Nitrogen induced DOC and heavy metals leaching: Effects of nitrogen forms, deposition loads and liming[☆]

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ABSTRACT

Atmospheric nitrogen (N) deposition is believed to accelerate dissolved organic carbon (DOC) production and could lead to increased heavy metal mobility into water resources. We sampled intact soil cores from the Isle of Skye with low background N deposition history and having Serpentine rock known for its higher heavy metal concentrations including zinc (Zn), copper (Cu), nickel (Ni) and lead (Pb). The effects of 16 (16kgN) and 32 kg N ha⁻¹ year⁻¹ (32kgN), and liming with 32kgN (32kgN+Lime) on soil solution chemistry and heavy metal mobilization were investigated over the 15-month study. Nitrogen in deposition load was added at five ammonium (NH₄⁺) to nitrate (NO₃⁻) ratios of 9:1, 5:1, 1:1, 1:5 and 1:9 along NO₃⁻ dominance. We found significant effects of load on Cu and NH₄⁺/NO₃⁻ ratio on pH, DOC and Zn in soil solution. However, under lime and ratio experimental factors, liming significantly influenced pH, DOC, Cu and Pb, and NH₄⁺/NO₃⁻ ratio pH, DOC, Ni and Zn whereas interactions between lime and ratio was significant for Ni and Cu. pH and DOC increased with N load, liming and NO₃⁻ dominance, and both correlated significantly positively. Liming under NH₄⁺ dominance enhanced DOC production due to supply of base cations in lime. Mobilization of Cu, Ni and Pb was driven by DOC concentrations and, therefore, increased with load, liming and NO₃⁻ dominance in deposition. However, in contrast, low pH and high NH₄⁺ dominance was associated with Zn mobilization in soil solution. On the contrary, despite of some patterns, heavy metals in soil HNO₃ extracts were devoid of any load, lime and NH₄⁺/NO₃⁻ ratio effects. Our study suggests that the effects of N load and forms in deposition on sites with high accumulated loads of metals need to be better quantified through soil solution partitioning models.

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1. Introduction

The global nitrogen (N) cycle has dramatically changed since the onset of industrial N fixation in 1909 (Galloway et al., 1995; Ussiri and Lal, 2013; Erismann et al., 2018). Total N deposition to non-forest ecosystems in the UK showed a larger spatial variation and ranged from high (25–35 kg N ha⁻¹ yr⁻¹) to very low (3–5 kg N ha⁻¹ yr⁻¹) (Andersson et al., 1994a). Nitrogen deposition in the

United Kingdom (UK) fell by about 15% between 1988 and 2008, but it was unclear if soil processes were recovering due to decrease in N deposition, as the highest rates of N emissions and deposition dominated for four consecutive decades in the 20th century (Fowler et al., 2004a; Oenema et al., 2011; RoTAP, 2012). According to the scenario analyses, the European areas at risk from the N deposition would decrease from 75% (in 1980) to 55% (in 2020) under the amended Gothenburg Protocol (Maas and Grennfelt, 2016). Thus, in 2020 more than 50% of the European Nature Information System (EUNIS) classified ecosystem areas are still expected to face the risk of excessive nutrient N deposition (EEA, 2014a). This decline appears to be late to prevent and fully converse the destruction caused by the elevated N deposition of 3000–5000 kg N ha⁻¹ from

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1900 to 2000 in the most N-polluted regions of the UK (Fowler et al., 2004b). Despite the considerable decreases in N emissions (Matejko et al., 2009), the future deposition rates modelled for 2020 suggests that critical N loads will continue to exceed in about half the area of the most sensitive ecosystems in the UK (Matejko et al., 2009; RoTAP, 2012; Bobbink et al., 2015).

Nitrogen deposition occurs in two major forms: a reduced, mainly as ammonia (NH_3) or ammonium (NH_4^+) and an oxidized form dominated by nitrate (NO_3^-) (Aneja et al., 2001). While, the emissions of oxidized N in the UK declined by 58% during the 1988–2008 period, those of reduced N only declined by 21% (RoTAP, 2012). High emissions of reduced N are associated with regions of intensive agricultural activities, while regions of high emissions of oxidized N are associated with high vehicular and/or industrial activities (Decina et al., 2020). Hence, the proportion of N deposition that is in the oxidized or reduced form shows strong spatial variations across the UK and other European countries, and is also changing over time (van den Berg et al., 2016). For example, the UK shows a large spatial range in $\text{NH}_4^+/\text{NO}_3^-$ deposition ratios, from 0.3 mol mol⁻¹ to 3 mol mol⁻¹ (Fowler et al., 2004a). It is, therefore, imperative to understand the effects of different forms of N deposition on soil biogeochemical processes, toxicity mechanisms and ecosystem functions (Sheppard et al., 2004; Huang et al., 2015; Chen et al., 2016). Such improved understanding would allow sites and habitats at greatest risk to be more reliably identified, and hence, provide a greatly effective evidence base to adopt better protection measures (Stevens et al., 2011; Li et al., 2016). Different N forms may have differential direct and indirect effects on N sensitive vegetation such as bryophytes and vascular plants resulting from preferential uptake mechanisms, toxicity and through changes in soil chemistry (Fleischer et al., 2015; Binkley and Hogberg, 2016).

