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RESEARCH ARTICLE

The effect of contrasting biosolids application strategies on soil quality

**M. J. Gutiérrez-Ginés · N. J. Lehto ·
E. Madejón · B. H. Robinson**

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Abstract
Purpose Incorporating biosolids into the soil improves plant yield compared with surface application, but it can result in the increased uptake of trace elements. However, there is a lack of knowledge about how different types of biosolids applications affect soil quality. We aimed to determine the effect of the type and rate of biosolids application on soil quality and the mobility of contaminants.
Methods Soil quality was determined by soil fertility (inorganic N, exchangeable P, Mg, Ca, K), exchangeable trace and non-essential elements (Al, Mn, Zn, Cu and Cd) and biological activity (dehydrogenase activity). We measured the properties of soil pore water, bulk soil and rhizosphere in a pot and a rhizobox experiment, with increasing concentration of biosolids (equiv. 16 t ha^{-1}, 48 t ha^{-1} and 145 t ha^{-1} dry weight), applied on the surface, incorporated to 25 cm, or incorporated into a patch.
Results and discussion The incorporation of biosolids into the soil increased the exchangeable Zn, Cu, Cr, Ni and Cd, compared with surface application. The surface application of biosolids increased the inorganic N in the soil compared with biosolids incorporation (680 mg kg^{-1} vs. 380 mg kg^{-1}), and decreased soil pH by 1.1 units. This aligned with solubilisation of Al (43 mg kg^{-1} vs. 6 mg kg^{-1}) and Mn (43 mg kg^{-1} vs. 33 mg kg^{-1}) and explains the decreased microbial activity in the soil compared with the unamended soil. Incorporating biosolids in the soil increased the biological activity, likely due to biosolids-borne microbes. The root systems significantly increased microbial activity, pH, and the concentration of NH_4^+, NO_3^-, and exchangeable P, S, Mg, Na, Zn, Cu and Ni, and significantly decreased exchangeable concentration of Mn and Fe.

Keywords Plant nutrients · Trace elements · Incorporation · Surface application · Root systems

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M. J. Gutiérrez-Ginés · N. J. Lehto · B. H. Robinson
Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, New Zealand

M. J. Gutiérrez-Ginés (✉)
Institute of Environmental Science and Research Ltd, Christchurch, New Zealand
e-mail: maria.gines@esr.cri.nz

E. Madejón
The Institute of Natural Resources and Agrobiology of Seville (IRNAS), CSIC, Sevilla, Spain

B. H. Robinson
School of Physical and Chemical Sciences, University of Canterbury, Christchurch, New Zealand

Introduction

Biosolids are a by-product from the treatment of wastewater. Biosolids can be used to improve soil through the addition of organic matter and plant nutrients, yet their reuse is limited by elevated concentrations of contaminants and pathogens (Gianico et al. 2021). Research on the beneficial reuse of biosolids started at Pennsylvania University in the early 1960s (Sopper and Kardos 1974), and in some U.S. jurisdictions, biosolids reuse became a common, but regulated practice since the 1970s (Lu et al. 2012). Agriculture is the preferred option for the reuse of biosolids in many countries: nearly 50% of biosolids are applied to agricultural soil in the EU and USA (Collivignarelli et al. 2019; LeBlanc et al. 2008), and up to 70% in Australia (ANZBP 2020). In contrast, in New Zealand (NZ) only 3% of biosolids are used in agriculture, with ~77% of biosolids being landfilled, monofilled, stockpiled or discharged into the ocean (Stantec 2019). Due to social and cultural concerns about using biosolids in food-production systems (Ataria et al. 2016), beneficial reuse of biosolids in NZ tends to be restricted to forestry (Xue et al. 2015), or restoration of degraded land into native ecosystems (Simcock et al. 2019).

Biosolids application into degraded soils increases soil organic matter and plant nutrient concentrations (Gravuer et al. 2019; Madejón et al. 2016; Wang et al. 2008) and improves infiltration and aeration (Gravuer et al. 2019; Lu et al. 2012). The application of biosolids for ecosystem restoration is different from the application to productive systems. Biosolids used for productive systems such as agriculture or forestry need a recurrent application (Alvarez-Campos and Evanylo 2019; Nicholson et al. 2018; Wang et al. 2004), which in NZ is limited to 200 kg N/ha/y, which equals to ~5 t ha^{-1} of biosolids depending on the N concentration (NZWWA, 2003). In the case of use in restoration, the most likely scenario is a one-off application of biosolids to enhance plant establishment (Fuentes et al. 2010; Newman et al. 2014), with no additional biosolids applications (Borden and Black 2011). When biosolids are used to establish vegetation in degraded areas, the underlying soil may be contaminated or entirely absent, such as in the case of disused mines (Borden and Black 2011), or quarries (Moreno-Peñaranda et al. 2004). The application rate is usually higher for ecosystem restoration than in agricultural use, and rates that have been researched vary between 10 and 500 t ha^{-1} (Borden and Black 2011; Fuentes et al. 2010; Moreno-Peñaranda et al. 2004). This has implications for the best application and management methods. High application rates can result in an initial pulse of nitrate leaching, which can be reduced by blending biosolids with other organic waste with a higher C:N ratio (Paramashivam et al. 2017). Similarly, nitrate leaching may be reduced by using species with biological nitrification inhibition (BNI) activity (Esperschuetz et al. 2017; Halford et al. 2021) showed both reduced nitrate leaching and nitrous oxide emissions in soils exposed to high rates of nitrogen inputs where *Leptospermum scoparium* (mānuka) was grown. *L. scoparium* is a pioneer plant species native to New Zealand (Stephens et al. 2005), which is frequently used in restoration plantings. In addition to the environmental benefits of growing *L. scoparium* on biosolid-amended soils, this species has both cultural and economic significance for honey and essential oil production (Stephens et al. 2005).

Biosolids application may alter ecological succession and result in ecosystems that are dissimilar to the restoration objectives (Borden and Black 2011; Moreno-Peñaranda et al. 2004; Newman et al. 2014; Simcock et al. 2019). Altering biosolids type and rate of application can address some of these challenges. Biosolids can be applied to the land as a surface application, where the biosolids are spread on the surface of the soil without further management, or they can be incorporated into the soil, where ploughing ensures the blending of the biosolids into the soil. Alternatively, biosolids can be blended with other substrates as soil substitution in mine or quarry rehabilitation. Incorporation of biosolids is usually a preferred option for reducing the potential spread of pathogens or contaminants via run-off (NZWWA 2003). However, for restoration, ploughing exacerbates the loss of existing vegetation, and potentially increases soil erosion (Alvarez and Steinbach 2009). Reis et al. (2017) showed in a pot experiment that surface application of biosolids decreased the plant uptake of certain trace elements compared with incorporation into the soil. The roots of some plants can forage patches of biosolids in the soil, showing similar growth rates as plants growing in surface applied or incorporated biosolids into the soil (Gutiérrez-Ginés et al. 2019; Reis et al. 2017). The rate of biosolids application can also be managed for achieving preferred outcomes. For example, Martínez et al. (2003) showed that grassland species richness decreased while plant cover increased with higher rates of biosolids. Gutiérrez-Ginés et al. (2019) and Seyedalikhani et al. (2019) showed that increasing rates of biosolids had beneficial effects on plant growth up to an optimum beyond which plant health started to be compromised.

