European Journal of Soil Biology 86 (2018) 72–80

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: www.elsevier.com/locate/ejsobi

Structural and functional characteristics of high alpine soil macro-invertebrate communities

Michael Steinwandter[a,b], Alexander Rief[b], Stefan Scheu[c,d], Michael Traugott[b], Julia Seeber[a,b,*]

[a] Institute for Alpine Environment, Eurac Research, Viale Druso 1, 39100, Bozen/Bolzano, Italy
[b] Mountain Agriculture Research Unit, Institute of Ecology, University of Innsbruck, Technikerstrasse 25, 6020, Innsbruck, Austria
[c] J.F. Blumenbach Institute of Zoology and Anthropology, University of Goettingen, Berliner Str. 28, 37073, Goettingen, Germany
[d] Centre of Biodiversity and Sustainable Land Use, University of Göttingen, Von-Siebold-Str. 8, 37075, Göttingen, Germany

ARTICLE INFO

Handling editor: Stefan Schrader

Keywords:
Alpine soil fauna
Isotopic niche
Mixing models
Diplopoda
Insecta larvae
DNA barcoding

ABSTRACT

Soil macro-invertebrates play an important role in the formation and functioning of soils, which makes them indispensable for all terrestrial ecosystems, including high alpine soils. However, in the latter, knowledge on species identity, diversity, and functionality of macro-invertebrate soil communities is scarce. Here, we address this knowledge gap by investigating the structural and functional composition of soil macro-invertebrate communities in high alpine sites of the European Alps that differ in sheep grazing intensity (low, medium, and high). Abundance data were combined with the analysis of natural variations in stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$) of food sources and soil animals, allowing insight into the trophic structure of the decomposer community. The presence of sheep significantly increased the abundance of Nematocera, but reduced the abundance of most other taxa. Diplopoda were found exclusively at the low elevation site with almost no sheep grazing, while Diptera larvae increased in numbers at higher elevation sites. Lumbricidae species were abundant at all sites except the highest site, which was intensively grazed by sheep and also used as a resting place. In contrast, trophic relations were not affected by sheep grazing intensity, four trophic groups were clearly distinguished, pointing to a relatively simple food web structure: (1) primary decomposers, (2) secondary decomposers and dung feeders, (3) root and fungal feeders, and (4) predators. We suggest that the shallow soils with high organic matter do not allow the formation of more complex food webs.

1. Introduction

Soil macro-invertebrates, including Lumbricidae, Diplopoda, and Insecta larvae, decompose litter material and physically alter the soil structure by mixing organic and mineral matter as well as creating secondary pores, thereby regulating nutrient and carbon cycling, soil aeration, and soil water balance [1]. Thereby, they are indispensable for the functioning of terrestrial ecosystems including those at high altitude. While the macro-invertebrate soil communities of low elevation European mountains (up to ca. 2000 m a.s.l.) have been studied previously [2,3], knowledge on species identity, diversity and functioning of soil macro-invertebrates of high alpine regions is scarce and this also applies to the Central European Alps (but see Ref. [4] for gastropods [5]; for oribatid mites).

In the high alpine area of the Central European Alps above 2500 m a.s.l., the vegetation growth period is short, as temperatures are low and the snow cover persists for most of the year [6,7]. Plant communities comprise alpine grasslands (Seslerio-Caricetum sempervirentis, Caricetum firmae), which contain species that produce a high number of secondary plant compounds such as polyphenols for their protection against radiation and other stressors [8,9], thus producing litter of poor food quality for detritivores [10]. Climate and litter quality shape the soil macro-invertebrate community [11,12], along with a reduced habitat structure in the shallow alpine soils of the European Alps, limiting the leeway of the soil animals [13]. At these elevations, Insecta larvae, especially soil-dwelling larvae of Brachycera and Nematocera, increase in number and biomass, while Lumbricidae and Diplopoda get less abundant as a consequence of unfavourable environmental [14,15] and nutrient conditions [16]. However, sheep might enable macro-invertebrates to extend their upper elevational limit by providing them with a favourable food source in form of dung [17]. Schon et al. (2008) found that the abundance of meso- and macro-invertebrates was promoted by low intensity grazing (i.e. small sheep flocks), until negative effects of intensive pasturing by larger flocks took over (e.g., trampling, soil compaction) [18].

