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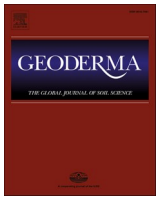

Field-based soil extractions capture more amino acids that are lost during short-term storage

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ABSTRACT

Aqueous soil extraction is a commonly used method to extract nitrogen (N) from soil. However, the disturbance of collection, transportation, and storage before extraction can potentially lead to mineralisation of extractable organic N pools, and as such may bias our interpretations of plant-available N towards inorganic N. Although disturbance through soil collection cannot be avoided, we evaluated the impact of short-term soil storage on water-extractable N pools, by extracting soils samples immediately after removal in the field, and again after overnight storage and extraction in the laboratory 24 h later. We chose five boreal forest soil sites within the Svartberget Research Area (northern Sweden). Soils were sampled across three seasonal time-points from June to September. We found that when measurements across all sites and time points were pooled, field-based extractions had significantly greater amino acid concentrations than lab-based extractions, contributing to greater soluble N concentrations (field extractions: 0.77 ± 0.07 μmol N/g soil DW; lab extractions: 0.17 ± 0.03 μmol N/g soil DW). Seasonal and site variation of amino acid concentrations was also much larger when soils were extracted in the field. Within sites, ammonium was often slightly elevated in lab-based extractions, but not to the same magnitude as reductions in amino acid concentrations, which we interpret as an overall N immobilisation effect during storage, likely through a combined effect of microbial utilisation of amino acids, and adsorption to the soil mineral phase. We found that negatively-charged and polar amino acid concentrations were most affected by storage – but the magnitude of loss of most amino acids was generally similar. Hydrolytic enzyme activity was correlated with total protein concentrations across all sites, this association was strongest in June, but was correlated equally with both lab and field extractions. In contrast, enzyme activity was not well associated with amino acids, regardless of extraction type, indicating that hydrolytic enzyme activity does not fully explain our observations of amino acids concentrations. We conclude that field extractions are a cheap and efficient way to capture higher resolution within organic N profiles of boreal soils during sampling, unmasking information that might be lost during storage.

1. Introduction

Organic nitrogen (N) is increasingly recognised as a critical pool of soil N that contributes to both microbial and plant N uptake (Daly et al., 2021; Farzadfar et al., 2021; Näsholm et al., 2009). In boreal soils, organic N forms like amino acids are a considerable source of N for plant species (Högberg et al., 2017), either directly or via mycorrhizal symbionts, given that net N mineralisation rates are outpaced by amino acid turnover rates (Jones and Kielland, 2012, 2002). It is then critical to ensure our measurements of soil N compounds are accurate representations of what is available for uptake by plant roots and soil microorganisms. Accurately measuring organic N requires sampling methods sensitive enough to evaluate availability without significant artefacts that might promote sample transformation.

Aqueous soil extraction (water extraction) is an efficient and common method for sampling dissolved N from soils, which is considered an active pool of soil N dynamically linked to sinks and sources within the microbial community, rhizosphere and greater soil environment. The forms and magnitudes of N sampled by water extractions differs greatly to that from salt extractions (e.g. potassium chloride, KCl, potassium sulfate, K_2SO_4), which are capable of sampling a much larger N pool available through ionic exchange at soil surfaces (Ros et al., 2009). Both water and salt extractions are easily producible and scalable with minimal cost outlay. However, extractions can be deceptively complex, with

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many variables resulting in differences in solute composition and abundance. Extraction time and temperature, soil-to-water ratios and soil pre-treatment (storage, sieving and air-drying) can have significant impacts on the amount of dissolved organic C and N extracted (Jones and Willett, 2006; Ros et al., 2009; Rousk and Jones, 2010). Changes in extractable N (with water and salt extractions) have been observed with greater soil pre-treatment (Bailey et al., 2021; Jones and Willett, 2006; Makarov et al., 2017; Sollen-Norrlin and Rintoul-Hynes, 2024), thought to be the result of soil structures being disturbed during sampling, increasing or decreasing solubility of absorbed N, which can be further transformed by the soil microbial community (Inselsbacher, 2014). In N-limited boreal soils, organic N forms such as amino acids can dominate available N pools, but are often masked by mineralisation promoted by soil disturbances (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012; Kielland et al., 2007; Rousk and Jones, 2010). *In situ* sampling tools like microdialysis (Buckley et al., 2020) circumvent many of the issues with soil disturbance, and subsequently have measured much higher fluxes of amino acids relative to inorganic N. This contrasts to a greater dominance of inorganic N in water extractions (Inselsbacher et al., 2011; Inselsbacher and Näsholm, 2012). However, *in situ* methods can be difficult to deploy at scale, and are far more costly – and so efficient ways of preserving the integrity of soil N profiles during sampling are desirable.

In this study, we evaluated whether organic N profiles can be preserved by extracting soils with water immediately after removal from the ground, in the field, using a short extraction time of 10 min to circumvent transformation promoted by lengthy processing and storage (Werdin-Pfisterer et al., 2009). We compared field-extracted N to paired samples that were transported back to the laboratory and extracted within 24 h, using the same extraction time and same water-to-soil ratios as in the field. Our study encompasses five boreal forest soil sites within the Svartberget Research Area (northern Sweden), sampled at three different timepoints during the active summer season (June, August, September), analysing organic N (total protein, 22 amino acids) and inorganic N (ammonium; NH_4^+; and nitrate; NO_3^-). We hypothesised that soils extracted in the laboratory would present with lower amino acid concentrations, and consequently higher inorganic N concentrations. Previous work has also highlighted strong correlations between amino acids and protease activity in several boreal soil sites (Kielland et al., 2007), and here we also investigate whether this relationship is apparent at our sites, and is affected by storage.

