increased with the N:P ratio for all dosages of digestate and that the effect of high N:P ratios increased slightly with dosage for both soils. As expected, red clover showed no response to the N:P ratio, but biomass production increased with digestate dosage. Residual effects of red clover on a

TABLE 2 Analysis of variance of the experiments with Westerwolds ryegrass (WR) and Red clover (RC): R^2 adj. = 96, 58, and 45%, respectively.

		WR		RC		WR with RC as preceding crop	
Source	DF	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Soil	1	49,65	0,000	27,07	0,000	27,63	0,000
Dose	2	1,691,42	0,000	18,91	0,000	2,70	0,087
N:P	3	351,05	0,000	0,35	0,557	0,41	0,530
Soil*Dose	2	5,41	0,005	0,73	0,490	0,51	0,608
Soil*N:P	3	2,46	0,064	0,0	0,964	4,11	0,054
Dose*N:P	6	23,66	0,000	1,90	0,164	0,24	0,786
Soil*Dose*N:P	6	3,26	0,005	2,90	0,068	0,05	0,955

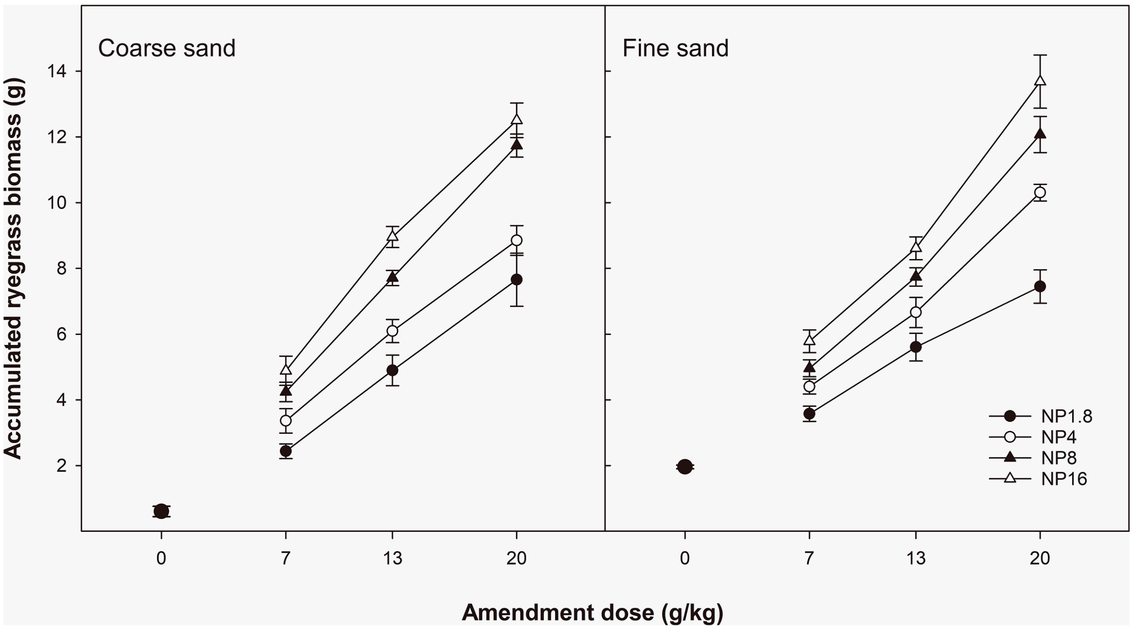

FIGURE 1
The responses of accumulated biomass of Westerwolds Ryegrass cv. Bartigra (means with 95% confidence interval) to increasing amounts of phosphorus to sandy soils (Coarse sand: Anthropic Regosol and Fine sand: Arenosol) at four N:P ratios.