To improve understanding of the soil macro-invertebrate

* Corresponding author. Institute for Alpine Environment, Eurac Research, Viale Druso 1, 39100, Bozen/Bolzano, Italy.
E-mail addresses: Julia.Seeber@uibk.ac.at, Julia.Seeber@eurac.edu (J. Seeber).

https://doi.org/10.1016/j.ejsobi.2018.03.006
Received 14 September 2017; Received in revised form 22 March 2018; Accepted 22 March 2018
Available online 09 April 2018
1164-5563/

community structure and its functioning in high alpine regions in Central Europe, their species composition as well as their trophic structure need to be studied. Functional aspects such as trophic relationships describe what organisms do in an ecosystem [19], and add significantly to the knowledge of the ecology of species [20]. A common method to assess the trophic ecology of animals is the dual stable isotope approach, which provides information on the main food resources (reflected by $^{13}C/^{12}C$ ratios) and the trophic position (reflected by $^{15}N/^{14}N$ ratios), i.e. their position in the food chain [21,22]. Displaying the animals' stable isotope ratios in a bi-plot provides information on the trophic structure of communities, as the heavier isotopes increase in abundance from lower to higher trophic levels [21,23]. Furthermore, the variation in carbon and nitrogen isotope ratios reflect the trophic niche of species or taxa [24], and isotope niches can therefore be used as metrics of the species' trophic ecology [25].

Food web studies using stable isotope analysis have been carried out successfully for several terrestrial as well as for aquatic ecosystems. However, studies on trophic relations of soil animals are still scarce as interpreting isotopic data from soil animals is complicated by a variety of factors: for example, the range of ^{13}C and ^{15}N of the litter material and organic matter, which form the basis of the soil food web, may span a large range of values, changes with time and decomposition status, and increases with soil depth. Most soil animals are omnivorous, feeding on a variety of food sources, making it difficult to pinpoint single sources and to assign them to a distinct trophic level [21]. In addition, microbes, which fractionate nitrogen isotopes, make up part of the nutrition of soil macro-invertebrates, which further complicates interpreting the stable isotope values of consumers. Nevertheless, novel insights into the structure and functioning of soil animal communities has been gained in food web studies (e.g. Refs. [26–28] and studies on trophic relations of individual species (e.g. Refs. [29–31])).

In this study, we assessed for the first time structural and functional aspects of a high alpine soil macro-invertebrate community of the Central European Alps. We expected that sheep grazing favours soil macro-invertebrates, especially Lumbricidae due to additional and easily available food sources (i.e. dung), allowing them to colonize high alpine soils. For this purpose we investigated community composition and community-environment relationships using the abundance of invertebrates from three high Alpine soils differing in sheep grazing intensity. In a second step we assessed natural variations in stable isotope ratios of these animals to delineate their trophic positions. For identifying juvenile Lumbricidae species, which lack morphological characters needed for species identification but form a significant proportion of the soil invertebrate community, we employed DNA barcoding [32].

2. Material and methods

2.1. Study area

The study was conducted on the Hoher Burgstall (2470–2600 m a.s.l.), a mountain top above Neustift in the Stubai Valley (Central Alps, Tyrol, Austria, 11.2810° E, 47.1334° N, Fig. A1, see supplementary data). The bedrock is calcareous; the dominating soil type is Rendzina with a high organic matter content and mull as the prevalent humus form. The experimental sites were located in part on strongly inclined slopes exposed to the south or south-west (Table 1). The four sites varied in location (slope S or top T) and sheep grazing intensity (low L, medium M, and H high): (i) SL – site located at the slope with almost no sheep present, (ii) SM – sheep regularly graze the mountain slope, (iii) TM – site at the mountain top which is regularly used by sheep for grazing, and (iv) TH – plane grassland at the mountain top which is, beside for grazing, used by the sheep as resting place during the day and overnight. The medium sheep grazing intensity was considered as the average situation found on a wide range of sites on the study area with approximately 50 sheep roaming the area daily from June to September (fam. Pfurtscheller, farmer and landowner, pers. commun.), whereas the low and high states represented exceptional conditions. We are aware that, with the exception of the medium intensity with two sites serving as true replicates, the soil samples are pseudoreplicated (i.e. the low and high sheep grazing intensity occurred only once), which hampers the generalization of our results beyond this case study. However, since it is difficult in the Central European Alps to find true replicates with the same environmental conditions and because data on alpine soil communities are scarce, we still find value in our study.