While the effect of biosolids application rate in the soil has been extensively studied (Fuentes et al. 2010; Griffith et al. 2020; Martínez et al. 2003), there is less information on soil responses to different types of biosolids application. There are studies of either incorporated biosolids (Bozkurt et al. 2006; Griffith et al. 2020), either surface applied biosolids (Jin et al. 2015; Moffet et al. 2005). However, few studies have specifically compared the effects of both types of application on soil characteristics. Gove et al. (2002) demonstrated in a column experiment with a sandy loam that the subsurface application of biosolids increased P and metal leaching compared with surface application, while nitrate losses were independent of the application method. Further research to compare the two types of biosolids application was more focused on plant growth and health. A field experiment with *Pennisetum purpureum* (elephant grass) and biosolids incorporated vs. surface applied (Castillo et al. 2011) demonstrated higher crop yields when biosolids were incorporated, however, the concentration of N and P in the leaves was not significantly different in both types of application. The same experiment showed that, at the same rate of N application, organic N mineralization was greater when biosolids were incorporated into the soil than when they were applied on the surface. Roman-Perez et al. (2021), in a field experiment with *Hordeum vulgare* (barley), demonstrated that incorporating biosolids led to higher N_2O emissions, and higher barley biomass and greenness, than surface application. By contrast, in two different pot experiments, Reis et al. (2017) and Gutiérrez-Ginés et al. (2019), did not find differences in biomass production by *Leptospermum scoparium* when the biosolids were incorporated or surface applied in the soil, nor did they find any differences in plant nutrient concentration in the leaves. However, the leaf concentrations of Mn, Zn, Cu and Cd were different depending on type of biosolids application, and the soil type. The soil properties that could have explained those changes in plant growth and plant composition were not investigated. There is a general lack of knowledge on how the type of biosolids application affects soil quality.

The objective of this research was to investigate the combined effects of type and rate of biosolids application in the quality of a low fertility soil. We aimed to determine whether changes in the soil by root systems of a potential BNI plant species (*Leptospermum scoparium*) could mitigate the loss of nutrients from a biosolids-amended soil. Based on the results by Castillo et al. (2011) and Roman-Pérez et al. (2021), we hypothesized that compared to surface application, the incorporation of biosolids into the soil would increase the soil fertility of the soil, as indicated by the bioavailability of plant nutrients, such as N, P and K. Based on Gove et al. (2002), Reis et al. (2017) and Gutiérrez-Ginés et al. (2019), we expected to see an increase in trace element availability in soils where biosolids were incorporated in the soil compared with surface application. Given the potential BNI by *L. scoparium* demonstrated by Esperschuetz et al. (2017), we hypothesized that the rhizosphere soil would have a higher $NH_4^+ : NO_3^-$ ratio than the bulk soil. To test these hypotheses, we determined the changes in soil and rhizosphere properties in the soils of two experiments set up in pots and rhizoboxes (Gutiérrez-Ginés et al. 2019).

Materials and methods

Collection of materials, experimental set up and harvesting

A detailed explanation of the materials, experimental set up of the pot and rhizobox experiments, and harvesting was described by Gutiérrez-Ginés et al. (2019) and is briefly summarized here. A naturally low fertility soil, a Craigieburn silt loam; NZ Soil classification - Typic Allophanic Brown Soil, Hewitt (2010), was collected from three soil horizons (Ah: 0–15 cm, Bw; 20–40 cm, and BC: 40–70 cm) and sieved to 12 mm. Anaerobically digested and thermally dried municipal biosolids were supplied in granular form by the Christchurch City Council. Given the concentration of Zn, Cu and Cd in the biosolids (Table 1), these are graded *b*, which means that their use is restricted (NZWWA 2003). The chemical properties of the soil and biosolids are shown

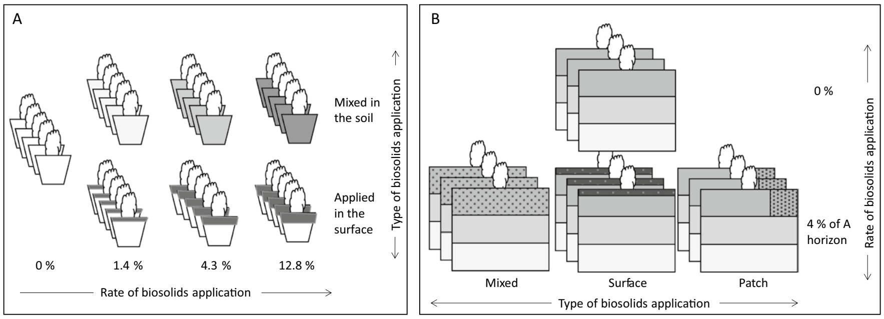


Fig. 1 Design of the **A** pot and **B** rhizobox experiments. The shaded areas represent the location of biosolids in the pots and rhizoboxes, with darker shading representing a higher concentration of biosolids

in Table 1. *Leptospermum scoparium* J.R.Forst. & G.Forst. seedlings, between 4 and 6 cm high, were obtained from the Department of Conservation of New Zealand's Motukarara Nursery.

A 35-pot experiment was set up with 3.2 kg of substrate per pot, consisting of a mixture of 4:1 of A and B soil horizons to which biosolids were applied at rates of 1.4%, 4.3% and 12.8% fresh weight (corresponding

Table 1 Chemical characteristics of the soils and biosolids in the pot and rhizobox experiments