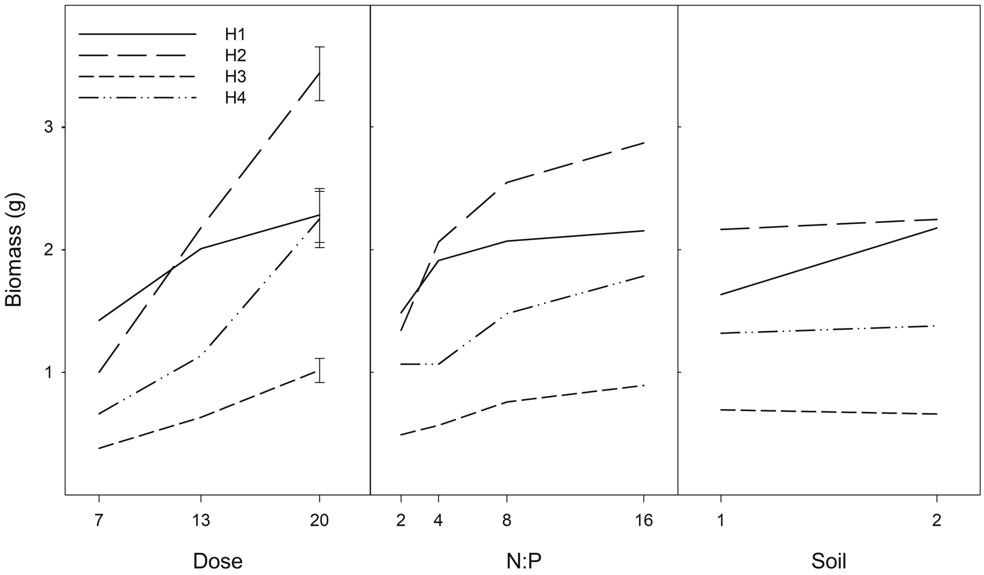

FIGURE 2
Estimated main effects of phosphorus dose, NP ratio, and soil on Westerwolds ryegrass yield over the four harvests (H1–H4). Error bars represent the estimated 95% confidence interval across treatments.

following ryegrass rotation were however marginal and were primarily dependent on original soil nutrient content.

The increase in Westerwolds ryegrass yield with an increase in N:P demonstrated the need for and effect of mineralized nitrogen. At a lower C:N ratio than that was used in our study, nutrients may be immobilized by microbes. Still, the increase in yield in both Westerwolds ryegrass and red clover in response to digestate dose shows that the nitrogen supply was not critically low in our experimental setup. It is well known that mineralization and release of N to plant growth are pronounced over a long time, even several years (Schröder, 2005; Webb et al., 2013; and references therein). In addition, the small differences in the effects of P dose and N:P ratio between the two soils in our experiment could possibly have been larger if the experiment had gone on for a longer period, given the large differences in N turnover between soils (Chen et al., 2014). The short-term responses that we found are nevertheless important since the establishment and the