Soil acidification and soil buffering capacity, measured as the acid neutralizing capacity (ANC), are considered to play an important role in defining the soil-plant responses to N deposition. Moreover, N mobility is partly driven by dissolved organic carbon (DOC), which is a potentially significant mechanism of C export from the soil system under highly organic conditions (Kaiser, 2001; Jiang et al., 2016). Atmospheric N deposition may be associated with the increased DOC production through increased microbial activity which can facilitate transport of metals through formation of DOC-metal complexes (Yang et al., 2013; Hu et al., 2018). Hence, increased N deposition may result in increased metal transport to deeper ground water layers (Stevens et al., 2009). The soils with high metal concentrations, slightly acidified and depleted of base cations, may be expected to experience higher metals concentrations in soil solution through both the decreased pH and the increased DOC concentrations (Tipping and Hurley, 1992; Jones, 1998; Goulding, 2016).

The objectives of this greenhouse mesocosm study were to investigate the effects of two N deposition loads, 16 (16kgN) and 32 kg ha⁻¹ year⁻¹ (32kgN) applied as five ammonium to nitrate ($\text{NH}_4^+:\text{NO}_3^-$) ratios and liming, at high N load (32kgN+Lime), on changes in soil solution chemistry and their influence on nickel (Ni), copper (Cu), zinc (Zn) and lead (Pb) in porewater. Our core hypothesis was that the changes in soil solution pH, DOC, NH_4^+ and NO_3^- would mobilize Ni, Cu, Zn and Pb differently because:

- 1) Cu and Pb are primarily associated with DOC and their concentrations in porewater are higher when NO_3^- dominates N deposition load; and,
- 2) Ni and Zn increase with acidity and, therefore, Ni and Zn concentrations in porewater are higher when NH_4^+ is dominant in deposition.

2. Materials and methods

2.1. Study site characteristics

The intact monoliths for this greenhouse study were selected from the Isle of Skye, Scotland (57.2736° N, 6.2155° W; UK grid ref: GR 408224). The site has long low N deposition history of receiving an estimated 1–3 kg N ha⁻¹ year⁻¹. The mean monthly temperature at the site is 8.5 °C and has 112 mm mean monthly rainfall whereas the total rainfall at the site is 1348 mm. The underlying rock is serpentine in the area known for higher concentrations of heavy metals including Cu, Ni, Zn and chromium (Alexander, 2004). This site, therefore, provided a unique opportunity to develop an experimental set-up to test our hypothesis that NH_4^+ and NO_3^- deposition have differential effects on heavy metal leaching through changes in soil solution pH and DOC.

2.2. Intact core samplings and greenhouse experiment

The mesocosms for this study were developed by collecting intact monoliths, including both the soil and vegetation, in April 2009 from a wet-heath site (NVC M15 wet heath) on the Isle of Skye, UK (57.2736° N, 6.2155° W). Within uniform patch of M15 vegetation area, 96 PVC cores (25 cm height and 20 cm diameter) were inserted into the peat while avoiding any damage to the vegetation. The monoliths were excavated to get intact peat cores with vegetation which were taken to greenhouse facilities at the York University (53.9461° N, 1.0518° W) for the mesocosm experiment. The greenhouse was selected to avoid outside variations in temperature and, because of generally warmer conditions in glass house, evaporation was controlled by watering with distilled water, especially in summer months. Soil monoliths, with intact vegetation, were fitted into a plastic bottom with outlet to maintain porewater levels at 5 cm below field level. Each core was fitted with a Rhizon samplers (Eijkelkamp, The Netherlands) at 10 cm below the surface of core. Nitrogen deposition was simulated using ammonium chloride (NH_4Cl) for NH_4^+ and sodium nitrate (NaNO_3) for NO_3^- in artificial rainwater which was applied three times a week. Artificial rainwater contained deionised water with macro- and micro-nutrients at background concentrations: 66 μmol L⁻¹ Na⁺, 77.5 μmol L⁻¹ Cl⁻, 7.5 μmol L⁻¹ Mg²⁺, 4 μmol L⁻¹ SO₄²⁻, 1.5 μmol L⁻¹ Ca²⁺ and 1.4 μmol L⁻¹ K⁺ (van den Berg et al., 2005). Five different $\text{NH}_4^+/\text{NO}_3^-$ ratios of 1:9, 1:5, 1:1, 5:1 and 9:1 (mol:mol) were tested in simulated atmospheric N deposition at two N loads of 16 (16kgN) and 32 kg N ha⁻¹ year⁻¹ (32kgN). These N deposition loads represented a gradient in ammonium dominance to nitrate dominance and corresponded to the critical N load and the highest ambient N deposition rates in the UK for this ecosystem. In addition, these five $\text{NH}_4^+/\text{NO}_3^-$ ratios were also tested at a fixed N deposition load of 32 kg N ha⁻¹ year⁻¹ with a lime (32kgN+Lime) (CaCO_3 80%, MgCO_3 10%) added at 100 g m⁻² rate as a powder to the soil surface at the start of the experiment. Lime was only added once, assuming that it was enough to compensate for base cation depletion throughout the experiment without changing the soil pH too much. In this fully factorial experimental design, each treatment was replicated six times and experiment consisted of 90 intact monoliths in total (Table S1).