Parameter	Biosolids	Soil for pots	Horizon Ah	Horizon Bw	Horizon BC
pH	6.78 ± 0.02	5.56 ± 0.01	5.57 ± 0.00	5.77 ± 0.04	5.89 ± 0.06
EC (dS m^{-1})	2.7 ± 0.032	0.036 ± 0.001	0.036 ± 0.001	0.017 ± 0.0007	0.008 ± 0.0003
C (%)	30 ± 0.03	1.59 ± 0.04	1.46 ± 0.18	1.03 ± 0.07	0.70 ± 0.05
N (%)	3.95 ± 0.00	0.22 ± 0.01	0.24 ± 0.00	0.12 ± 0.00	0.07 ± 0.00
NH_4^+ -N	2375 ± 14	< 0.01	< 0.01	< 0.01	< 0.01
NO_3^- -N	3.56 ± 0.23	< 0.01	0.04 ± 0.02	0.29 ± 0.04	0.17 ± 0.03
Olsen P	506 ± 5.9	18 ± 1.1	15 ± 0.37	15 ± 0.37	6.2 ± 0.23
P (T)	16,250 ± 10.2	720 ± 6.4	750 ± 16	650 ± 17.9	450 ± 4.3
S (T)	14,000 ± 90	380 ± 2.4	380 ± 8.5	270 ± 3.5	230 ± 3.3
S (E)	1807 ± 21	< 0.01	< 0.01	< 0.01	< 0.01
K (T)	2160 ± 19	3510 ± 51	3430 ± 88	3750 ± 112	4200 ± 85
K (E)	879 ± 40	102 ± 15	87 ± 12	36 ± 9	23 ± 6
Ca (T, %)	3.0 ± 0.02	0.48 ± 0.006	0.50 ± 0.01	0.37 ± 0.01	0.42 ± 0.004
Mg (T)	5020 ± 24	5620 ± 30	5570 ± 60	5650 ± 109	6040 ± 52
Mg (E)	916 ± 9	163 ± 14	134 ± 14	57 ± 12	9.1 ± 1.8
Na (T)	650 ± 5.9	210 ± 8.4	210 ± 7	220 ± 8.4	210 ± 3.7
Na (E)	564 ± 15	15 ± 2.9	13 ± 2	18 ± 4.2	8.2 ± 1.9
Fe (T, %)	2.2 ± 0.01	2.8 ± 0.005	2.7 ± 0.02	2.8 ± 0.05	2.2 ± 0.03
Fe (E)	62 ± 9.7	1.4 ± 0.05	1.3 ± 0.13	0.49 ± 0.12	0.11 ± 0.01
Al (T, %)	1.1 ± 0.02	4.2 ± 0.04	4.1 ± 0.02	4.3 ± 0.13	3.1 ± 0.05
Al (E)	168 ± 27	8.4 ± 0.06	5.3 ± 0.67	13.1 ± 2.9	4.5 ± 0.8
Mn (T)	410 ± 2.2	510 ± 1.9	520 ± 2	440 ± 6.3	340 ± 6.2
Mn (E)	11 ± 0.3	4.1 ± 0.8	4.8 ± 0.6	1.2 ± 0.5	0.4 ± 0.0
Cu (T)	291 ± 2.4*	8.4 ± 0.1	8.5 ± 0.22	8.5 ± 0.26	10.7 ± 0.14
Cu (E)	2.8 ± 0.06	0.02 ± 0.00	< 0.01	< 0.01	< 0.01
Zn (T)	993 ± 1.8*	94 ± 1.4	90 ± 1.3	104 ± 2.6	79 ± 0.7
Zn (E)	5.1 ± 0.54	0.22 ± 0.07	0.13 ± 0.05	0.02 ± 0.01	0.06 ± 0.04
Ni (T)	27 ± 0.08	10 ± 0.07	10 ± 0.07	11 ± 0.32	13 ± 0.24
Cd (T)	1.6 ± 0.01*	0.32 ± 0.03	0.3 ± 0.03	0.29 ± 0.03	0.15 ± 0.04
Pb (T)	54 ± 0.6	26 ± 0.6	26 ± 0.6	25 ± 0.6	20 ± 0.6

The reported values are averages ± standard errors ($n = 5$) and given as mg kg^{-1}, unless indicated otherwise. Total (T) and exchangeable (E) concentrations shown for major cations and trace elements

*exceeds guideline for Grade A biosolids (Cu = 100 mg kg^{-1}, Zn = 300 mg kg^{-1}, Cd = 1 mg kg^{-1} (NZWWA 2003)).

to approx. 16 t ha^{-1}, 48 t ha^{-1} and 145 t ha^{-1}, respectively). These rates are within the range of application investigated previously by other authors (Borden and Black 2011; Fuentes et al. 2010; Moreno-Peñaranda et al. 2004). The highest application rate intended to identify the concentration at which plant health is impacted, as demonstrated by Gutiérrez-Ginés et al. (2019), and the effects in soil quality, which is the objective of this work. The biosolids were either applied to the surface or homogeneously mixed into the soil. There were five replicates of each treatment and a control without biosolids (Fig. 1A).

Twelve large rhizoboxes (80×80×2.5 cm) were filled with Craigieburn silt loam with the three soil horizons repacked in 25 cm layers. Biosolids were applied at a rate of 4% (wt/wt) of the A horizon (150 g per rhizobox) using one of three modes of application: surface (T); mixed (M) in the A horizon; or as a concentrated patch (P) in one third of the A horizon (Fig. 1B).

Both experiments ran for four months in a greenhouse with an overall mean temperature of 20.2 °C (min. – max. range: 9.8–32 °C). The pots and rhizoboxes were watered daily to field capacity. One week before the end of the experiment, soil pore water in the pots was collected with Rhizon samplers (Eijkelkamp Soil & Water, Giesbeek, The Netherlands) placed vertically near the centre of the pot. The soil pore water in each pot was sampled daily over a 3-day period, approx. 1 h after watering. A 50 mL syringe was used to create a vacuum for approx. 2 h in each collection time and to collect the sample. The three collected samples in each pot were combined into a single sample. The samples were stored in the dark at 4 °C and analysed within one week of collection.

At the end of the experiments, the soil in the pots was collected and divided into rhizosphere soil (attached to the roots of *L. scoparium*) and bulk soil (soil not attached to the roots after gentle shaking). In the rhizobox experiment, the soil from each horizon was collected separately. Each horizon was divided into three quadrants of the same width (see Fig. 5). On some occasions, the quadrants were further divided to potentially identify gradients from the biosolids (see Fig. 5). Bulk soil and rhizosphere soil were also separated in each of the quadrants. The biosolids applied on the surface of both experiments were sampled separately from the soil. Soil samples were stored at 4 °C until they were processed within 48 h.

Chemical analysis

Concentrations of mineral nitrogen species (NH_4^+ and NO_3^-) in the soil pore water were analysed within two days of collection with a Flow Injection Analyser (FOSS FIAstar 5000). pH and electrical conductivity (EC, as a measurement for salinity) were analysed with a Toledo pH and EC meter. Elements were analysed using an ICP-OES (Varian 720-ES).

The collected fresh soil samples were sieved through a 4 mm sieve for homogenization, and subsamples were taken for each analysis. Soil was extracted with 2 M KCl (Clough et al. 2001) for determining NH_4^+ and NO_3^- concentrations with the Flow Injection Analyser. Dehydrogenase activity (DHA) was analysed spectrophotometrically by the transformation of 2,3,5-triphenyl-tetrazolium chloride into triphenylformazan, as described by Gutiérrez-Ginés et al. (2017).

Sub samples of soil and biosolids were oven-dried at 60 °C for one week and sieved through a 2 mm stainless steel sieve. These were used for analyses of pH and EC (1:5 w:v soil-water ratio (Blakemore et al. 1987)) and exchangeable element concentrations. Exchangeable elements were determined using a 0.05 M $Ca(NO_3)_2$ extraction (McLaren et al. 2005), and analysed by ICP-OES. Total-extractable element concentrations were determined in the soils and biosolids prior to the experiment. The samples (0.5 g) were digested using a CEM MARS Xpress microwave digestion with 4.0 mL of 30% H_2O_2 and 4.0 mL of 69% HNO_3, and analysed by ICP-OES.