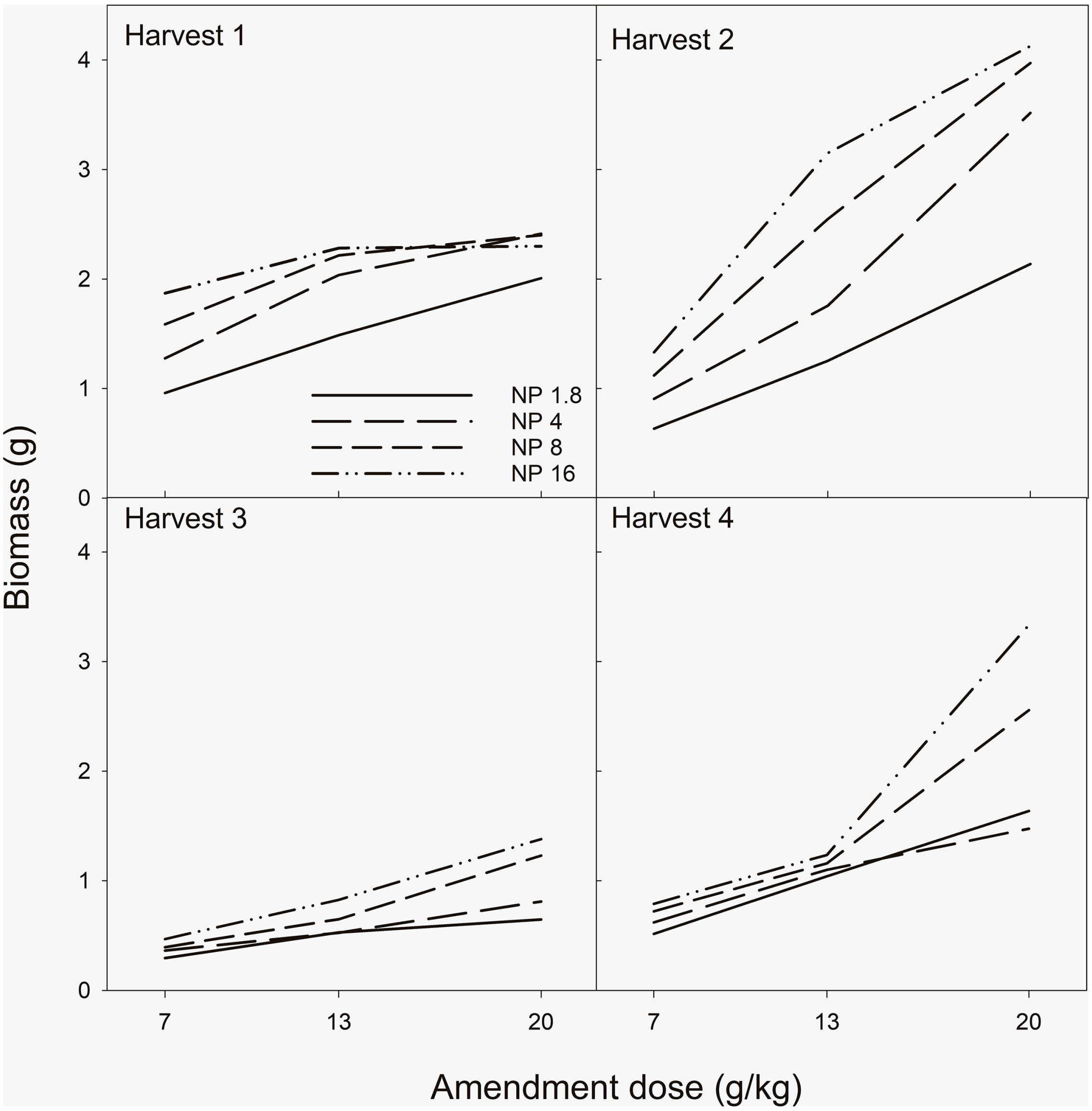

FIGURE 3
Estimated interaction effects of phosphorus dose and N:P ratio on Westerwolds ryegrasss yield over the four harvests (Harvest 1–Harvest 4).

start of crop growth may be affected, as well as the final yield. The ratio between N supplied by manure, digestate, or other organic materials, on the one hand, and mineral fertilizer, on the other hand, could also affect the N:P ratio effects. A meta-study with rice (Hou et al., 2023) showed good effects on partial replacement of mineral fertilizers with manure, but 100% replacement decreased yields, probably due to the immobilization of nitrogen by microbes. After mineralization, this nitrogen could again be available. Increasing the digestate dose will add more nutrients to the plant–soil system, but effects on yield may also differ between cropping systems and soil differences beyond those evaluated here. Kokulan et al. (2022) found that the yields in annual (canola and barley rotation) and perennial (timothy/orchard grass) cropping systems were affected by pig manure, with either N-based or P-based application quantities. However, the effects were different for different cropping systems and the different species. Barley had the largest yield at N-based manure supply, whereas canola yield was not different at N-based and P-based applications of pig manure. Chen et al. (2018) observed higher yields of crops fertilized with organic amendments and inorganic fertilizers only in soil with sandy texture and near-neutral pH and in soils with low initial fertility, but not under less stressful conditions. Accordingly, the positive dosage effect of digestate on the Westerwolds ryegrass and red clover yield in our study might be more representative for sandy soils than for other soils.

The red clover plants were young and probably less efficient in supplying N to the Westerwolds ryegrass than what can be expected later in the season, when the plants have more biomass above and below ground (Tzanakakis et al., 2017). Nitrogen fixation also depends on temperature (Pandey et al., 2017), and at high latitudes, grasses compete strongly with legumes in leys if the N supply is sufficient (Tzanakakis et al., 2017). Under such conditions, nitrogen fixation in red clover can be rather low (Hatch et al., 2007; Paynel et al., 2008; Pandey et al., 2017). However, the supply of nitrogen from N-fixing plants in crop rotations with leys could contribute significantly to later yields.

Although the threshold level of dosage on red clover yield was similar in the two soils, differences in responses between soils have been observed in other studies (Pandey et al., 2017). It is well known that nitrogen fixation in soils is inhibited when soils have significant nitrate concentrations (Voisin et al., 2002). The increase in red clover biomass from the lowest to the medium and further decrease from the medium to high dose in our experiments indicates an increased supply of nutrients. For this species, at the high dose, the N-supply from the digestate was probably larger than what was needed for the N-fixation to be efficient, which aligns with previous studies (Kebede, 2021). Moreover, the positive effect of the dose of digestate on Westerwolds ryegrass, which was preceded by red clover and soil freezing, indicates that the amendment had an effect that lingered beyond the season when they were applied. In a study of red and white clover intercropped with barley, oat, pea, and oilseed radish as cover crops, only a little recovery of N from legume cover crops the following season after the winter was found (Langelier et al., 2021). However, wheat yields increased significantly following the preceding season with cover crops, especially in treatments with clover and barley. This was likely due to the transfer of biologically fixed nitrogen (Paynel et al., 2008) and the improvement of the soil quality.