2. Materials and methods

2.1. Sampling site descriptions

Soil samples were collected from the organic horizon during the summer of 2020 at five different locations in the area of the Svarberget Experimental Forest, northern Sweden. Three of the sites (Svartberget A, B, C) were along an elevation moisture gradient at Högsvartberget (64°24′N, 19°75′E), representing moist (Svartberget A), mesic (Svartberget B) and dry soil (Svartberget C), located at the lowest, middle and top of the gradient. The vegetation at these sites were late successional mixed conifer forest (*Picea abies* (L.) Karst. and *Pinus sylvestris* (L.)) of dwarf shrub type with ericaceous shrubs ((*Vaccinium myrtillus* (L.) and *Vaccinium vitis-idaea* (L.)) (Larson et al., 2023). The fourth site was a ~100-year-old *Pinus Sylvestris* (L.) heath forest with dwarf shrubs, (*Vaccinium myrtillus* (L.) and *Vaccinium vitis-idaea* (L.)) at the Rosinedal Research area (64°16′N, 19°79′E) (Lim et al., (2015)). The fifth site was a >50-year-old *Betula pubescence* (Ehrh.) stand with understorey vegetation dominated by forbs and grasses near Storsandsjön (64°31′N, 19°74′E). All soils were podzols. The organic horizon at the conifer sites was composed of mor and the birch forest of moder. The annual mean precipitation at these sites is approx. 600 mm and the annual mean air temperature is 2 °C (Laudon et al., 2013). The soils contained 29–48 % C, 0.8–1.6 % N, measured using air-dried and ground soil samples on an Elemental Analyser – Isotope Ratio Mass Spectrometer (EA-IRMS, DeltaV, Thermo Fisher Scientific) (Werner et al., 1999). Soil pH was between 3.8 and 5.5 (Table 1), measured with pH electrode in a 1:5 soil: water (w/w) soil slurry. Mean soil air temperature and soil temperature (for the three sampling timepoints/seasonal mean) ranged between 17.2–21.1℃ and 11.0–12.7 °C (Table 1). See Supplementary Figs. 1 and 2 for detailed seasonal measurements for water content, air and soil temperature.

2.2. Soil sampling and extraction

At each soil site and sampling timepoint, a 1 m x 1 m quadrat was randomly placed on the forest floor. The quadrat had string evenly tied at 33 cm intervals along each perpendicular edge; sampling points were identified at each point where strings intersected another string or the frame, resulting in 16 sampling points. An additional three sampling points were identified by sampling at 1 m intervals along the plane of one edge of the quadrat – resulting in a total of 19 sampling points for each site and timepoint. To prepare for sampling, the superficial moss layer was carefully removed exposing the organic layer and the samples were collected from the top 3–5 cm of the organic layer with an apple corer (Cuisipro, Markham, Canada).

At each of the 19 sampling points within the quadrat, a pooled sample was removed and briefly homogenised in a plastic cup, before two 1 g of fresh soil samples were taken – one for immediate extraction (field extraction), and the other to be transported back to the laboratory for extraction the day after (lab extraction). This allowed for a paired comparison of each extraction type at the same sampling point. All samples were weighed using a portable digital scale (0.1 g resolution, max load 1000 g; Luxorparts, Kjell & Co., Sweden). For the field extraction, 1 g of fresh soil was placed in a 50 mL centrifuge tube and extracted with 7.5 mL (for Svartberget A and Birch), or 15 mL (for Svartberget B, Svartberget C, Rosinedal) of deionised high-purity water (MilliQ, Millipore). Different extraction volumes were chosen during sample collection at the first sampling timepoint (in June), as we discovered that some sites contained bulkier soils that required larger water volumes for extraction during shaking. Volumes for each site were maintained throughout each sampling timepoint. Samples were extracted for 10 min on a portable shaker (Heidolph DSG 304, Schwabach, Germany), at approx. 700 rounds per minute (Werdin-Pfisterer et al., 2009), powered by a small car battery via a 12 V power inverter. After extraction, samples were filtered using 0.2 μm syringe filters, with aqueous extractions stored on ice until freezing at −20 °C after transportation back to the laboratory.

Soil for lab extractions was stored on ice during transportation back to the laboratory and stored overnight at 4 °C in a cold room. Samples were extracted the following day (approximately 24 h after collection), in a similar manner to field extractions using the same scale and shaker as used in the field – however, filtered extract solutions were frozen at −20 °C immediately after processing.

2.3. Enzyme activity

Enzyme activity was measured the day after field collection at our laboratory, with soil samples stored overnight at 4 °C. Protease activity was measured as per Kandeler (1996), with some modifications. 0.5 g of fresh unsieved field-moist soil was mixed with 2.5 mL Tris-(hydroxymethyl)-aminomethane (THAM) buffer (50 mM, pH 8.1) and 2.5 mL of casein substrate solution (2 % w/v in THAM buffer) in 50 mL centrifuge tubes. Soil solutions were then incubated for two hours at room temperature (22 °C). Control soils (without substrate solution) were also included. After incubation, 2.5 mL of substrate solution was added to controls, with 2.5 mL of trichloroacetic acid (TCA) added immediately to all samples, which were then centrifuged for 3 min at 4000 RPM. 50 μL of supernatants from each sample were mixed in a microplate well with 75 μL alkali reagent and 50 μL of Folin-Ciocalteu's phenol reagent

Table 1
Site information for five sites used in this study. Sv A = Svartberget A; Sv B = vartberget B; Sv C = Svartberget C. Water content and pH values represent means of measurements across three seasonal timepoints (June, August, September). All values are ±SE.