Data analysis

The results of the two experiments were analysed separately. Two-way ANOVA was used to determine differences in each of the parameters based on rate of biosolids application and type of application. Homoscedasticity was verified with the Levene's test, and normality with the kurtosis and skewness of the data distribution. When assumptions were not fulfilled by the data, transformations (ln or square root) were applied. Principal Component Analyses (PCA) were also performed on the standardized data of soil pore water and soils (separately) from the pot experiment, and from the rhizobox experiment. For these analyses, all the results were treated together, without distinguishing between bulk and rhizosphere soil. The rhizosphere effect was assessed by a pairwise T-test. Due to the high variability in the results, this analysis was performed for all the results of each parameter, instead of separating them between treatments. The results are, however, shown for each treatment (Fig. 6). The software Statgraphics Centurion was used for the calculations. The graphics were created in Microsoft Excel 10, except for the graph in Fig. 2, which was created with the *ggplot2* package (Wickham 2016), using RStudio (R Core Team 2021).

Results

Effect of rate and type of biosolids application on soil quality

The results of soil quality parameters in the pot experiment are represented in Fig. 2 and in Suppl. Material. The concentrations of key plant nutrients (including P, NH_4^+, NO_3^-, K, Ca, Mg and S) significantly increased with higher rates of biosolids application. The increase in exchangeable P, S and Mg was significantly higher when biosolids were mixed into the soil than when they were applied on the surface, and this difference was more pronounced in the highest rate of biosolids application. In the soil with the highest biosolids concentration (Eq. 145 t ha^{-1}), exchangeable P was 6 mg/kg when biosolids were mixed into the soil vs. 0.7 mg kg^{-1} when they were applied on the surface. This difference, although significant, was less pronounced at a rate of Eq. 48 t ha^{-1}: 1.7 mg kg^{-1} vs. 0.7 mg kg^{-1}. The inorganic nitrogen, on the other hand, was significantly higher when biosolids were applied on the surface. NH_4^+ increased from 8 mg kg^{-1} in unamended soil to 316 mg kg^{-1} in the highest biosolids application when applied on the surface, compared with 43 mg kg^{-1} when biosolids were mixed into the soil. At lower application rates (Eq. 16 t ha^{-1} and 48 t ha^{-1}), this difference was less pronounced (see Suppl. Material Table S1 and S2). The exchangeable concentration of micronutrients and non-essential elements was also significantly higher with increasing rates of biosolids application, but this increased depended on the type of application in some of the elements. Exchangeable Na, Zn

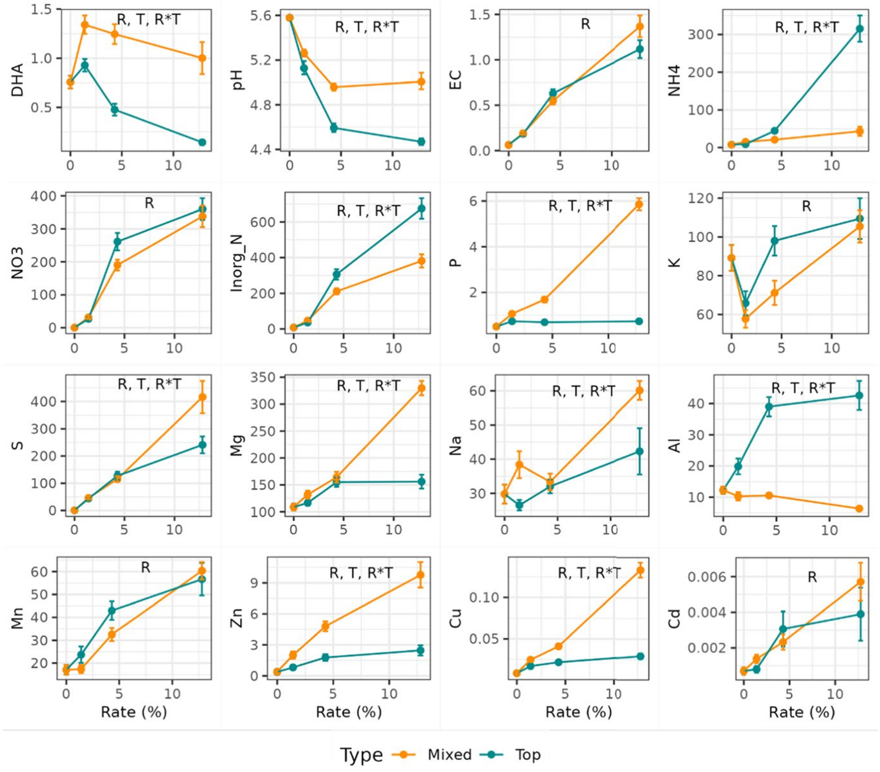


Fig. 2 Average and standard errors of soil biological and chemical parameters (average of bulk soil and rhizosphere) analysed in the pot experiment, and factors significantly (ANOVA, $p < 0.05$) affecting the results (R: rate of application, T: type of application, R*T: interaction between both factors). The elemental results refer to extractable concentration in mg kg^{-1}. DHA: dehydrogenase activity (μg g^{-1} h^{-1}). EC: electrical conductivity (dS m^{-1}). NH4: NH_4^+ -N, NO3: NO_3^- -N, and Inorg. N: inorganic N (mg kg^{-1})

and Cd increased significantly more when biosolids were mixed into the soil than when they were applied on the surface. On the other hand, exchangeable Al only increased when biosolids were applied on the surface, being 3.5 higher in soil with the highest rate of biosolids compared with unamended soil. Salinity (measured by EC) was also significantly higher as the rate of application increased, but the results were not affected by the type of application. pH significantly decreased with increasing rates of biosolids application, and this decrease was significantly lower when biosolids were applied to the surface; in the highest rate, soil pH was 5 when biosolids were mixed into the soil, compared with 4.5 when they were applied on the surface. The dehydrogenase activity (DHA) in the soil was very variable with rate and type of biosolids application. When the biosolids were mixed into the soil, DHA increased almost twice with the lowest application rate compared with unamended soil, and decreased 30% with the highest application rate, but it was still higher than in the unamended soil. On the contrary, when biosolids were applied on the surface, DHA slightly increased 20% with the lowest biosolids application, but decreased five times compared with unamended soil, when biosolids were applied at Eq. 145 t ha^{-1}.

The results of soil pore water showed similar trends to the exchangeable element concentration, with similar results of the Principal Component Analysis (Fig. 3), so they are only represented in Supp. Material. The first two components of the PCA explained a cumulative 67.1% of the variance in the soil results (Fig. 3A), and 73.5% of the variance of the soil pore water results (Fig. 3B). The first component mostly organized the results with increasing biosolids application rates in the negative part of the component, which was correlated with plant nutrients (NO_3^-, P, Mg, and S), EC and Na, and trace elements (Cu, Zn, Cd, Ni). The second component separated the results based on the type of biosolids application. The results of mixed biosolids had a positive value of the second component, which was mostly due to weights of DHA, pH, and trace elements. The results of surface applied biosolids were in the negative part of the second component, which were mostly related to Al, Fe, NH_4^+ and Mn.