TABLE 3 Summary of fixed effects from a mixed effect model to investigate interactions between treatments and time on the temporal patterns of biomass yield in Westerwolds ryegrass.

Fixed effects	Estimate	SE	df	*T*	*p*
Intercept	−1.128	0.680	458.100	−1.659	0.098
Time	−0.212	0.277	295.300	−0.767	0.444
Dose	0.930	0.315	458.100	2.956	0.003
N:P	0.162	0.074	458.100	2.203	0.028
Soil	0.892	0.430	458.100	2.075	0.039
time:dose	0.472	0.128	295.300	3.680	0.000
time:N:P	0.006	0.030	295.300	0.192	0.848
dose:N:P	−0.123	0.034	458.100	−3.612	0.000
time:soil	0.130	0.175	295.300	0.742	0.459
dose:soil	−0.385	0.199	458.100	−1.935	0.054
N:P:soil	−0.025	0.047	458.100	−0.536	0.592
time:dose:N:P	0.035	0.014	295.300	2.486	0.013
time:dose:soil	−0.005	0.081	295.300	−0.061	0.951
time:N:P:soil	−0.014	0.019	295.300	−0.714	0.476
dose:N:P:soil	0.040	0.022	458.100	1.834	0.067
time:dose:N:P:soil	0.001	0.009	295.300	0.088	0.930

The soil amendments affect the plants and soil systems in complex ways, depending on the soil amendment properties and climatic factors, especially soil temperature and soil water relations, yielding different mineralization patterns and possibly also run-off incidents over time. The benefits of using digestate, manure, or other organic amendments often increase with time, both in the season of application and over the years, because mineralization of nutrients takes some time to establish, depending on the type of organic amendment and microbes present in the soil and climatic variables (Webb et al., 2013; Luo et al., 2018). Che et al. (2018) observed that similar amounts of added P through either super phosphate or in organic soil amendments yielded different soil responses. The mineral fertilizer did not result in larger available P in degraded grassland soil, probably because of precipitation and binding of P to other minerals. The organic supply, on the other hand, gave more plant available P in the soil.

Long-term effects need to be studied to have a more complete picture of the effects of organic soil amendments, and further studies

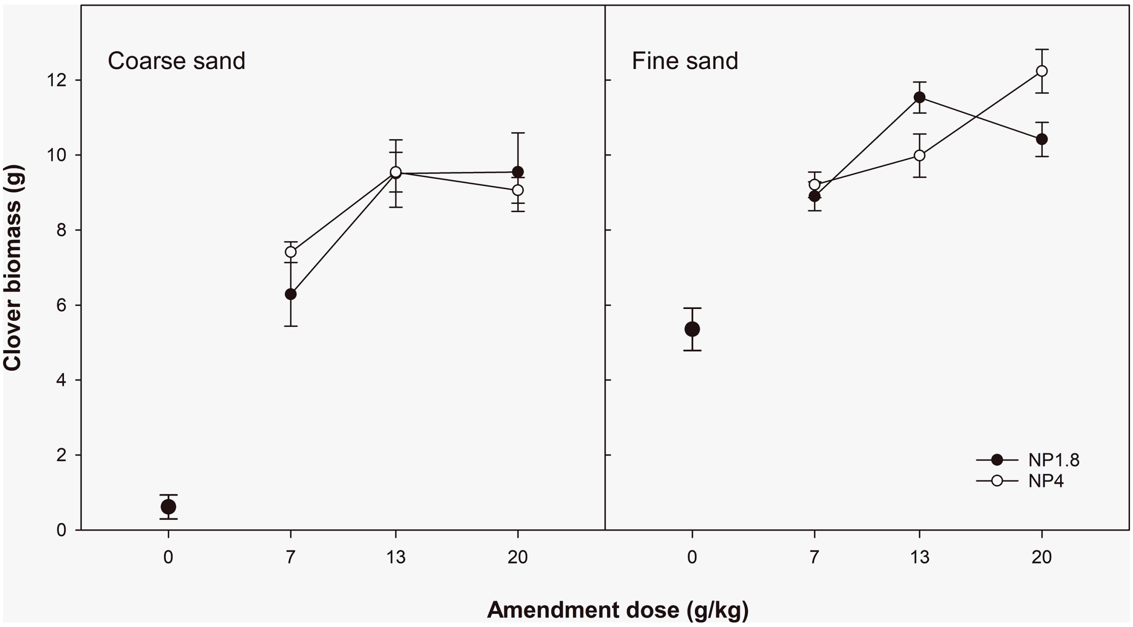

FIGURE 4
Clover biomass per pot (means with 95% CI) at increasing doses of composted swine digestate and manure at two N:P ratios in two sandy soils.