2.3. Soil solution sampling and analysis

Porewater samples were taken with rhizone samplers, fitted at 10 cm depth, six times (June 2009, September 2009, January 2010, April 2010, July 2010, and September 2010) over the 15-month experiment from June 2009 to Sep 2010. Soil solution pH were measured with a pre-calibrated Thermo Orion 420 pH meter (Riaz

et al., 2009).

The porewater samples were analyzed for NH_4^+-N and NO_3^--N concentrations on an auto-analyzer (Bran + Luebbe Auto Analyzer 3, Germany) using a matrix-matched standards protocol within 24 h of collection. Ammonium nitrate standards of 0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1} were prepared in distilled water and 2.0 ppm standard was always used as drift standard.

Concentrations of DOC in soil solution samples were determined using a TOC elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Prior to analysis, samples were filtered through 0.45 μm Whatman membrane filter papers. Basic stock solution containing 500 mg L^{-1} TIC (sodium carbonate as TIC standard) and 500 mg L^{-1} TOC (potassium phthalate as the TOC standard) was prepared. Intermediate stock solutions were prepared to produce calibration standards having 1, 2.5, 5, 10, 20, and 50 mg L^{-1} DOC concentrations.

The soil solution samples for metal analysis were acidified by adding 100 μL 70% nitric acid (HNO_3) to every 10 mL sample so precipitation in the samples was avoided and samples were storing at 4 $^\circ\text{C}$ until analysis.

2.4. Soil sampling and digestion

Soil samples from the 36 cores received 16kgN, 32kgN and 32kgN + Lime at 9:1 and 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratios were taken in October cutting at least 70 g fresh soil from the top 15 cm of peat in each core. The soil samples were transferred to the laboratory in an ice box and stored at 4 $^\circ\text{C}$ until analysis. Soils were stored in airtight plastic bags, from which as much air as possible was removed. The roots and shoots were removed and soils were mixed thoroughly by hand for homogenized samples to avoid internal variation between sub samples. Ten-gram of these soil subsamples were dried in an oven at 105 $^\circ\text{C}$ and ground into fine-powder using a Retsch MM400 Ball Mill (Retsch GmbH, Germany). Acid digestion was carried out by adding 4 mL 65% nitric acid (HNO_3) and 1 mL 30% hydrogen peroxide (H_2O_2) to 200-mg of each sample in a Milstone microwave (type MLS 1200 mega, Milstone Inc., Sorisole, Italy) following procedure described by Zia et al. (2018). After filtering through Whatman#42 filter papers, digests were diluted with deionised water to 100 mL volume and kept at 4 $^\circ\text{C}$ until analysis.

2.5. Analysis of heavy metals

Concentrations of Ni, Cu, Zn and Pb were analyzed on ICP-MS and ICP-OES. Zn, was determined using an ICP Spectrometer (IRIS Intrepid II, Thermo Electron Corporation, USA) whereas Ni, Cu and Pb were analyzed using ICP Spectrometer (X series; Thermo Fisher Scientific, Waltham, MA, U.S.A). Blanks were used to correct for extraction buffer quality and reference solutions for quality checks on the concentrations. Where the blank values were significantly above zero, they were subtracted from the measured values. The drift and QC control were achieved by the ICP-computer program which was set at a level of 2.5%. If the ICP found that the standards and reference solutions diverted by more than 2.5%, it gave a "fail" message and the samples were reanalyzed consequently. The instrumental drift was also corrected. The standards used were multiple element stock standards of a specific concentration (2, 10 and 50 ppb for Ni, Cu, and Pb; 50 ppb and 500 ppb for Zn).

2.6. Quality control (QC) and quality assurance (QA) protocols

In addition to the already mentioned QC measures, standard procedures were always adopted appropriately to maintain QC and QA throughout the analysis. Chemical reagents and procedural blanks were analyzed in parallel to the samples using similar

procedures. Calibration curves were optimized by application of quality control standards at each step during analysis. All chemicals and reagents used in analyses were of analytical grade and purchased from Merck (Germany) or BDH (UK) with 99.9% certified purity.