	Sv A	Sv B	Sv C	Rosinedal	Birch
DominantVegetation	*P. sylvestris* *P. abies*	*P. sylvestris* *P. abies*	*P. sylvestris* *P. abies*	*P. sylvestris*	*B. pendula*
C content (%)	29.4 ± 0.8	43.0 ± 0.49	47.8 ± 0.29	41.0 ± 1.1	47.6 ± 0.48
N content (%)	1.0 ± 0.03	1.0 ± 0.02	1.1 ± 0.02	0.8 ± 0.03	1.6 ± 0.02
C:N ratio	29.3 ± 0.36	43.1 ± 0.5	45.3 ± 0.72	50.5 ± 1.7	30.3 ± 0.46
$pH_{(H2O)}$	5.5 ± 0.03	4.3 ± 0.02	3.8 ± 0.02	4.4 ± 0.03	4.0 ± 0.02
Water Content (g H_2O g^{-1} soil DW)	2.65 ± 0.71	1.23 ± 0.2	0.77 ± 0.18	1.17 ± 0.27	1.86 ± 0.28
Air temperature (seasonal mean °C)	17.2	20.7	20.1	21.1	19.1
Soil temperature (seasonal mean °C)	11.0	11.7	12.7	12.2	12.3

(20 % v/v) and left to stand at room temperature for 30 min before measuring absorbance with a spectrophotometer at 700 nm. Tyrosine was used as a standard to estimate concentrations of amino acids hydrolysed from protein in samples.

Fluorescein diacetate (FDA) hydrolysis activity was measured as per Green et al. (2006) with some modifications. 0.5 g of fresh unsieved field-moist soil was combined with 10 mL of THAM Buffer (50 mM, pH 8.1) in 50 mL centrifuge tubes. 100 μL of an FDA solution (12.01 μM FDA mL^{-1}) was added to each tube, and incubated at room temperature (22 °C) for two hours, before centrifuging for 3 min at 4000 RPM. No acetone was added to halt hydrolysis, instead 200 μL of supernatant from each sample were directly added to a microplate, with absorbance measured on a spectrophotometer at 490 nm, with concentrations measured using a standard curve generated from fluorescein stock solution (602 μM, in 10 mL acetone & 40 mL 50 mM THAM, pH 8.1).

2.4. Measurement of extracted solutes

Nitrate (NO_3^-) was measured via the reduction of NO_3^- to NO_2^- with vanadium (III) chloride, followed by the Griess reaction, as described by Miranda et al. (2001). Total protein was measured using a modified Lowry assay as described by Redmile-Gordon et al. (2013).

Ammonium (NH_4^+) and 22 amino acids (Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, GABA-aminobutyric acid, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Ornithine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine) were measured using reverse-phase liquid chromatography as per Svennerstam and Jämtgård, 2022, using an Agilent HPLC system equipped with a triple-quad mass spectrometer.

2.5. Statistical analyses

To gather a generalised perspective of the influence of field versus lab extractions, we first grouped all sites and timepoints into one dataset, pairing measurements of field and lab extractions. Field and lab extractions were then directly compared using paired two-tailed t-tests, with significance differences identified at $p < 0.05$. Seasonal and site differences were analysed using a two-way repeated measures ANOVA to determine whether differences between field and lab extractions were detectable over time. This analysis was followed by a Šídák's multiple comparisons test to detect differences between lab and field extractions at each sampling timepoint. For seasonal means for individual solutes, we use two-sided t-tests to determine significant differences between lab and field extractions. Spearman correlations were used to compare relationships between protease activity and N compounds in lab and field extractions, using two-tailed P values. Confidence intervals (95 %) were used to determine whether r values were different between lab and field extractions.

We defined the percent loss in individual amino acids after lab extraction as:

$$\%Loss = (Lab_{AA} - Field_{AA})/Field_{AA} \times 100$$

3. Results

Across all sites and timepoints, we observed that field extractions had significantly greater water-soluble N (0.77 ± 0.07 μmol N/g soil DW) than lab extractions (0.17 ± 0.03 μmol N/g soil DW) (paired two-way t-test; $p < 0.0001$) (Fig. 1; A). In field extractions, amino acids contributed the most to total LMW-N (71.3 % ± 4.2), but their contribution was much lower in lab extractions (43 % ± 1.6), whereas inorganic N forms increased in their contribution (Fig. 1; B).