Soil and environmental properties were measured at each site and on each sampling date. These are: (i) pH of the soil (with a pH Meter, Metrohm, Herisau, Switzerland), (ii) total carbon and nitrogen content as well as C/N ratio of soil and litter material (using an organic elemental analyser, FlashEA 1112, Thermo Fisher Scientific, Waltham, Massachusetts), (iii) soil organic matter (SOM, using the dry combustion method by incinerating the material at 500 °C for 4 h in a muffle furnace, Nabertherm, Lilienthal, Germany), and (iv) inclination of the plots.

2.2. Soil sampling and identification of soil invertebrates

Six soil core samples (i.e. soil with vegetation and litter, diameter 15 cm, soil depth 15 cm if possible, but at least 12 cm) per site and sampling date were taken randomly at least 5 m apart from any other sample. The vegetation was cut to 1 cm on-site and was not used for further analyses. Sampling areas varied between 400 (TH) and 700 m^2 (TM). The samples were taken in mid-June 2010, end of July 2010, and end of September 2010 to cover the different activity periods of the soil fauna groups within the vegetation period [13,33]. All soil core samples were transferred to the soil laboratory of the University of Innsbruck on the sampling day.

Soil animals were heat-extracted alive for 12 days (damp tissue was placed into the collection bowls) using a modified Kempson apparatus [34], sorted and counted daily, and stored individually at −28 °C until further analyses. Daily collection of live soil animals and freezing them was done to prevent changes in isotope signatures resulting from decay of body tissue and preservation liquids [35]. Insects (adults and larvae) were identified to family level, Lumbricidae (partly by DNA barcoding, see below) and Diplopoda to species level. The frozen soil animals were identified using a dissection stereo-microscope with petri dishes kept constantly on cooling elements. Taxonomic identification of Lumbricidae followed Csuzdi & Zicsi (2003) [36], that of Diplopoda Pedroli-Christen (1993) [37], and that of all other taxa Schaefer (2009) [38].

2.3. DNA barcoding

Juvenile Lumbricidae were identified to species level using DNA barcoding. DNA was extracted from the first two segments (without gut content) using the peqGOLD Tissue DNA Mini Kit (peqlab, Erlangen, Germany), following manufacturer's instructions. Part of the mitochondrial cytochrome *c* oxidase subunit one gene (COI) was amplified using the universal invertebrate primers described by Folmer et al. (1994) [39]. PCR was carried out in 10 µl reaction volumes containing 0.2 mM dNTPs (Gencraft), 1 µM of each primer, 1 × buffer (Biotherm), 3 mM $MgCl_2$, 5 µg of bovine serum albumin (BSA), 0.375 U *Taq* Polymerase (BioTherm), 3.525 µl of PCR-grade RNase-free water and 1.5 µl of DNA extract. Thermal cycling comprised 94 °C for 2 min, 35 cycles at 94 °C for 20 s, 46 °C for 30 s, 72 °C for 45 s and a final elongation at 72 °C for 3 min. PCR products were purified using ExoSAP-IT (USB Corporation) and sequenced in forward direction. The sequences were aligned with BioEdit (Version 7.0.9.0, Hall, 1999) and compared to existing species sequences from the research site [40].

2.4. Stable isotope analysis

Litter (0 to +1 cm) and soil (−15 to 0 cm) material was air-dried,