2.7. Statistical analysis

Data were tested for normality and homogeneity of distribution. If required, data were log-transformed prior to applying parametric statistical analyses. The effects of load, ratio and their interactions on soil solution chemistry and heavy metal contents in HNO_3 extracts, were tested using a two way-analysis of variance (ANOVA) procedure. A similar analysis was conducted for the effects of liming, ratio and their interactions. This design meant that the data for the 32kgN treatment were included in both ANOVAs, but it was deemed to be the most effective way of testing the original hypotheses. However, we also compared the soil solution chemistry parameters and heavy metals in soil HNO_3 extracts at each ammonium/nitrate ratio between 16kgN, 32kgN and 32kgN+Lime using one-way ANOVA. In addition, means of soil solution chemistry were tested for significance of differences between 16kgN, 32kgN and 32kgN+Lime treatments following one-way ANOVA. Tukey's HSD post-hoc test was used for multiple mean comparison techniques, when required. Bivariate correlation plots were developed to study the relationships between soil solution chemistry variables and heavy metal concentrations. All statistical analysis was carried out in SPSS for Windows v. 19 software.

3. Results

3.1. Effects on soil porewater chemistry

Table 1 shows the two-way ANOVA results concerning the effects of N load, ratios and their interactions on soil solution chemistry. N load had significant effects on Cu concentrations only whereas N ratios significantly altered pH, DOC and Zn concentrations in porewater. However, interactions between load and ratio were not significant for any porewater chemistry parameters. Two-way ANOVA indicated that effects of liming were significant on pH, DOC, NO_3^- , Cu and Zn concentrations, and for those of ratio were significant on pH, DOC, Ni and Zn (Table 2). Interactions between lime and ratio were significant for Ni and Cu concentrations only.

Data about the effects of 16kgN, 32kgN and 32kgN+Lime on soil solution pH, DOC, NH_4^+ and NO_3^- concentrations at each $\text{NH}_4^+/\text{NO}_3^-$ ratio are shown in Fig. 1. Liming significantly increased pH than 16kgN and 32kgN at each $\text{NH}_4^+/\text{NO}_3^-$ ratio and the highest pH was noted at 32kgN+Lime treatment at 1:1 $\text{NH}_4^+/\text{NO}_3^-$ ratio (Fig. 1a). Effects of 16kgN and 32kgN on pH were generally non-consistent, however, at 1:5 and 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratios, pH increased with N

Table 1

Analysis of variance of effects of N load, ratio and their interactions on soil solution pH, DOC, NH_4^+-N , NO_3^--N and metal concentrations over the period from June 2009 to September 2010.

Treatment		pH	DOC	NH_4^+-N	NO_3^--N	Ni	Cu	Zn	Pb
Load	F	0.28	1.22	1.39	1.03	0.24	3.96	3.12	1.76
	P	0.60	0.27	0.24	0.32	0.63	0.05	0.08	0.19
Ratio	F	12.9	5.82	0.66	1.23	0.70	1.85	4.82	0.15
	P	0.00	0.00	0.62	0.31	0.60	0.14	0.00	0.96
Load*Ratio	F	2.20	2.05	0.89	1.52	1.19	1.80	2.42	1.66
	P	0.09	0.10	0.48	0.21	0.33	0.15	0.06	0.18

Bold values indicate level of significance at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Table 2
Analysis of variance of effects of liming, ratio and their interactions soil solution pH, DOC, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and metal concentrations over the period from June 2009 to September 2010.

Treatment		pH	DOC	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	Ni	Cu	Zn	Pb
Lime	F	35.6	6.37	0.22	5.89	1.45	8.88	8.56	0.02
	P	0.00	0.01	0.64	0.01	0.23	0.01	0.00	0.90
		***	*		*		*	**	
Ratio	F	11.3	3.19	1.40	1.48	3.22	0.45	10.0	0.41
	P	0.00	0.02	0.25	0.23	0.02	0.77	0.00	0.80
		***	*			*		***	
Lime*Ratio	F	1.76	0.74	0.08	1.96	2.79	4.09	1.05	1.41
	P	0.15	0.57	0.99	0.12	0.03	0.00	0.39	0.24
						*	**		

Bold values indicate level of significance at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Fig. 1. Effects of ammonium/nitrate ratios on soil solution a) pH, b) DOC (mg L^{-1}), c) $\text{NH}_4^+\text{-N}$ ($\mu\text{g mL}^{-1}$) and d) $\text{NO}_3^-\text{-N}$ ($\mu\text{g mL}^{-1}$) in 16kgN, 32kgN and 32kgN+Lime treatments over the period June 2009 to September 2010. Values are the means across the 16kgN, 32kgN and 32kgN+Lime. Error bars indicate standard errors of means (n = 30). Bars under each ratio with different letter show significant differences between treatments at $P < 0.05$.

load. pH was low at NH_4^+ dominance and increased when NO_3^- dominated N loads particularly for the 32kgN and 32kgN+Lime treatments. Liming also increased DOC concentrations compared to no-limed N treatments at all NH_4^+ and NO_3^- ratios except for 1:5 ratio, however, effects were significant at 9:1 ratio only (Fig. 1b). DOC concentrations were higher towards NO_3^- dominance mainly for 16kgN and 32kgN but the trend in effects was not very consistent in 32kgN+Lime treatment. NH_4^+ concentrations in soil solution increased with N loads and liming with exceptions to the 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratio where reverse effects were found (Fig. 1c). However, due

to larger variations in the data, the comparisons between $\text{NH}_4^+/\text{NO}_3^-$ ratio were not significant and conclusive. Variations in NO_3^- concentrations were similar to those observed for NH_4^+ , however, higher NO_3^- concentrations in 32kgN+Lime were significantly different than 16kgN and 32kgN treatments at 9:1 and 5:1 $\text{NH}_4^+/\text{NO}_3^-$ ratios (Fig. 1d). Moreover, excluding 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratio, NO_3^- concentrations were always higher in 32kgN+Lime treatments.