3.1. Site and seasonal changes

When considering seasonal changes of solutes at each sampling site, there were site and time-dependent differences observed (Fig. 2). Amino acids concentrations were often found to be greater in field extractions than in lab extractions, with some exceptions; for instance, the Rosinedal site showed no significant differences at any timepoint (Fig. 2, AA-N, Rosinedal). Svartberget C and Birch sites showed the highest amino acid concentrations (observed in field extractions), and the greatest differences between field and lab extractions, with seasonal means that were 0.86 μmol g^{-1} soil DW (Svartberget C), and 1.16 μmol g^{-1} soil DW (Birch) greater in field extractions than those from the lab. We also noted that the magnitude of amino acid variation between sites throughout the season was much larger when using lab extractions. Concentrations of NH_4^+ were much lower than amino acids, but some sites showed a significant increase in NH_4^+ concentrations in lab extractions at some timepoints, and within seasonal means (Fig. 2, NH_4^+). No differences in NH_4^+ concentrations were observed at the Birch site (Fig. 2, NH_4^+, Birch). Some significant differences were also observed for both NO_3^- and total protein concentrations (Fig. 2, NO_3^- and Birch). We also noted that sample measurement variation was generally greater in field extractions, than in lab extractions – particularly for amino acids. (Fig. 2, AA-N).

3.2. Enzyme activity

There were some significant seasonal differences in protease activity at the Svartberget A, Birch and Rosinedal sites – but most notably at a strong peak in activity in August at Birch and Rosinedal sites (Fig. 3, A). Seasonal differences in FDA hydrolysis activity were detected at Svartberget A and B sites, with the highest activity detected at the Svartberget A site in June (2002 ± 120 μg fluorescein g^{-1} soil DW) (Fig. 3, B).

Over the season, protease activity (Fig. 4) was most strongly correlated with total protein in June (Protease: Field $r = 0.63$; Lab $r = 0.66$; $p < 0.05$, Spearman correlations), and weakened as the season progressed. Although lab-extracted protein was often less strongly correlated with

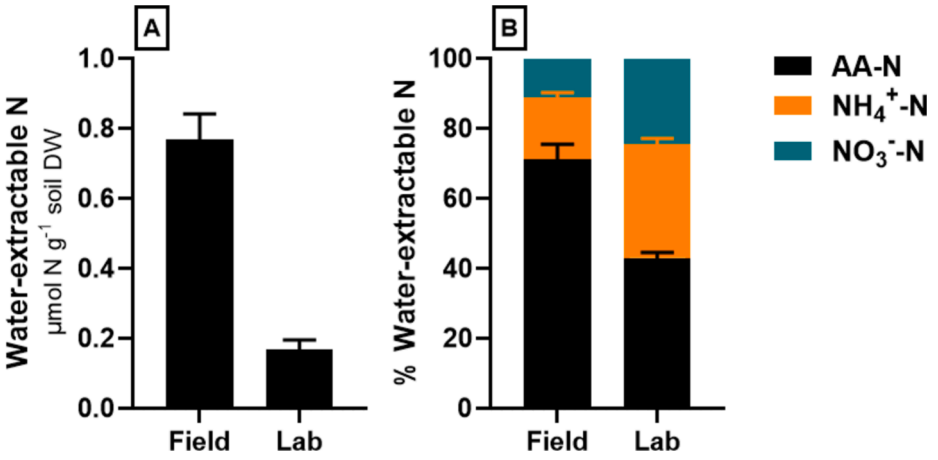

Fig. 1. A) Means of analysed water-extractable N concentrations (sum of amino acid-N, NH_4^+-N and NO_3^--N), in extracts performed immediately after soil sampling and extraction in the field, or after transportation and extraction in the laboratory. Error bars denote ± SE, n = 285. B) Proportional contributions of Amino Acid-N (AA-N; in black), NH_4^+-N (in orange), and NO_3^--N (in green) to total LMW-N after field and lab extraction. Error bars denote ± SE. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

protease activity, 95 % confidence intervals overlapped, indicating this difference is not significant. In contrast, enzyme activity had little seasonal correlation with amino acids, regardless of extraction or enzyme type (Supplementary Table 2) – however we did note that in June, there were significant negative correlations between enzyme activity and amino acid concentrations extracted in the field.

3.3. Effect of extraction location on individual amino acids

As amino acids represent a diverse pool, we evaluated the effect of extraction location on individual amino acids, pooling all soils and timepoints (Fig. 5). The median relative loss for all amino acids in lab extractions (relative to field extractions) was 72.8 % − with Asparagine (92 ± 2.2 % SE), Glutamic Acid (87.3 ± 3.5 %), and GABA (85.5 ± 3.3 %) reduced the most, and with Ornithine (55 ± 20.8 %) Cystine reduced (40.81 ± 13.6 %) the least.

4. Discussion

Our study shows that field-based soil extractions capture a large pool of amino acids that are lost during storage. In contrast, our lab-based soil extractions resulted in a significant reduction in amino acid concentrations compared to field-based extractions, with only a small increase in inorganic N concentrations, which we interpret as indirect evidence of microbial immobilisation during overnight storage. Although loss of amino acids was expected after lab extraction, the lack of substantial mineralisation was unexpected, as a previous study using soil from similar sites within the region have shown that lab extractions resulted in mineralisation of organic N, producing more inorganic N as a result (Inselsbacher, 2014). Studies from other biomes and land uses also suggest that transport and storage of soils can further increase the magnitude of this transformation (Bailey et al., 2021). Our shorter extraction time (10 min, compared to 60+ minutes, (Werdin-Pfisterer et al., 2009)), and overnight storage may have contributed to differences from other studies, providing less time for microbial mineralisation to occur during extraction and extended storage (Bailey et al., 2021; Jones and Willett, 2006; Makarov et al., 2017; Ros et al., 2009). Field extractions also sampled greater variability in amino acid concentrations between sites and across the season, which may indicate that short-term storage and lab-based extraction may mask a large source of variation that could be useful for differentiating site-specific ecological processes. Our findings emphasises the care needed when sampling and interpreting N data acquired from soil extractions after transportation and storage – as even limited handling of soils could lead to dramatically altered outcomes for soluble N measurements. Consequently, this may lead to conclusions that do not reflect the real nature of our study sites, or the size and composition of soil N solutes that are available to plants and soil microbes throughout the season. Field extraction thus may provide us with a simple way of capturing a fuller resolution and magnitude of soluble soil N, and with some planning could be easily incorporated into field work schedules. We recognise that our method requires the transportation of additional tools and equipment (e.g. a balance, shaker, battery, distilled water), all of which can be difficult if study sites are remote, and have no vehicular access. However, slight modifications may be possible that can help reduce carry weight and bulkiness if, for instance, an alternative shaking method can be used that guarantees reproducibility, eliminating the need for a shaker and a battery.