As reflected by the representation of the PCA, the results of the soil quality in the rhizobox experiment follow a similar pattern than in the pot experiment (Fig. 4). The two first components explained 63.1% of the variance. The first component was mostly determined for the variation in plant nutrients and trace elements, while the second component was determined by a positive weight by Al, and a negative weight by pH. The distribution of the results as a scatterplot showed the difference in the results from three soil horizons; the results of the A horizon were separated based on type of biosolids application. The results of the Patch application were both similar to the results of the Control (for the quadrants without biosolids) and similar to the mixed application (for the quadrant where the biosolids were concentrated).

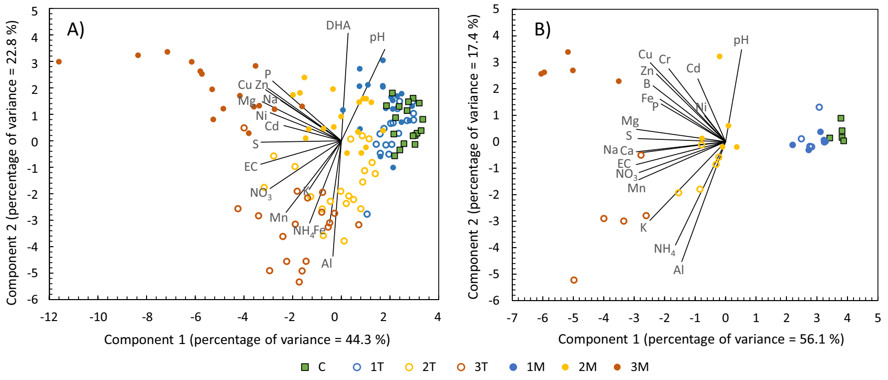


Fig. 3 Bi-plot of the Principal Component Analysis (PCA) and the distribution of the results in the two components. Results of soil samples (**A**) and in soil pore water (**B**) in the pot experiment. Results of the scatterplot are grouped based on the three treatments: (C) control, (T) surface application of biosolids and (M) biosolids mixed with the soil, with the numbers 1–3 reflecting the increasing rates of biosolids application

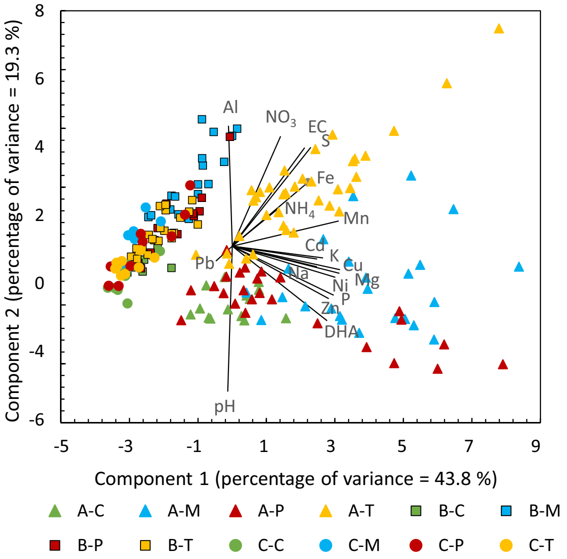


Fig. 4 Bi-plot of Principal Component Analysis (PCA) and the distribution of the results of the rhizobox experiment in the first two components. The scatterplot shows results grouped based on soil horizon (first letter A to C) and treatment (second letter C – control, T – top application, M – mixed into the A horizon, P – patch in the A horizon)

The chemical characteristics of the B and C horizons also changed based on types of biosolid application (Fig. 4), with the results of mixed treatment presenting higher values in PC1 and PC2 than those of surface applied.

Figure 5 shows the spatial distribution of the main plant nutrients and pH in the rhizoboxes, with the complete set of results shown in Supp. Material. Exchangeable P was higher in the areas with biosolids, or in the horizons immediately below the biosolids compared with the respective horizons in the control rhizoboxes. However, the concentration was similar to the control in the deeper horizons, like in the C horizon in all the treatments, and in the B horizon on the surface application. NO_3^- can be detected in all horizons of the rhizoboxes where biosolids were applied, while it was not detected in any horizon of the control rhizoboxes. In the deepest horizon, C, NO_3^- was higher when biosolids were mixed into the soil (58 mg kg^{-1}), or in the area under the patch (48 mg kg^{-1}), than when they are applied on the surface (20 mg kg^{-1}). The NH_4^+ was similar in the A horizon when biosolids were applied on the surface (27 mg kg^{-1}) than when they were mixed into the A horizon (21 mg kg^{-1}). However, it was the highest in the B horizon immediately below the path of biosolids (87 mg kg^{-1}).

Effect of the rhizosphere on soil properties with contrasting biosolids applications

The rhizosphere had a significant effect in most of analysed soil properties (Fig. 6). Although these effects appear different depending on biosolids rate and type of application (Fig. 6), the difference between rhizosphere and bulk soil was only analysed for the whole set of results, due to the high variability of the results. The DHA was significantly higher in the rhizosphere than in bulk soil. Similarly, pH and exchangeable P, S, Mg, Na, Zn, Cu and Ni were significantly higher in the rhizosphere than in bulk soil. Although both NH_4^+ and NO_3^- were significantly higher in rhizosphere, the ratio of both was also different with higher proportion of NH_4^+ compared with NO_3^- in rhizosphere than in bulk soil (average ratio 1.2 vs. 0.4). The ratio could not be calculated in control pots because there was no detectable concentration of NO_3^- in the soil. Contrary effect was detected for Fe and Mn, which were significantly lower in the rhizosphere than in the bulk soil.

Discussion

Rate and type of biosolids application affect soil quality

As hypothesized, increasing the rate of biosolids application increased nutrients, but also the exchangeable concentration of trace elements in the soil. Incorporation increased the exchangeable Zn, Cu, Cr, Ni and Cd, and P at the highest application rate (Eq. 145 t ha^{-1}) compared with surface application. These results explained the higher plant uptake of these elements by *L. scoparium* plants grown in the pots with the highest rate of biosolids application mixed into the soil compared with the rest of the treatments (Gutiérrez-Ginés et al. 2019).