Differences in Ni concentrations in soil solution between 16kgN, 32kgN and 32kgN+Lime treatments at different $\text{NH}_4^+/\text{NO}_3^-$ ratios were not consistent (Fig. 2a). Ni concentrations in 16kgN and

Fig. 2. Effects of ammonium/nitrate ratios on soil solution a) Ni ($\mu\text{g mL}^{-1}$), b) Cu ($\mu\text{g mL}^{-1}$), c) Zn ($\mu\text{g mL}^{-1}$) and d) Pb ($\mu\text{g mL}^{-1}$) in 16kgN, 32kgN and 32kgN+Lime treatments over the period June 2009 to September 2010. Values are the means across the 16kgN, 32kgN and 32kgN+Lime. Error bars indicate standard errors of means ($n = 30$). Bars under each ratio with different letter show significant differences between treatments at $P < 0.05$.

32kgN+Lime treatments increased with NO_3^- dominance until 1:5 $\text{NH}_4^+/\text{NO}_3^-$ ratio. However, the effects of 1:5 and 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratios were not similar. Cu concentrations were higher in 32kgN+Lime than 16kgN and 32kgN treatments at 9:1, 5:1, 1:1 and 1:5 $\text{NH}_4^+/\text{NO}_3^-$ ratios but differed significantly at 5:1, 1:1 and 1:5 $\text{NH}_4^+/\text{NO}_3^-$ ratios only (Fig. 2b). Cu concentrations also increased with NO_3^- dominance in 32kgN treatments in contrast to those in 16kgN with little variations and values below $30 \mu\text{g L}^{-1}$ at all $\text{NH}_4^+/\text{NO}_3^-$ ratios. Zn concentrations decreased with NO_3^- dominance and N load and liming at each $\text{NH}_4^+/\text{NO}_3^-$ ratio (Fig. 2c). Effects of N load and liming

became more pronounced and significant at 1:1 and 1:5 $\text{NH}_4^+/\text{NO}_3^-$ ratios. Between 16kgN, 32kgN and 32kgN+Lime treatments at each $\text{NH}_4^+/\text{NO}_3^-$ ratio, 32kgN + Lime always had low Zn concentrations in soil solution. Concentrations of Pb showed varied influence of 16kgN, 32kgN and 32kgN+Lime, and $\text{NH}_4^+/\text{NO}_3^-$ ratios (Fig. 2d). At 9:1, 5:1 and 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratios, Pb concentrations in 32kgN were higher than 16kgN and 32kgN+Lime but significantly different at 9:1 $\text{NH}_4^+/\text{NO}_3^-$ ratio only. For the 16kgN and 32kgN+Lime treatment, NO_3^- dominance increased Pb concentrations up to 1:5 and 1:1, respectively.

Changes in soil solution pH, DOC, NH_4^+ , NO_3^- , Ni, Cu, Zn and Pb averaged over five $\text{NH}_4^+/\text{NO}_3^-$ ratios between 16kgN, 32kgN and 32kgN+Lime treatments are shown Fig. 3 pH in 32kgN+Lime was significantly higher than 16kgN and 32kgN, however, DOC concentrations varied significantly between 16kgN and 32kgN+Lime treatments (Fig. 3a and b). NH_4^+ and NO_3^- concentrations increased sharply with N load and liming but only 32kgN+Lime significantly increased NO_3^- concentrations (Fig. 3c and d). Concentrations of Ni, Cu and Pb were higher in 32kgN+Lime treatment and Zn was the lowest in this treatment (Fig. 3e,f,g,h). Cu concentrations increased

strongly with N load and liming in contrast to Zn which followed opposite trend (Fig. 3f and g).

3.2. Relationship between porewater variables

Bivariate correlation plots showed that soil solution pH was significantly positively correlated with DOC ($R^2 = 0.52$, $P < 0.01$; Fig. 4a) and NO_3^- concentrations ($R^2 = 0.41$, $P < 0.01$; Fig. 4c). Similar plots were drawn to show the relationships of soil solution pH, DOC, NH_4^+ and NO_3^- with Ni, Cu, Zn and Pb concentrations (Fig. 5).

Fig. 3. Mean values in the three load and lime treatments of a) pH, b) DOC (mg L⁻¹), c) NH_4^+-N ($\mu\text{g mL}^{-1}$), d) NO_3^--N ($\mu\text{g mL}^{-1}$), e) Ni ($\mu\text{g mL}^{-1}$), f) Cu ($\mu\text{g mL}^{-1}$), g) Zn ($\mu\text{g mL}^{-1}$) and h) Pb ($\mu\text{g mL}^{-1}$) over the period June 2009 to September 2010. Values are the means and contain standard error of means. Bars with different letters indicate significant treatment effects at $P < 0.05$.

