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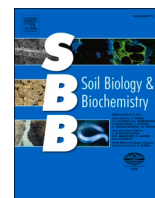
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Responses of soil hexapod communities to increasing nitrogen in a subarctic grassland

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ABSTRACT

The warming of boreal ecosystems accelerates decomposition and increases nitrogen (N) availability. The impact of increased N on subarctic soil fauna communities, however, remains poorly understood. We investigated the response of soil hexapods to a N addition experiment in a subarctic grassland. We characterized the soil hexapod communities using environmental DNA metabarcoding and analyzed the levels of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial carbon (Cmic), and microbial nitrogen (Nmic). N addition increased DON and Nmic, while DOC and Cmic pools remained unchanged. Furthermore, N addition caused shifts in soil hexapod community compositional diversity between control and N plots in herbivore and microbivore taxa. The levels of DON and Nmic strongly correlated with these shifts, explaining 54% and 45% of the compositional variability, respectively. This study demonstrates a clear link between N availability and shifts in soil hexapod communities, associated to changes in microbial and dissolved N pools in subarctic grasslands.

The subarctic region harbors the largest pool of terrestrial carbon (C) on Earth (Scharlemann et al., 2014). Increasing temperatures in these ecosystems are expected to accelerate the decomposition of soil organic matter (SOM) eventually leading to a higher availability of nitrogen (N) due to the higher N mineralization rates of soil microbes (Marañón-Jiménez et al., 2018; Walker et al., 2018). Yet, the impacts of this potential increase in N availability on the communities of soil fauna in subarctic ecosystems remain largely unknown. This is of particular importance due to our increasing appreciation of the interactions between microbes and soil fauna, which typically accelerate SOM decomposition (Handa et al., 2014), and how these relationships may be mediated by overall nutrient availability at an ecosystem level (Peguero

et al., 2019).

Arthropod-mediated decomposition is a prominent example of our knowledge gap regarding the impact of N addition. Soil hexapods facilitate decomposition and mineralization via litter fragmentation and habitat transformation (Bardgett and van der Putten, 2014; Filser, 2002), but little is known about their response to shifts in N availability. Most detritivore hexapods are adapted to low-N diets, and microbial N (Nmic) is their main path for N assimilation (Douglas, 2009). Consequently, N availability may drive a bottom-up control on microbivore soil hexapods mediated by microbial communities (i.e. food resources), ultimately regulating hexapods' abundance, diversity and activity (Chahartaghi et al., 2005; Fiedler et al., 2007; Filser, 2002; Hyodo et al.,

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2011; Traugott et al., 2008). However, to our knowledge no previous research has assessed the validity of this trophic cascade involving the response of hexapod communities to shifts in nutrient availability and microbe-derived C and N, which may be particularly sensitive at high-latitude ecosystems.

To better understand the impact of changes in carbon (C) and nitrogen (N) availability on soil hexapod communities in subarctic ecosystems, we conducted a N fertilization experiment (+150 kg N ha⁻¹) in a natural grassland in Iceland. We characterized soil hexapod communities by means of environmental DNA (eDNA) metabarcoding, and we analyzed the levels of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), microbial carbon (Cmic), and microbial nitrogen (Nmic). Additionally, we investigated whether these environmental variables could account for the compositional dissimilarities observed among hexapod communities. We hypothesized that N addition would affect the structure and compositional diversity of soil microbivore hexapod communities through indirect effects arising from alterations in microbial C and N.

We conducted this study at the ForHot research site in Iceland (Sigurdsson et al., 2016) from August 2017 to June 2018 (64°0'N, 21°11'W) (see the supplementary materials for further information on the sampling site). Since 2014, the experimental plots had received annual treatments of 150 kg N ha⁻¹, applied in three doses as NH₄-NO₃. We collected soil cores using an auger to a depth of 10 cm from five replicate plots (2 × 2 m) per N level (control vs. N addition) in four seasons (2 treatments × 5 replicates × 4 seasonal samplings; N = 40) (see Table S1 for the nil impact of seasonality over arthropod communities). We characterized the soil hexapod communities using molecular Operational Taxonomic Units (mOTUs) obtained through eDNA metabarcoding of the 16S mitochondrial rDNA region (see supplementary materials for detailed protocol information). We quantified dissolved organic C (DOC) and dissolved organic N in all soil samples in 1 M KCl extracts. We determined soil Cmic and Nmic using the chloroform-fumigation extraction method, followed by 1 M KCl extraction. We analyzed all extracts for DOC and DON concentrations using a TC/TN-Analyzer (Shimadzu, TOC-VCPH/CPNTNM-1 analyzer). The units for all environmental variables are concentrations in parts per million (ppm). We conducted data handling, visualization, and statistical analyses using R v4.0.6 (R Core Team, 2020) (see the supplementary materials for further details on the statistical analyses).