Contrary to our hypothesis, the surface application of biosolids increased the fertility of the soil (i.e. higher inorganic N) more than when they were incorporated into the soil. Surface application also increased the exchangeable concentrations of Al and

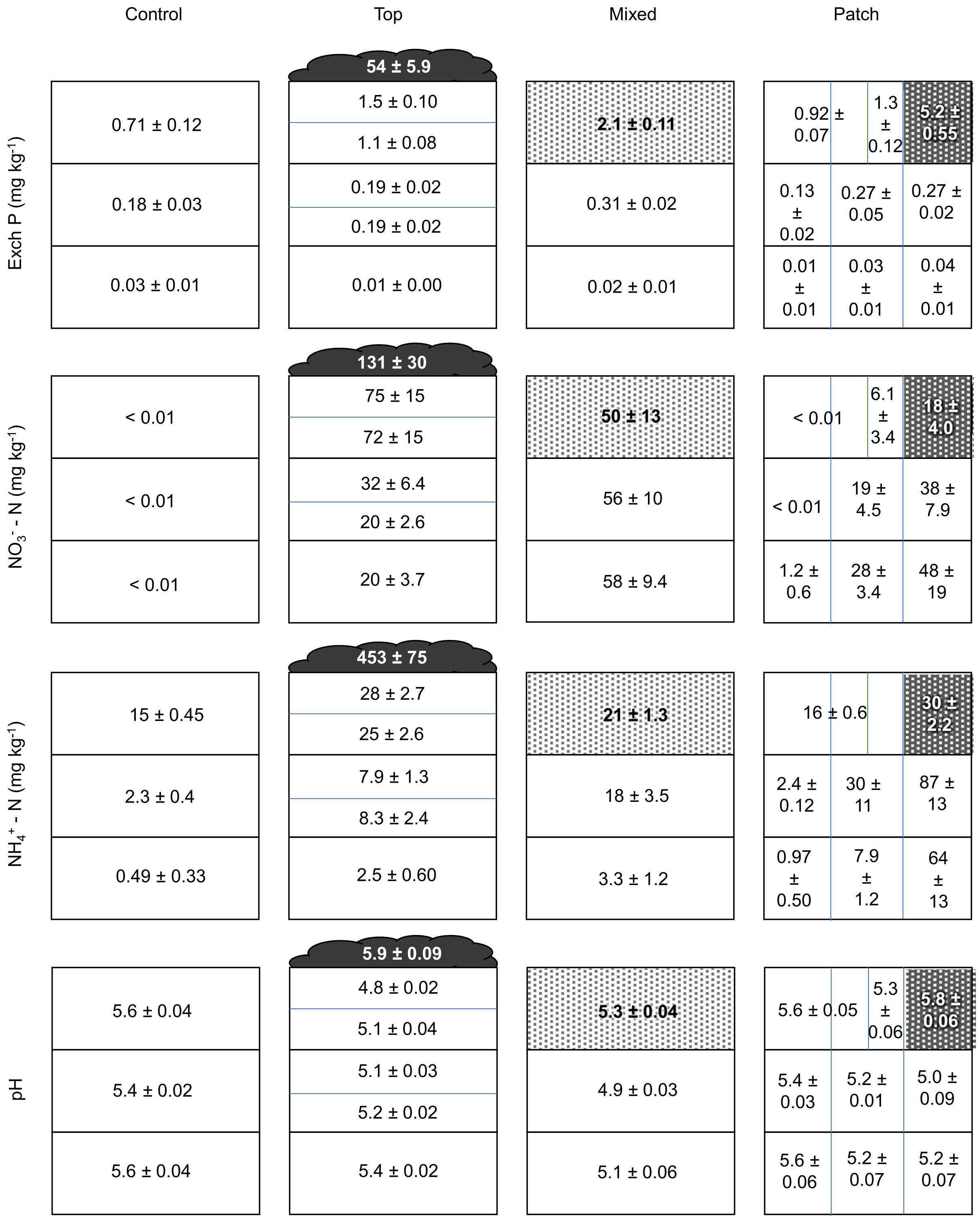
Mn in the soil and pore water, compared with biosolids incorporation. This may be due to the significant decrease in soil pH. These effects were more pronounced in the highest rate of application (Eq. 145 t ha^{-1}). The differences in inorganic N were not reflected by N foliar concentration of *L. scoparium*, which was similar in all the treatments, and only significantly different between control and the treatment with the highest application rate mixed into the soil (3 M) (Gutiérrez-Ginés et al. 2019). The increased fertility of the soils with surface application of biosolids contrasts with previous results showing better N mineralization and plant growth when biosolids were incorporated (Castillo et al. 2011; He et al. 2003; Roman-Perez et al. 2021) also observed higher inorganic N in the soil over time when biosolids were applied on the surface, compared with incorporation into the soil. However, those authors were unable to give an explanation to those findings, mostly due to the contrast with their results. They assumed the higher mineralisation and N_2O emissions, and higher yield and health of the crops when biosolids were incorporated into the soil, were due to higher N bioavailability. Quemada et al. (1998) found higher NH_4^+ in soil when biosolids were incorporated, but higher NO_3^- in the soil and significantly higher NH_3 volatilization when they were applied on the surface. They assumed a higher mineralization when biosolids were applied on the surface. However, given the high concentration of NH_4^+ in their biosolids, NH_3 might have volatilized from NH_3 directly or from the initial load of NH_4^+, rather than from N mineralization. The high amount of organic matter concentrated on the surface locally may have induced highly reducing conditions (Mench et al. 2003). The low pH and reducing conditions could explain the increased NH_4^+ and higher exchangeable Mn concentrations.

Sullivan et al. (2006) showed a significant decrease in soil pH (from 6.3 to 5.5) when the rate of biosolids application was increased (from 2.5 to 30 t ha^{-1}) in a long-term experiment of surface applied biosolids. This was despite the biosolids having a pH of 7.3. Xue et al. (2015) also showed a decrease in soil pH (5.4 to 4.8) with an increasing rate of biosolids application (0 t ha^{-1}, 3 t ha^{-1} and 6 t ha^{-1}, applied every 3 years) in a long-term experiment of repeated surface applied biosolids in a pine plantation, even with alkaline biosolids with pH of 8.5. The biosolids in those experiments, as well as those in our experiment, had high concentrations of NH_4^+ that could have potentially driven a reduction in pH as it underwent nitrification. The decrease in pH may be linked to the observed increases in exchangeable Mn, Al, and Fe, as these elements are rarely soluble in soils above pH 5.0 (Sposito 2008). However, the extent of acidification was lower when the biosolids were incorporated into the soils, and this is likely to be due to an increase in the soil cation exchange capacity (CEC) in the mixed and patch treatments. Welikala et al. (2018) determined the CEC for these same biosolids as 31 cmol kg^{-1}, which was 5 times higher than the CEC of soil collected from the same formation as our experiment (Craigieburn soil, 6.5 cmol kg^{-1}; Welikala et al. 2021) and mixing them may have resulted in an overall increase in the soil CEC. This agrees with previous research that showed that incorporation of biosolids into the soil increased the CEC (Gardner et al. 2010; Price et al. 2015). Increased CEC in the mixed treatments may have mitigated acidification both directly, through buffering against pH change, and indirectly by binding NH_4^+ and reducing its availability to nitrifying bacteria, when compared to the top treatment (cf. lower NO_3^- concentrations in the mixed treatments).

DHA in the soil increased with increased biosolids application rate if they were incorporated into the soil. These results are consistent with findings from previous research (i.e. Gardner et al. 2010; Gutiérrez-Ginés et al. 2017). Interestingly however, when biosolids were applied on the surface, DHA only increased with the lowest application rate (Eq. 16 t ha^{-1}) but significantly decreased with higher application rates (Fig. 2). This indicates that indigenous microbes of the soil were negatively affected by low pH, increased EC, increased exchangeable Al and Mn, and potentially anoxic conditions. The addition of biosolids in the soil might shift the microbial communities to favour biosolids-borne microbes at the expense of the soil indigenous ones. Sullivan et al. (2006), Hu et al. (2019), and Wang et al. (2020), amongst others, demonstrated that biosolids change the soil microbial communities.