Fig. 4. Relationships of pH with a) DOC (mg L^{-1}), b) $\text{NH}_4^+\text{-N}$ ($\mu\text{g mL}^{-1}$) and c) $\text{NO}_3^+\text{-N}$ ($\mu\text{g mL}^{-1}$) based on 15 different treatments in soil porewater. Data are mean values of 5 ratios within each of the 16kgN, 32kgN and 32kgN+Lime treatments.

Soil solution pH correlated significantly positively with Cu ($R^2 = 0.30$, $P < 0.05$; Fig. 5b) and significantly negatively with Zn ($R^2 = 0.66$, $P < 0.001$; Fig. 5c) whereas positive correlation with Ni (Fig. 5a) and Pb (Fig. 5d) were not significant. Ni concentrations showed significant positive correlation with DOC ($R^2 = 0.26$,

$P < 0.05$; Fig. 5e), however, significant negative correlation was observed between Zn and DOC concentrations ($R^2 = 0.41$, $P < 0.05$; Fig. 5g). None of the metals showed significant relationships with NH_4^+ concentrations (Fig. 5i,j,k,l). However, Cu was the only metal showing significant positive correlation with NO_3^- concentrations ($R^2 = 0.38$, $P < 0.05$; Fig. 5n).

3.3. Soil analysis

Two-way ANOVA results revealed non-significant effects of ratio, load, liming or their interactions on acid digest concentrations of any of the four metals (Table 3). As evident from ANOVA, the mean data showed no overall patterns in the effects of 16kgN, 32kgN and 32kgN+Lime treatments at 9:1 and 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratios on heavy metal concentrations in soil digests (Table 4). However, it can be deduced that Ni, Cu and Zn were higher in 32kgN treatment at 9:1 and 32kgN+Lime at 1:9 $\text{NH}_4^+/\text{NO}_3^-$ ratios, respectively but Pb concentrations, in contrast, were devoid of such patterns.

4. Discussion

In this study, the $\text{NH}_4^+/\text{NO}_3^-$ ratios significantly altered soil solution pH and with a high NH_4^+ to NO_3^- ratio, pH was lower than when NO_3^- ratios was dominant. This effect of a high NH_4^+ to NO_3^- ratio on soil solution pH has been reported earlier, and is more pronounced under acid conditions because the greater release of H^+ in soils decrease soil pH (Lucassen et al., 2003; van den Berg et al., 2005). During the nitrification process, two H^+ ions are release, which can accumulate and significantly reduce the pH of the soil. The NH_4^+ , therefore, decreases soil pH to a greater extent due to the nitrification process (Booth et al., 2005). However, at 1:1 $\text{NH}_4^+/\text{NO}_3^-$ ratios, pH in 32KgN was lower than 16kgN, an observation supported by Pilkington et al. (2005) who showed that soil pH decreased with N load containing balanced NH_4^+ and NO_3^- . This seems also important observation because in acidic grasslands nitrification process can be sustainably reduced and NH_4^+ can accumulate in soils (Carroll et al., 2003; Riaz et al., 2008; Mian et al., 2009). A recent global meta-analysis also predicted inhibition in nitrification with increase of $\text{NH}_4^+/\text{NO}_3^-$ ratios in N deposition which would result in NH_4^+ dominance and reduction in soil pH (Yan et al., 2019). As reported in our study, soil pH increased with N load and NO_3^- dominance in acidic ecosystems (van den Elzen et al., 2018). As expected, liming increased pH, however, this effect was somewhat lower when NH_4^+ dominated in N load which was also reflected from the significant effects of liming and $\text{NH}_4^+/\text{NO}_3^-$ ratios on soil solution pH. This was probably because liming was done only once, at the start of the experiment. The higher nitrification rates caused by a high $\text{NH}_4^+/\text{NO}_3^-$ ratios in deposition, and the fact that NO_3^- is less likely to be retained in the soils, is likely to result in greater soil acidification, and a greater reduction in the initial effect of the applied lime over time (Lazof et al., 1994; Corre et al., 2003; Ghnaya et al., 2007). Bäckman and Klemetsson (2003) also reported an increase in N availability and nitrification in acid coniferous soils under liming and high N deposition.

Increase in DOC concentration under the influence of N deposition in our study is consistent with previous findings (e.g. Bragazza et al., 2006; Smemo et al., 2007; Lu et al., 2011). Findlay (2005) also suggested that changes in pH from N deposition were potential drivers to explain observed increases in DOC export from ecosystems. The underlying mechanisms remain hypothetical as there are reports of decreased activity of oxidative enzymes in soils receiving N amendments which would result in greater production of intact phenolic compounds in the bulk DOC pool potentially available for export (Sinsabaugh et al., 2002). One consequence of such a change in carbon degradation patterns in soils would be a

Fig. 5. Relationships of pH, DOC (mg L⁻¹), NH₄⁺-N (µg mL⁻¹) and NO₃⁻-N (µg mL⁻¹) with Ni (µg mL⁻¹), Cu (µg mL⁻¹), Zn (µg mL⁻¹) and Pb (µg mL⁻¹) based on 15 different treatments in soil porewater (5 ratios within each of the 16kgN, 32kgN and 32kgN+Lime treatments).