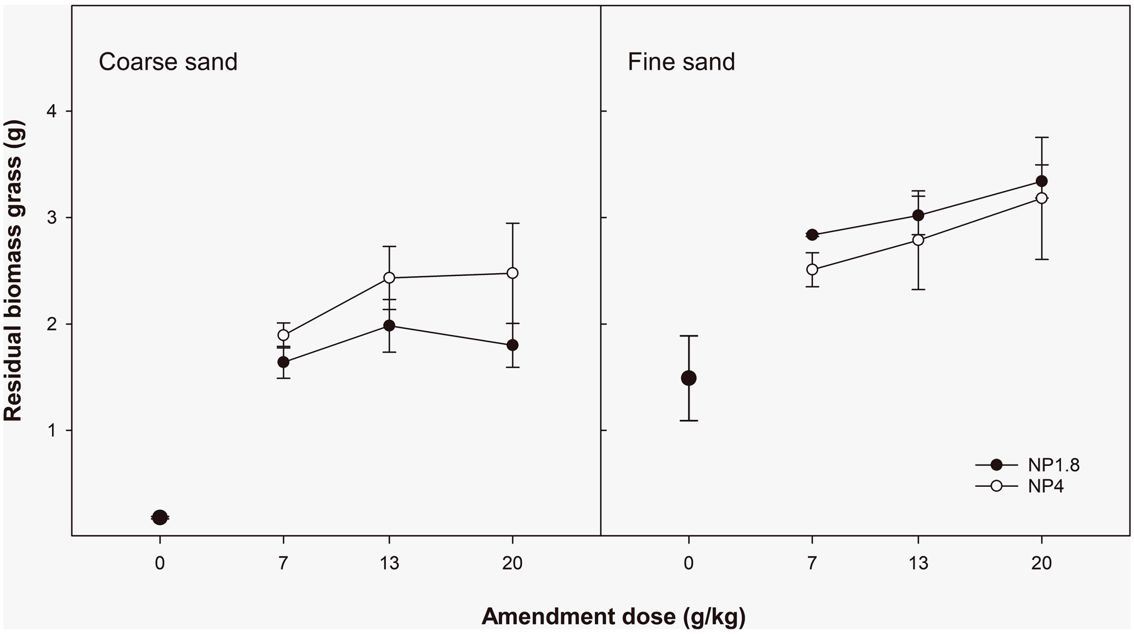

FIGURE 5
Biomass per pot of ryegrass (means with 95% CI) at increasing doses of composted swine digestate of manure at two N:P ratios in two sandy soils low in nutrients after a cycle of red clover growing in the same pots.

TABLE 4 Regression analysis of the relationships between the biomass of preconditioned red clover, soil type, amendment dosage, and amendment N:P ratio on the biomass yield of Red Clover//Westerwold ryegrass.

Source	df	*F*	*p*
Regression	5	7.07	0.000
Clover biomass	1	0.75	0.394
Soil	1	13.75	0.001
Dose	2	0.87	0.428
N:P	1	0.38	0.541

R^2 adj = 47 and error df = 30.

should be made with N-fixing species. However, the dilemma of deciding the dose of organic fertilizers and soil amendments, based on either N-content or P-content, remains. However, if we are to replace mineral fertilizers with digestate, manure, and other organic rest products, because of economic as well as ecological concerns, the best solution would be to reduce P-content in the parent materials, e.g., by precipitation of struvite or other inorganic compounds (Tao et al., 2016; Persson and Rueda-Ayala, 2022) to a level that reduces the risk of phosphorus losses. If this is done, the dosage of fertilizers and soil amendments can be decided based on the N content.

5 Conclusion

The yield of the Westerwolds ryegrass increased with an increase in the dose of digestate and the N:P ratio, while red clover also gave a higher yield at a lower dose of digestate and lower N:P ratios. The effect of added mineral N in higher N:P ratios on accumulated biomass was primarily additive, with no indications of P deficit at the higher N dosages, although the temporal dynamics across harvests indicated a switch from excess N at the first harvest to increasing N deficit at the final harvest. Hence, targeted N management is required to benefit from the P-rich digestate in grass cultivation, while the red clover, as expected, maintained productivity at lower N:P ratios. The long-term effects of red clover on soil N status using P-rich digestates still need further investigation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AS: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. TP: Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. PS: Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. HH: Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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