Due to limitations in our study, we are unable to determine why water-soluble amino acid concentrations are so affected by overnight storage. However, there are likely several contributing mechanisms. For instance, soil disturbance and storage can decrease hydrolytic enzyme activity over time (DeForest, 2009; Peoples and Koide, 2012; Turner and Romero, 2010), which could slow hydrolysis of proteins and peptides reducing the production of free amino acids. If microbial uptake of amino acids is greater than protein depolymerisation rates, then this would result in a decrease in extractable amino acids. If protein hydrolysis was disrupted here, we might also expect an accumulation of soluble protein in lab extractions – however, we did not observe significant differences in protein concentrations within sites (Fig. 2, Total Protein) which could indicate that protein is less affected by storage, or that newly-produced protein faces a fate similar to amino acids – for instance, direct utilisation of peptides by microbes (Farrell et al., 2013), or adsorption to the mineral phase. Soil sampling also excludes living roots, and the supply of fresh C through root exudation – this may disrupt microbial relationships and alter microbial activity (Averill and Hawkes, 2016; Blagodatskaya et al., 2014; Lang et al., 2021), with microbes potentially shifting towards soil-derived organic compounds such as amino acids as alternative C sources. However, over the short term (within a day after soil disturbance), this abrupt root exclusion and structural environmental disturbance may be more akin to a physical shock stress response within the microbial community. Although the effects of acute short-term sampling disturbance are rarely evaluated, there is much evidence that microbial communities can be dramatically altered by physical disturbance events such as those caused by harvesting, fire, weather and experimentally-induced disturbance (Cho et al., 2017; Choi et al., 2017; Holden and Treseder, 2013; Kataja-aho et al., 2011; Smenderovac et al., 2023), and changes to C and N