Table 3
Analysis of variance of effects of load, ratio and their interactions, on metal concentrations in HNO₃ extracts of soils at the end of the experiment.

Factors	Statistics	Ni	Cu	Zn	Pb
Load	F	0.56	0.65	1.38	0.08
	P	0.47	0.43	0.26	0.78
Ratio	F	1.96	1.97	0.68	0.17
	P	0.18	0.18	0.42	0.69
Load*Ratio	F	0.59	1.44	0.03	2.36
	P	0.45	0.25	0.86	0.14
Lime	F	0.01	0.06	0.05	0.01
	P	0.91	0.81	0.83	0.91
Ratio	F	0.03	0.75	0.00	0.64
	P	0.87	0.40	0.97	0.43
Lime*Ratio	F	2.08	3.91	0.99	1.38
	P	0.17	0.06	0.33	0.26

decrease in decomposability for this pool of dissolved organic matter. No effects of N load on DOC concentrations were found in this study despite of significant effects of NH₄⁺/NO₃⁻ ratios on DOC

concentrations. While discussing the historical trends of DOC export to rivers in UK, [Worrall and Burt \(2007\)](#) also could not find clear relationships between N deposition and DOC concentrations whereas air temperature and atmospheric CO₂ concentrations seemed relatively more important drivers. [Evans et al. \(2008\)](#) reviewed data from 12 European and North American field N manipulation experiments and found inconsistent effects of N load to DOC, however, they reported strong control of N forms on DOC and showed that NO₃⁻ dominance consistently enhanced DOC in contrast to NH₄⁺ which generally decreased DOC. In another study, [Sawwicka et al. \(2017\)](#) used data from six sites from two monitoring networks across UK from 1993 to 2010 and employed MADOC model to predict that DOC concentrations would increase as consequence of N pollution. Liming increased DOC in our study, an observation consistent with other findings e.g. [Curtin and Simillie \(1983\)](#) noted positive effects of liming on soil organic matter concentrations in soil solution, and [Andersson et al. \(1994a, 1994b\)](#) found the similar effects under laboratory incubation experiment. Both liming and NH₄⁺/NO₃⁻ ratios had significant effects on soil solution pH and DOC in our study and, similar, positive correlation

Table 4

Effects of ammonium/nitrate ratio (9:1 and 1:9) on HNO_3 soil extract concentrations ($\mu\text{g kg}^{-1}$) of Ni, Cu, Zn and Pb at the end of experiment in the 16kgN, 32kgN and 32kgN + Lime treatments.

Treatments	Ni ($\mu\text{g kg}^{-1}$)		Cu ($\mu\text{g kg}^{-1}$)		Zn ($\mu\text{g kg}^{-1}$)		Pb ($\mu\text{g kg}^{-1}$)	
	9:1 ratio	1:9 ratio	9:1 ratio	1:9 ratio	9:1 ratio	1:9 ratio	9:1 ratio	1:9 ratio
16kgN	27.7 (4.72) a	20.9 (3.90) a	37.2 (5.84) a	35.4 (6.14) a	42.2 (2.66) a	38.3 (5.32) a	66.9 (4.89) a	55.9 (5.18) a
32kgN	44.1 (18.6) a	20.6 (5.84) a	53.6 (12.3) a	32.3 (5.41) a	50.4 (9.09) a	44.3 (4.86) a	54.7 (9.20) a	73.8 (14.7) a
32kgN + Lime	27.4 (8.47) a	39.9 (12.6) a	36.9 (4.72) a	45.3 (5.65) a	45.5 (3.85) a	52.1 (6.35) a	64.9 (7.84) a	61.3 (5.88) a

Standard errors of means are enclosed in parenthesis. For each metal, values under each ratio columns with different letters differ significantly at $P < 0.05$.

was found between soil solution pH and DOC which explained the increase in DOC concentrations along the NO_3^- dominance in N load. This also signified the negative effects of acidity on DOC production when NH_4^+ dominated N load. Changes in soil solution pH is the fundamental mechanism linked to increase in DOC concentrations after liming and N deposition (bAndersson et al., 1994b; Wang et al., 2016). Therefore, effects of rising pH and declining ionic strength on DOC solubility could have been potentially dominant mechanisms.