The rhizosphere effect and potential to reduce export of contaminants

As expected, the rhizosphere had a significant positive effect in the availability of most plant nutrients and microbial activity. These results mostly agree


Control
Top
Mixed
Patch
Exch P (mg kg-1)
0.71 ± 0.12
54 ± 5.9
1.5 ± 0.10
1.1 ± 0.08
2.1 ± 0.11
0.92 ± 0.07
1.3 ± 0.12
5.2 ± 0.55
0.18 ± 0.03
0.19 ± 0.02
0.19 ± 0.02
0.31 ± 0.02
0.13 ± 0.02
0.27 ± 0.05
0.27 ± 0.02
0.03 ± 0.01
0.01 ± 0.00
0.02 ± 0.01
0.01 ± 0.01
0.03 ± 0.01
0.04 ± 0.01
NO3- - N (mg kg-1)
< 0.01
131 ± 30
75 ± 15
72 ± 15
50 ± 13
< 0.01
6.1 ± 3.4
18 ± 4.0
< 0.01
32 ± 6.4
20 ± 2.6
56 ± 10
< 0.01
19 ± 4.5
38 ± 7.9
< 0.01
20 ± 3.7
58 ± 9.4
1.2 ± 0.6
28 ± 3.4
48 ± 19
NH4+ - N (mg kg-1)
15 ± 0.45
453 ± 75
28 ± 2.7
25 ± 2.6
21 ± 1.3
16 ± 0.6
30 ± 2.2
2.3 ± 0.4
7.9 ± 1.3
8.3 ± 2.4
18 ± 3.5
2.4 ± 0.12
30 ± 11
87 ± 13
0.49 ± 0.33
2.5 ± 0.60
3.3 ± 1.2
0.97 ± 0.50
7.9 ± 1.2
64 ± 13
pH
5.6 ± 0.04
5.9 ± 0.09
4.8 ± 0.02
5.1 ± 0.04
5.3 ± 0.04
5.6 ± 0.05
5.3 ± 0.06
5.8 ± 0.06
5.4 ± 0.02
5.1 ± 0.03
5.2 ± 0.02
4.9 ± 0.03
5.4 ± 0.03
5.2 ± 0.01
5.0 ± 0.09
5.6 ± 0.04
5.4 ± 0.02
5.1 ± 0.06
5.6 ± 0.06
5.2 ± 0.07
5.2 ± 0.07

◀**Fig. 5** Representation of mean ± standard error of exchangeable P, NO_3^--N, NH_4^+-N (mg kg^{-1}), and pH in each of the soil horizons (A: upper layer, B: middle layer, C: lower layer) and quadrants of the rhizobox experiment. The shaded areas represent the location and relative concentration of the biosolids in each rhizobox. Note that samples were collected and analysed separately per quadrant, but quadrants with similar results are represented together for simplicity (i.e. each horizon in Control, Top and Mixed, and adjacent quadrants in A horizon in Patch)

with Liu et al. (2022) who showed a generally higher concentration of available nutrients and microbial biomass in the rhizosphere of most plants. Microbial activity around plant roots is usually higher than in bulk soil, due to the synergies between plants and associated microorganisms (McNear Jr 2013). This explains the higher DHA in the rhizosphere vs. bulk soil (Fig. 6). Higher concentration of NH_4^+ and NO_3^- in the rhizosphere were also similar to previous works (Liu et al. 2022) and agree with observations of increased mineralization of organic matter and associated nutrient release in the rhizosphere of plants (Keiluweit et al. 2015; Liu et al. 2022). Our results confirm the hypothesis of a higher NH_4^+:NO_3^- ratio in the rhizosphere indicating a slower nitrification around *L. scoparium* roots compared with bulk soil, as reported by Esperschuetz et al. (2017). Higher exchangeable P in the rhizosphere of *L. scoparium* contrast with general trends for plants (Liu et al. 2022) with high demand for P, however it was similar to other trees and herbaceous plants (Liu et al. 2022). This higher mobilization of nutrients around the roots can explain the significantly higher EC in the rhizosphere compared with bulk soil. Notably, the pH was significantly higher in rhizosphere than in bulk soil. This contrast with previous results showing that the roots of *L. scoparium* can decrease 1 unit soil pH compared with *Lolium perenne*. However, given the low pH of the soil and soils amended with biosolids in our experiment, the increase of pH by *L. scoparium* roots is consistent with the meta-analysis of Liu et al. (2022), who demonstrated that the differences in pH in rhizosphere compared with bulk soil were dependent on the original soil pH, and that plants had a neutralizing effect both in acidic and alkaline soils. Lower concentrations of exchangeable Fe and Mn in the rhizosphere can indicate a depletion of these micronutrients, indicating a high demand by *L. scoparium*, also occurs for P in some plants (Liu et al. 2022). The high Mn demand by *L. scoparium* was indicated by the high foliar Mn concentration in plants grown in these experiments (Gutiérrez-Ginés et al. 2019), and previous ones (Reis et al. 2017).

Even with the strong rhizosphere effect shown by these experiments, it is unlikely that only roots can manage the export of contaminants from biosolids amended soil. As demonstrated by the rhizobox experiment (Fig. 5 and Supp. Material), NO_3^-, NH_4^+, S and Al can reach deeper soil horizons. Gove et al. (2002) demonstrated no difference in NO_3^- leaching between types of biosolids application in their column experiment. Our rhizobox results partially supported those observations with similar NO_3^- concentrations in the horizons immediately below the biosolids. However, the results in the deeper horizons showed that when biosolids were mixed on the surface - either homogeneously or concentrated in a patch - the NO_3^- was higher under these treatments than when the biosolids were applied on the surface. On the contrary, Gove et al. (2002) showed an increased P leaching when biosolids were applied in the subsurface compared with surface application, which is opposite to our results showing that the exchangeable P in the deeper soil horizons was similar in all treatments including control.

Insights for management of biosolids land application

Our experiments showed that both rate and type of biosolids application can profoundly affect soil quality. Although it is not possible to extrapolate these results to field conditions to provide guidance for biosolids application, they are still valuable to raise concerns about effects on soil quality that they have not been demonstrated previously.