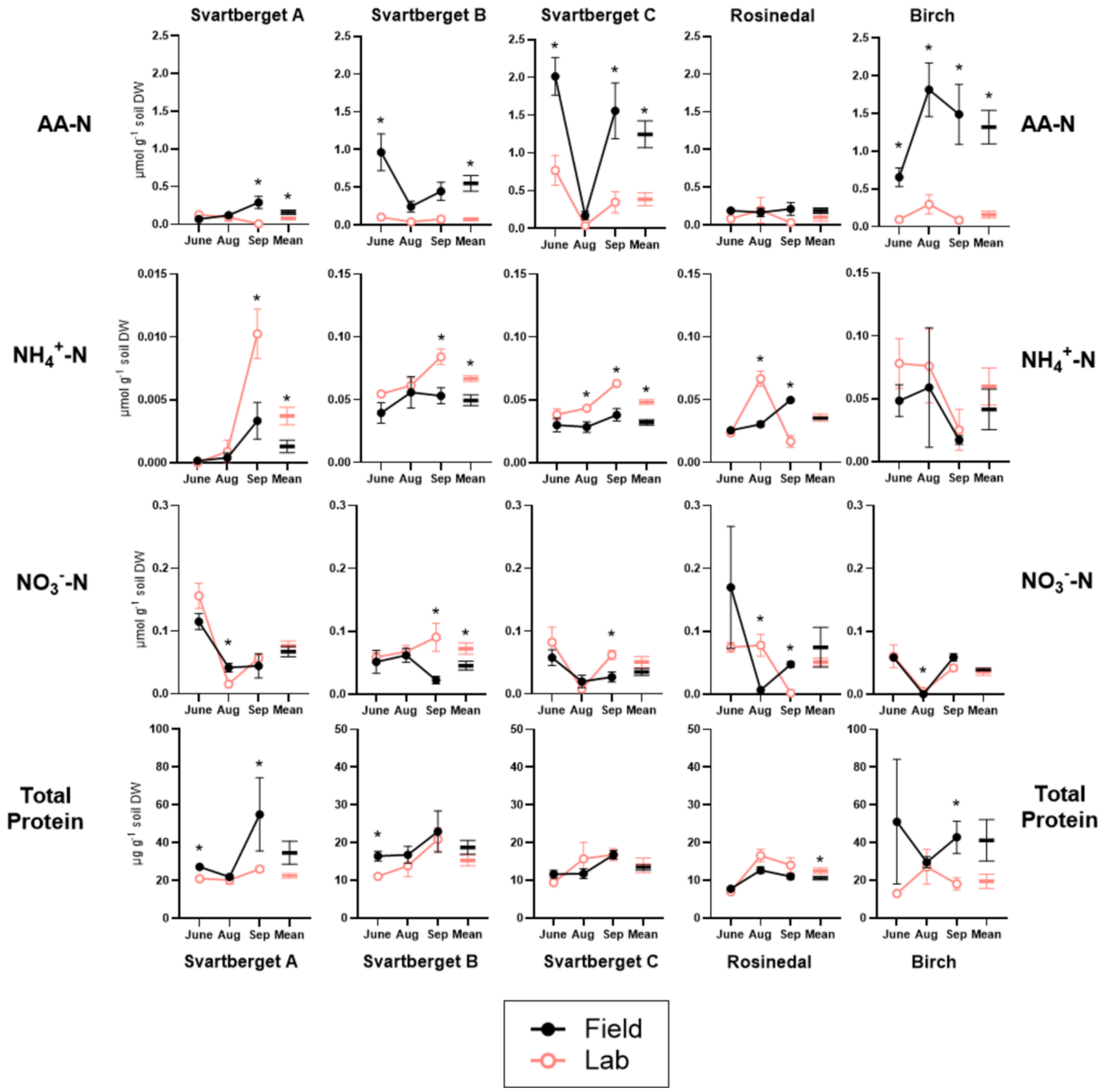

Fig. 2. Mean concentrations (±SE, n = 19) of solutes in soil samples extracted in the field (black dots and lines) and in the lab (red dots and lines) after storage and transportation, sampled at five soil sites, and at three timepoints (June, August, September). Seasonal means (across all timepoints) are also shown. Solutes included are Amino acids (AA-N), ammonium (NH_4^+-N), nitrate (NO_3^--N), and total protein. Asterisks denote significant differences between means at each timepoint (Two-way repeated measures ANOVA, Šídák post-hoc multiple comparisons test; $p < 0.05$), and between seasonal means (2-way *t*-test; $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

uptake by microbes are likely to accompany these alterations. Adsorption of amino acids may also explain a decrease in amino acid concentrations, and it is possible that disturbance and storage could enhance this process by exposing new soil sites for association. If so, we might expect that positively-charged and/or non-polar / hydrophobic amino acids would be most affected (Buckley et al., 2017; Vieublé Gonod et al., 2006). However, we observed that polar or negatively-charged amino acids were most affected by storage (except for isoleucine) (Fig. 5). This may simply be the result of the sampling biases of a water-based extraction technique, in that the most water-soluble compounds are easiest to sample, and thus easiest to detect differences between treatments. However, we also recognise that more work is needed to implicate these potential pathways, including studies that can directly measure microbial N immobilisation and N adsorption before and after storage. Ideally, future studies should also include conventional salt extractions (such as KCl), which will help compare such findings to the majority of soil studies measuring exchangeable soil N.

We must also highlight that there is a chance these findings are yet another artefact of soil disturbance – perhaps a short-term outcome caused by the lysis of root cells or microbial cells, producing a flush of

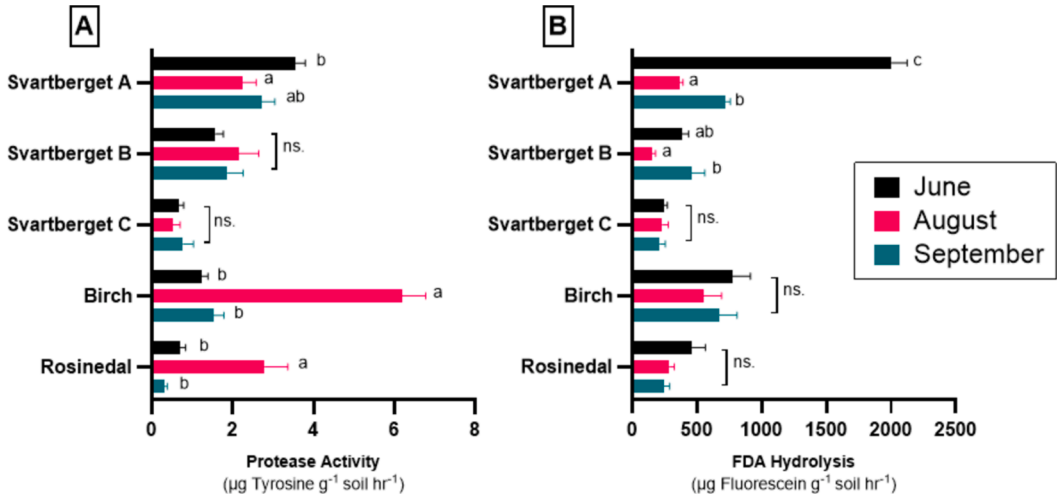

Fig. 3. A: Mean protease activity (µg tyrosine g^{-1} soil DW hr^{-1}) in soils collected from five soils sites, over three sampling timepoints. Error bars indicate ± SEM. Letters denote significant groupings after Tukey's post-hoc comparison test, within sites ($p < 0.05$). B: Mean fluorescein diacetate (FDA) hydrolysis activity (µg fluorescein g^{-1} soil DW hr^{-1}) in soils collected from five soils sites, over three sampling timepoints. Error bars indicate ± SEM. Letters denote significant groupings after Tukey's post-hoc comparison test ($p > 0.05$).