The soil solution pH readily affects chemistry of metals in soils, which influences the availability and plant uptake of micro-nutrients. Hence, it is clear that both the complexing capacity of organic acids and low pH are major factors related to mobilization of metals in soil (Kashem and Singh, 2001; McCauley et al., 2009). Therefore, soil solution pH determines mobility and bioavailability of heavy metals including Zn, Ni, Cu and Pb, and decrease in soil pH dramatically transform heavy metals from solid-phase to soil solution (Badawy et al., 2002; Zhao et al., 2010). In our study, for example, Zn partitioning between soil and soil solution was governed primarily by changes in pH because we found higher Zn concentrations at lower pH values that were recorded when NH_4^+ dominated in N load. However, the addition of lime decreased Zn concentrations in porewater due to less solubilization at higher pH values. In addition, increased NH_4^+ deposition affects the concentration of essential base cations such as K^+ , Mg^{2+} and Ca^{2+} by exchanging them on the cation exchange sites on the soil and, at low pH, by decreasing base cation uptake by plants (Boxman et al., 1991; Gloser and Gloser, 2000), and similar effects may apply to Zn in soil solution in our study. Leaching of base cations under the influence of N deposition is frequently reported, especially when NH_4^+ dominates deposition load (e.g. White and Cresser, 1998; Horswill et al., 2008; Cusack et al., 2016). Nickel, in contrast, showed no significant effect of N load but $\text{NH}_4^+/\text{NO}_3^-$ ratios and, lime and $\text{NH}_4^+/\text{NO}_3^-$ ratio interactions significantly affected Ni concentrations. There was also significant positive association of Ni with DOC and positive but non-significant correlation with pH. Similar to our observation, Rinklebe et al. (2016) found strong correlations between Ni and DOC concentration and predicted this association due to Ni-DOC complexes formation in serpentine-enriched fluviosol. As evident from significant N load, lime and, lime and $\text{NH}_4^+/\text{NO}_3^-$ ratio interactions effects on Cu concentrations and also positive but weak and non-significant correlations between Cu and DOC, Cu has been suggested to make stronger complexes with organic ligands compared to any other heavy metal (Lehman and Mills, 1994; Milne et al., 2003; Cuske et al., 2017). Moreover, Cu concentrations in soil solution in this study were significantly higher in cores when NO_3^- dominant deposition load and at higher concentrations of DOC in the 16kgN, 32kgN and 32kgN+Lime treatments. However, the same effects of DOC were not observed for Pb concentrations, for which no significant effects of N load, liming or $\text{NH}_4^+/\text{NO}_3^-$ ratios were found, despite of the positive insignificant correlations between DOC and Pb concentrations. This could be due to relatively low concentrations of both Pb and DOC in this experiment. We found that varying the $\text{NH}_4^+/\text{NO}_3^-$ ratios significantly affected both pH and

DOC concentrations in soil solution, however, these changes were not always associated with strong changes in heavy metal concentrations.

Analysis of the heavy metals data in HNO_3 soil extracts showed some patterns but no significant effects of N load, $\text{NH}_4^+/\text{NO}_3^-$ ratios, liming and their interactions. Therefore, we deduced that any treatment-related variations in soil metal concentrations had no significance for the interpretation of the soil solution data.

5. Conclusions

Our study showed that soil solution pH and DOC were lower when NH_4^+ dominated the N load and increased with N load and lime, and both pH and DOC increased with NO_3^- dominance and liming. pH and DOC also showed significant positive correlation. Application of base cations in the form of lime (Ca^{2+} and Mg^{2+}) resulted in larger increase of DOC leaching in those treatments where NH_4^+ was dominant and liming generally increased both pH and DOC in soil solution. Zn concentrations were significantly higher when NH_4^+ dominated and without liming suggesting pH as the major driver controlling its soil solution concentrations and evidence was further supported by the negative relationship between Zn and DOC. However, Cu concentrations were significantly higher when NO_3^- dominated N load and with liming, and a strong association was observed with DOC in soil solution. Metal mobilization of Ni, Cu and Pb was driven by DOC concentrations and, therefore, found to be higher in cores where NO_3^- was dominant in deposition load. On the other side, low pH and high NH_4^+ in N load were related to Zn mobility. Mean values in three N load and liming treatments showed their positive effects on soil solution pH, DOC, Ni, Cu and Pb whereas Zn concentrations decreased at higher N load and liming. However, despite some patterns in the effects of N loads and liming on heavy metal concentrations in soil HNO_3 extracts, none of them showed clear and significant effects. Our study concludes that both oxidized and reduced N species in deposition load could mobilize heavy metals in soil solution in low N input ecosystems and, liming ecosystems with historical N deposition, could further exacerbate the risk of heavy metal pollution of surface and groundwater resources. Therefore, the effects of N load along oxidized and reduced forms in deposition are required to be applied in a wider research programme that includes sites with high accumulated loads of metals so the impacts of N deposition forms are better quantified through soil solution partitioning models.

Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Afia Zia: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization, Writing -

review & editing, Funding acquisition. **Leon van den Berg:** Methodology, Resources, Writing - review & editing. **Muhammad Riaz:** Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Muhammad Arif:** Writing - review & editing. **Dania Zia:** Visualization, Writing - review & editing. **Shawana J. Khan:** Visualization, Writing - review & editing. **Muhammad Nauman Ahmad:** Visualization, Writing - review & editing. **Attaullah:** Writing - review & editing. **Mike Ahsmore:** Conceptualization, Resources, Supervision, Project administration.

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Appendix A. Supplementary data

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