Rate of biosolids application was the most important factor affecting the quality of soil, as demonstrated by the results on fertility, exchangeable trace elements, and biological activity (DHA). We agree with Borden and Black (2011) and Fuentes et al. (2010), to suggest that application rates <45 t ha^{-1} are recommended. Higher application rate in this experiment (Eq. 145 t ha^{-1}) produced acidification of the soil and solubilisation of Al when biosolids were applied to the surface, decreasing biological activity in the soil. However, when biosolids were mixed into the soil, the exchangeable concentration of P, Zn and Cu could be deleterious for plant health. Given that

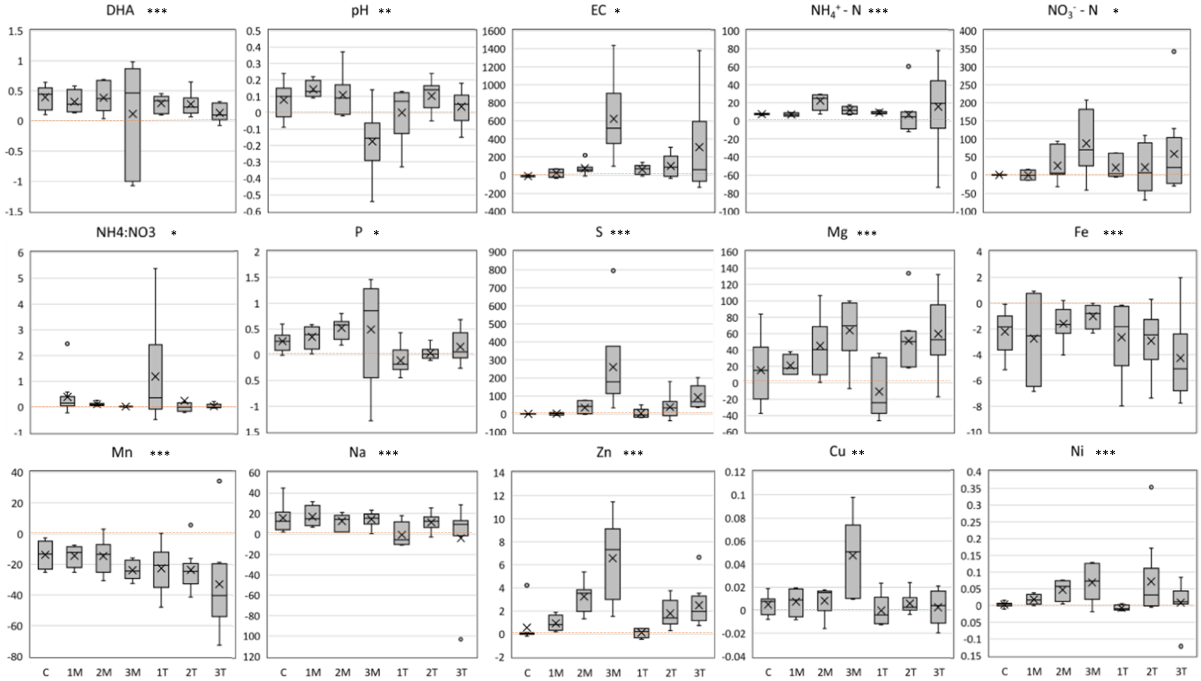


Fig. 6 Boxplots of the results of pairwise comparisons between rhizosphere soil and bulk soil (rhizosphere - bulk) in each of the treatments in the pot experiment. The *x*-axes show the different treatments: (C) control without biosolids, (M) biosolids incorporated into the soil, (T) biosolid applied on the surface, with the numbers 1–3 reflecting the increasing rates of biosolids application. Where significant differences between rhizosphere and bulk in the pairwise t-test were identified, they are represented by * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) next to the analysed parameter

L. scoparium decreased plant growth in the highest application rate (Eq. 145 t ha^{-1}) compared with lower rates (Eq. 16 t ha^{-1} and 48 t ha^{-1}), regardless of type of application (Gutiérrez-Ginés et al. 2019), it is obvious the deleterious effects on soil quality caused by the two types of application were similarly negative for this plant species.

Given the low nutrient requirements by *L. scoparium* (Stephens et al. 2005), or New Zealand native plants in general, the lowest application rate (Eq. 16 t ha^{-1}) would ensure the maintenance of a good biological activity in the soil regardless the type of application, still producing a significant increase in the plant biomass (Gutiérrez-Ginés et al. 2019). At the lowest application rate there were not significant differences in soil quality between surface or mixed application of biosolids (Fig. 2 and Supp. Material). Alternatively, heterogeneous application of biosolids in distinct zones in the soil (Fig. 5), through soil management practices such as strip tilling, could provide the benefits of incorporated biosolids and the maintenance of areas of low fertility and indigenous microbial community. Previous research (Gutiérrez-Ginés et al. 2019) showed that the root foraging behaviour of *L. scoparium* favours this heterogeneous application, still supporting an increased growth of this plant compared with unamended soil. This compromise may be more beneficial for restoring natural habitats by creating a variety of niches (Simcock et al. 2019). However, the results of the rhizobox experiment showed that this type of application may favour the movement of N and other nutrients such as K, Mg, S and Mn to deeper horizons by the high concentration of biosolids in a small area. Further research in real conditions is needed to better understand how different distribution of biosolids can affect soil quality and contribute to a more realistic restoration of natural ecosystems (Simcock et al. 2019). For creating guidance about the best methods for applying biosolids to restore degraded land, the effect of rainfall or irrigation need to be considered to better assess the real movement of contaminants to groundwater.

Conclusions

This is the first work that compare the effects of rate and type of biosolids application on soil quality in the same experiment. Our results showed that the surface application of biosolids increased the fertility of the soil (higher inorganic N in soil and soi solution, and K and P in soil solution), and significantly decreased soil pH compared with the treatments when biosolids were incorporated into the soil. The acidic conditions might have mobilised Al, as shown by higher exchangeable Al, and inhibited nitrification, increasing the accumulation of NH_4^+. Anaerobic microsites created by accumulation of organic matter on the surface of the soil, could also be linked to the high NH_4^+ concentration and Mn mobilisation. The potential increase in CEC when biosolids were incorporated might have buffered the pH reduction seen on the surface applied biosolids treatments. These changes in soil chemistry can help to explain the decrease in DHA when biosolids were applied on the surface compared with the unamended soil. The contrast with the increase of DHA when biosolids were mixed indicates an enhanced biological activity by biosolids-born microbes. Soil microbial activity was higher in the rhizosphere compared with bulk soil, where the concentration of plant nutrients and most of trace elements was also higher. Better understanding the biogeochemical changes happening in these biosolids altered systems requires further research.

Although the results of higher NH_4^+: NO_3^- ratio in rhizosphere than in bulk soil support the hypothesis of inhibition of denitrification by these root systems, this potential was not enough to prevent mobilization of these compounds to deeper soil horizons.

These results could inform decisions about biosolids application for ecological restoration purposes. The results suggest that application rates of Eq. 16 t ha^{-1} would provide an increase in fertility and biological activity in the soil, without the negative effects of increased soil acidity if biosolids were surface applied, or increased exchangeable Zn, and Cu if they were mixed into the soil. Heterogeneous distribution of biosolids could be a good method to increase microsites to favour diversity in the soil. However, potential export of nutrients to deeper soil horizons is a concern. Future research should focus on comparing these types of biosolids application in real conditions to better assess the benefits in recovering soil biodiversity and plant growth while minimizing contaminant exports to groundwater or plant uptake.

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Author contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by María J Gutiérrez-Ginés, with support from Niklas Lehto, Engracia Madejón and Brett Robinson. Data analysis and the first draft of the manuscript were prepared by María J Gutiérrez-Ginés and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The original data is provided as Excel sheets in Supplementary Material.

Declarations

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

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