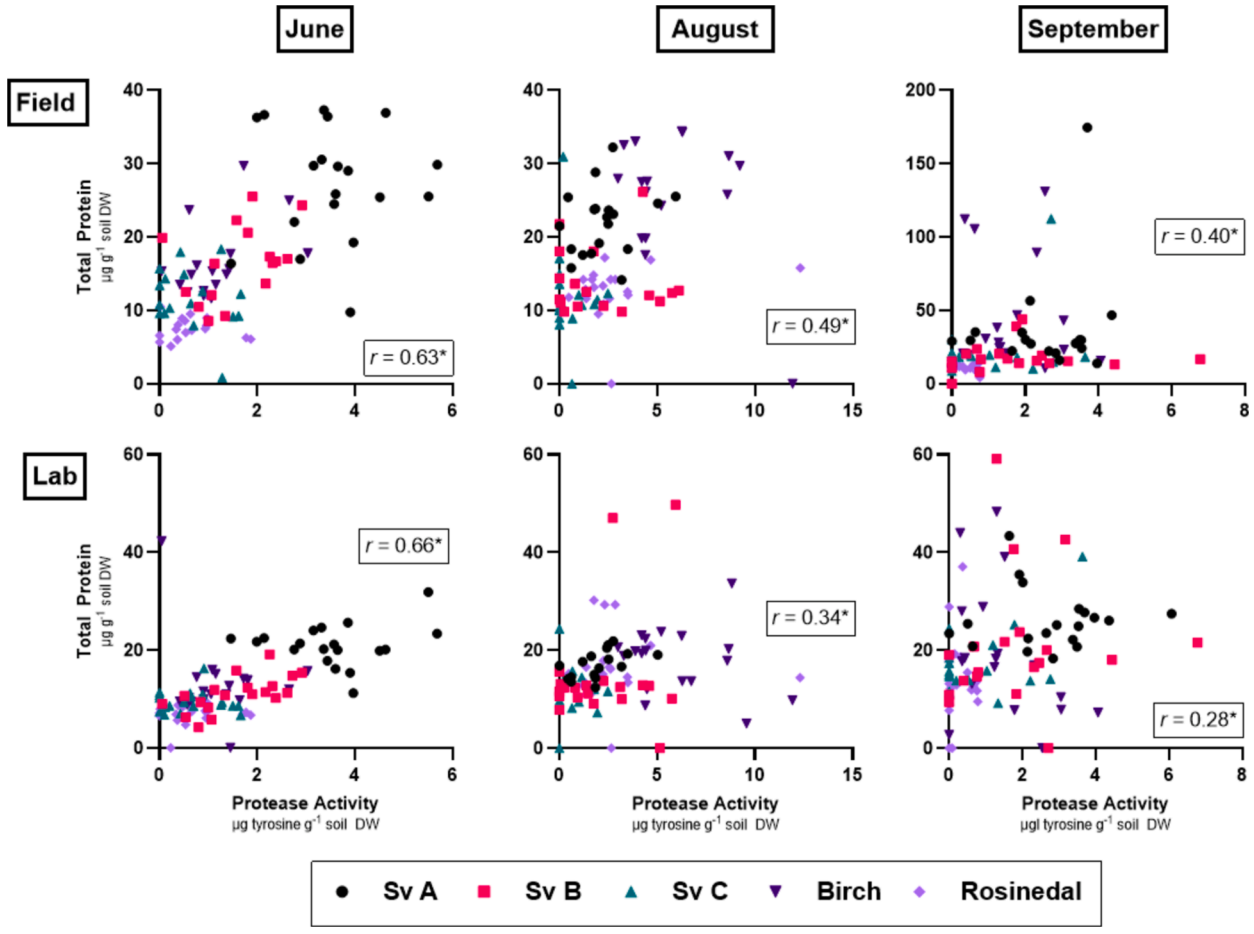

Fig. 4. Spearman correlations (*r*) of protease activity in relation to total protein concentrations in field and lab extractions at three seasonal timepoints (June, August, September) at five sites (Sv A = Svartberget A; Sv B = Svartberget B; Sv C = Svartberget C; Birch; Rosinedal), pooled into the same dataset. Dashes denote non-significant correlations. Asterisks represent significant correlations: * $p < 0.05$.

previously unavailable amino acids, particularly given these extractions take place closer in time to the initial source of disturbance. However, our study shares similarities with field-based, low disturbance *in situ* studies using lysimeters and microdialysis, which have also observed a dominance of amino acids in comparison to lab-based soil extractions (Inselsbacher et al., 2014, 2011; Inselsbacher and Näsholm, 2012). This indicates that higher concentrations of amino acids should be expected in boreal soils – and the differences we see between field and lab-based