Principal component analysis (PCA) showed that DON and Nmic were the most significant environmental variables distinguishing the control and N treatment groups (Fig. 1), with non-overlapping confidence ellipses in the environmental PCAs between the treatments. General linear models (GLMs) confirmed that N addition increased DON and Nmic, while reduced the microbial C:N ratio ($P < 0.01$, < 0.05 and < 0.05 , respectively; Table 1). The number of eDNA reads and mOTU richness did not differ between plots with or without N addition ($P = 0.84$ and 0.57 , respectively; Fig. S1 in the supplementary materials). However, we identified significant differences between the soil hexapod communities in the control and the N plots ($P < 0.01$, explained variance 15%; Fig. 2) based on a sparse partial least squares discriminant analysis (sPLS-DA). A higher score in the first variate of the sPLS-DA indicated a greater compositional dissimilarity with the control plots. The compositional dissimilarities driven by N addition were primarily influenced by certain species, notably the collembolans *Protaphorura armata*, *Sminthurinus bimaculatus*, and *Megalothorax minimus*, as well as the plant hopper *Javesella obscurella* (Fig. 2). In contrast, the collembolan *Pogonathellus flavescens*, the rove beetle *Philhygra debilis*, and the crane fly *Tipula cockerelliana* stood out as the more distinct taxa under control conditions (Fig. 2). The first sPLS-DA variate was then subjected to GLMs against the set of environmental variables to assess to what extent these predictors could account for compositional variability. The values of this first variate positively correlated with the amount of Nmic and soil DON ($P < 0.05$ and < 0.01 , respectively) and also marginally correlated with the amount of DOC ($P = 0.06$; Table 2). Thus, this indicated that the

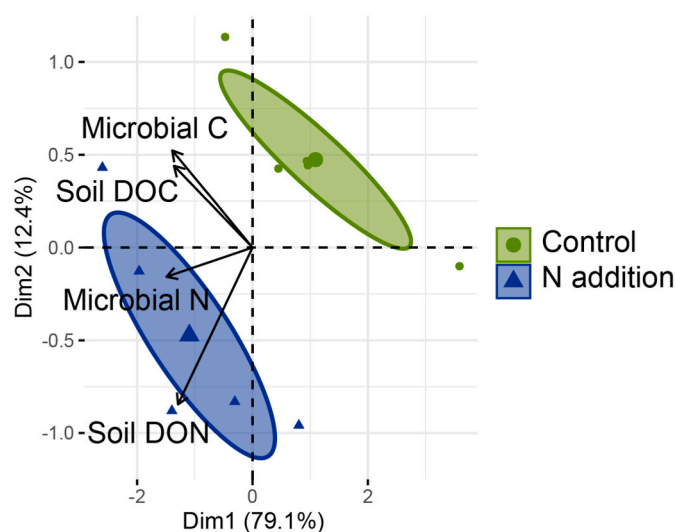


Fig. 1. Soil environment. Principal component analysis of the variables describing the soil environment (microbial carbon, microbial nitrogen, soil dissolved organic carbon and nitrogen -DOC and DON, respectively-). Ellipses denote 95% confidence envelopes for the control and the nitrogen addition plots.

higher the amount of Nmic, DON and to a lower extent of DOC, the greater was the compositional dissimilarity of the soil hexapod communities between the control and the N plots.

Nitrogen addition had a clear impact on the compositional diversity of soil hexapod communities in our studied grassland in the subarctic, which correlated with the increased level of N both in the soil solution and the microbial pool (Table 2). The extreme variations in N requirements among trophic levels and across phylogenetic lineages preclude generalizations of the effect of N for the whole subphylum Hexapoda (Fagan et al., 2002). Previous research has shown that increased N concentrations in the soil can benefit the fitness of insect herbivores via lower C:N ratio in plant tissues (Mattson 1980; Zechmeister-Boltenstern et al., 2015). For instance, the increase of the phloem-sucking *J. obscurella* after our N-addition experiment may underscore the end of the N limitation (Firn et al., 2019; Gargallo-Garriga et al., 2021). Similarly, lower C:N ratio in leaf-litter may favor microbial biomass and ultimately detritivore populations (Table 1) (Gargallo-Garriga et al., 2021). Therefore, it comes as no surprise that –since *S. bimaculatus* can be classified as an epigeic primary decomposer feeding on recently fallen litter, and *P. armata* and *M. minimus* are euedaphic secondary decomposers (Chahartaghi et al., 2005; Potapov et al., 2016)– detritivore collembolan species prefer N rich soils.

Interestingly, certain taxa thrived in conditions of lower N availability. A notable example is *T. cockerelliana*, whose larvae demonstrated a competitive advantage when nitrogen was scarce due to their ability to address nitrogen deficiency through microbial nitrogenase activity (Kostina et al., 2020). Additionally, the microbivore

Table 1
Effect of nitrogen (N) addition over dissolved and microbial carbon and N.

Response variable	Effect estimate	F	P-value	Adjusted R ²
Soil DOC	40 ± 30	1.34	0.27	0.03
Soil DON	60 ± 10	41.4	0.001	0.81
Microbial C	230 ± 230	0.93	0.36	0
Microbial N	170 ± 60	7.74	0.02	0.42
Microbial C:N	-0.66 ± 0.22	8.78	0.01	0.46

Effect estimates (± standard error) were calculated by means of separate general linear models for each environmental variable. The intercepts (not shown) are the ambient conditions. Units of response variables are in parts per million (ppm). All models have 8 degrees of freedom.