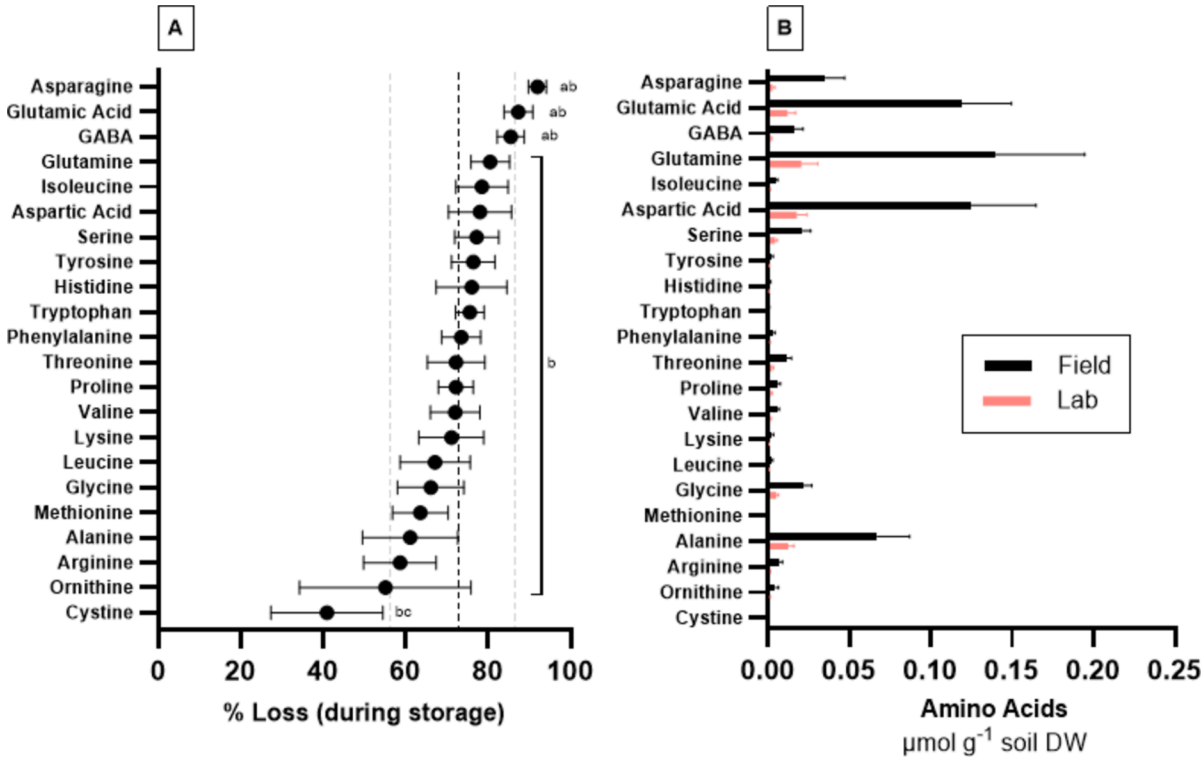

Fig. 5. A) Percent (%) loss of extracted amino acids from all soils after lab extraction, relative to field extraction. Error bars indicate ± SE. Black dotted line marks the median value of all amino acids (72.8 %); grey dotted lines denote the 10th and 90th percentile. Letters denote outcome of one-way ANOVA followed by Tukey's post-hoc test. B) Concentrations of individual amino acids (µmol N/g soil DW) as extracted from soils in the field (black bars) and in the lab (red bars). Error bars indicate ± SE. GABA = gamma-aminobutyric acid. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extractions may not be simply a methodological artefact of soil disturbance. We suggest that field-based soil extractions might provide a fast, simple and cheap alternative to *in situ* techniques – which are generally more difficult to deploy and costly to scale. Additional studies in a broader range of soils (e. g. higher pH, other soil types) will be of importance to evaluate the generality of our findings.

A previous study using field-based extraction observed strong positive relationships between organic N concentrations (amino acids and protein) and protease activity in boreal soils (Kielland et al., 2007), and there is a possibility that differences between lab and field-based extractions could strengthen or weaken these relationships. We observed a similar positive relationship between protein and protease activity (when pooling sites), with stronger correlations earlier in the season (Fig. 4), but this relationship was not present when compared with amino acids (Supplementary Table 2). This may indicate that hydrolytic enzyme activity may be a poor indicator of soluble amino acid availability in these boreal soils, which contrasts to previous work (Kielland et al., 2007). Interestingly, we observed a negative correlation between amino acids and enzyme activity in June when pooling sites (Supplementary Table 2) – a striking contrast to the positive correlations between protease and protein, and could further point to the rapid utilisation of amino acids during the early part of the season by soil microbes. This latter finding was only observable in field-extracted amino acids, perhaps due to the greater resolution and variability of amino acid measurements when using our field extractions. But overall, we noted little difference between correlations of enzyme activity to either lab or field extractions, suggesting that for comparisons with lab-based enzyme assays, the location and timing of N extraction matters less (at least within 24 h after collection). It is also likely that other key exoenzymes, such as oxidases, may be more critical to making organic N compounds accessible for hydrolysis, particularly in polyphenolic-rich boreal soils which may rapidly compound with protein (Adamczyk et al., 2009, 2008; Bonner et al., 2019). It is very possible that their activity may be variably impacted by soil disturbance and storage (Dadenko et al., 2009; DeForest, 2009). *In situ* temperature is another factor influencing enzyme activity differently for different enzymes and hence would be important to investigate in the context of *in situ* availability of organic N in future studies (Wallenstein and Weintraub, 2008).

5. Conclusion

We show that by extracting boreal soils in the field shortly after sampling, a large pool of amino acids can be captured that would otherwise be lost during storage and extraction in our lab. Perhaps more importantly, field extractions also showed larger seasonal variation between sites which is masked by storage and lab-based extraction, which ultimately skews our perception of the seasonal availability of organic N forms at each site. As soil analyses shift towards including organic N as part of the suite of solutes we routinely measure in soils, there is great potential for simple improvements to commonly used techniques to provide better data about seasonal soil N profiles in many different soil types and biomes, unmasked by methodological artefacts.

CRediT authorship contribution statement

Scott Buckley: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sandra Jämtgård:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2025.117163.

Data availability

Data will be made available on request.

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