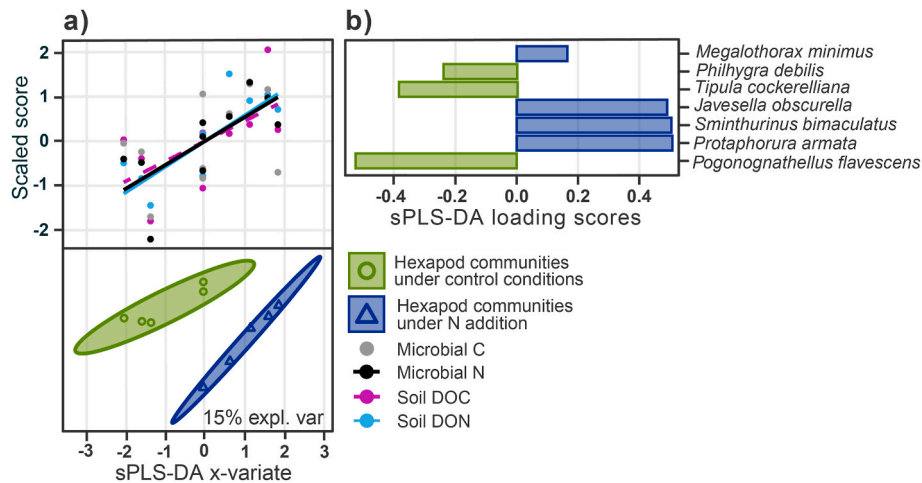


Fig. 2. Soil hexapod communities. (a) Compositional variation of the soil hexapod communities based on a single-component sparse partial least squares discriminant analysis (sPLS-DA) between control and N addition plots. The amount of explained variance by the x-variate is shown in the inset. Confidence ellipses are set at 95%. The linear relationships between the x-variate scores with the corresponding soil environmental variables (microbial carbon, microbial nitrogen, soil dissolved organic carbon and nitrogen -DOC and DON, respectively-) are shown on the top of the sPLS-DA ordination. Solid and dashed lines show significant and marginally significant slope parameters ($P < 0.05$ and $P < 0.1$, respectively). See Table 2 for further information on the linear models. (b) Identity of the main hexapod species-level mOTUs driving the compositional differences between the treatments. Only species-level mOTUs with a loading score >0.1 in the corresponding discriminant analysis are shown.

Table 2

Relationship between the compositional similarity of the soil hexapod community with dissolved and microbial carbon and nitrogen.

Explanatory variables	Effect estimate	F	P-value	Adjusted R ²
Soil DOC	0.017 ± 0.007	4.63	0.06	0.28
Soil DON	0.02 ± 0.008	11.8	0.008	0.54
Microbial C	0.001 ± 0.001	1.83	0.21	0.08
Microbial N	0.007 ± 0.002	8.53	0.02	0.45

Compositional variability of soil hexapod communities was synthesized as the x-variate from an sPLS-DA. The higher the value of the x-variate (response) the greater was the dissimilarity between control and nitrogen-addition communities (see Fig. 2). The effect estimates (\pm standard error) of each environmental variable were calculated by means of separated general linear models. Units of response variables are in parts per million (ppm). All models have 8 degrees of freedom.

collembolan *P. flavescens* also flourished in lower N concentrations, supporting a previous study that suggested enhanced fitness for this species in environments with reduced microbial nitrogen (Chagnon et al., 2001). Regrettably, there are no published studies that shed light on the preference of the rove beetle *P. debilis* for N-poor soils.

In contrast to N, microbial C did not influence the compositional diversity of soil hexapod communities and only the increase of C in the soil solution appeared to have a minor role (Table 2 and Fig. 2). The breadth of the isotopic signature of detritivores in soil meso and macrofauna is narrower for C than for N (Korobushkin et al., 2014; Potapov et al., 2016). This suggests a relatively small variation of C sources in soil detritivores that could eventually result in lower sensitivity to variations of this resource in the soil environment. Yet, the experimental addition of N barely affected the pools of C in the soil solution and in the microbial communities. Nonetheless, N addition might influence nutrient availability beyond C pools. For instance, previous research show how a large N supply favors phosphatase synthesis and increases P availability increasing plant productivity and ultimately modifying soil quality (Deng et al., 2016; Marklein and Houlton, 2012). Overall, this study provides a clear linkage between the availability of N and shifts in the compositional diversity of soil hexapod communities, which are related with changes in the microbial and dissolved N pools in subarctic grasslands.

Further research is needed to elucidate the specific mechanisms

underlying these responses, with a closer examination of the trophic interactions between subarctic hexapods and soil microorganisms and plant traits, and additionally, assessing the potential feedbacks of the observed changes at a community-level for ecosystem functioning.

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.

Data availability

The data supporting the results of this contribution will be published and freely available under CC by 4.0 license in the figshare repository. [ferrinetal_nmediatenitrogenimpactonsoilhexapods](https://doi.org/10.1016/j.soilbio.2023.109228) (Original data) (Figshare)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2023.109228>.

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