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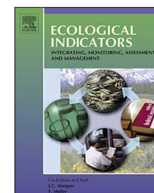

Original Articles

Ground cover and slope exposure effects on micro- and mesobiota in forest soils

María Gómez-Brandón[a,1,*], Judith Ascher-Jenull[a,b,1], Tommaso Bardelli[a,b], Flavio Fornasier[c], Giacomo Sartori[d], Giacomo Pietramellara[b], Paola Arfaioli[b], Markus Egli[e], Anneke Beylich[f], Heribert Insam[a], Ulfert Graefe[f]

[a] Institute of Microbiology, University of Innsbruck, Technikerstraβe 25d, 6020 Innsbruck, Austria
[b] Department of Agrifood and Environmental Science, University of Florence,Piazzale delle Cascine 18, 50144 Florence, Italy
[c] Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo (C.R.A.-R.P.S.), Via Trieste 23, 34170 Gorizia, Italy
[d] Museo Tridentino di Scienze Naturali, Via Calepina 14, 38100 Trento, Italy
[e] Department of Geography, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland
[f] IFAB Institut für Angewandte Bodenbiologie GmbH, Tornberg 24a, 22337 Hamburg, Germany

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ABSTRACT

The interrelation of Alpine topography with the micro − and mesobiota is still poorly understood. We investigated the effects of ground cover type and slope exposure on the soil microbial biomass (double-stranded DNA, *ds*DNA) and abundances (real time PCR, qPCR); hydrolytic enzyme activities; and enchytraeid community structure in top soils (2.5-cm increments depth) in subalpine forests in the Italian Alps. Dominant ground covers were grass, moss, litter and woody debris at the north- and the south-facing slopes. The autochthonous soil microbiota (bacteria, fungi and archaea) was quantified by qPCR in the extracellular (eDNA) and intracellular fraction (iDNA) of the total soil DNA pool. A higher eDNA/iDNA ratio indicative of lower microbial activity was recorded in the deepest layer of the grass plots at the north-facing slope. This can be related to a lower degradation of eDNA and/or to an accumulation of eDNA with increasing depth as a result of leaching. The exposure effect was enzyme-specific and higher activities occurred under woody debris primarily at the south-facing slope. These plots also showed a higher nutrient content and a greater microbial biomass assessed as *ds*DNA yields. Total microannelid abundance was elevated on north-facing slopes on account of strong acidity indicator species. This was related to soil pH being one unit lower compared to the south-facing slope. The thickness of the organic layer (OL + OF + OH) was elevated at the north-facing slope due to a considerably thicker OH-horizon. The vast majority of microannelids at this slope occurred in the organic layer, while at south exposure they were almost evenly distributed between the organic layer and the mineral soil (A-horizon). Exposure was found to be more determinative for the composition of microannelid assemblages than the ground cover type.

1. Introduction

Mountain ecosystems are predicted to experience a rapid warming in the future with distinct consequences for soil organic matter (SOM) quality and quantity (Mountain Research Initiative EDW Working Group, 2015). According to Beniston et al. (1997), an increase of approximately 2 °C of the annual minimum temperature has been observed in the European Alps during the 20th century. Changes in the abiotic environment due to rising temperatures may affect the microbial community structure, its activity and diversity, as well as vegetation composition (A'Bear et al., 2014), with implications for both ecosystem regulation and carbon feedbacks (Allison et al., 2010).

Soil microorganisms and in turn their enzymatic capabilities are further influenced by the activities of soil fauna that lives along them (Bardgett and Wardle, 2010). Enchytraeidae (Clitellata: Oligochaeta) are considered a keystone group responsible for the maintenance of decomposition processes and the functioning of detrital food webs (Didden, 1993; Karaban and Uvarov, 2014). The response of enchy-

* Corresponding author.
E-mail addresses: maria.gomez-brandon@uibk.ac.at, mariagomez@uvigo.es (M. Gómez-Brandón).
[1] Both authors contributed equally to this paper.

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traeids to abiotic factors was found to be species-dependent (Graefe and Beylich, 2003; Beylich and Graefe, 2009). Species assemblages of soil microannelids can exhibit site-specific differences as shown by Beylich et al. (1995); Jänsch et al. (2005); and Ascher et al. (2012). These latter authors observed that both the slope exposure and the altitude − and consequently the thermal conditions − exerted an interactive effect on the microannelid population in a forested Alpine ecosystem. They found that the species richness of microannelid assemblages was higher under warmer conditions (south-exposure and lower altitudes).

Slope aspect determines the amount of solar irradiation, and thus energy received. This does not only influence the soil temperature, but also the soil water retention and availability, nutrient dynamics (Egli et al., 2006, 2009), composition and activity of soil microbial communities (Kang et al., 2003; Ascher et al., 2012) and soil fauna (Ascher et al., 2012). However, to date the interrelation of Alpine topography with the micro − and mesobiota is still poorly understood. We assume that humus forms represent these interactions in a generic way. Humus form thickness and horizontation were studied to detect relations with depth distribution of soil biota.

We hypothesise that the influence of slope aspect is modified by factors being effective on a smaller scale, such as shadowing by trees or higher water retention under woody debris. Therefore, we tested the effects of ground cover type and slope exposure on the soil microbial biomass and abundances; hydrolytic enzyme activities; and enchytraeid community structure in top soils of subalpine forests in the Italian Alps. The autochthonous soil microbiota (bacteria, fungi and archaea) was quantified in the extracellular (eDNA) and intracellular fraction (iDNA) of the total soil DNA pool. The eDNA/iDNA ratio was also calculated as a proxy of microbial activity.

2. Material and methods

2.1. Study area and soil sampling

We studied two sites located at a subalpine altitude of 1600 m a.s.l. in Val di Rabbi (Trentino, Italy) on a north- and south-facing slope, respectively (N3: 46°24′08″N; 10°48′46.2″E; and S8: 46°22′41.4″N; 10°55′19.3″E). Both subalpine sites are on acidic paragneiss or morainic material consisting of paragneiss, predominated by Norway spruce (*Picea abies* (L.) Karst) (Petrillo et al., 2015). The soils are classified as Cambisols to Umbrisols according to Egli et al. (2006). The sampling was performed in June 2013, where the annual air temperature at site N3 was 3.5 °C and at site S8 5.5 °C (Fravolini et al., 2016).

The dominant ground covers were i) grass, moss and accumulation of branches at the north-, and ii) grass, organic litter and accumulation of branches at the south-facing sites. At each study site and for each ground cover three adjacent plots (5 × 5 m) were set up at 5 m distance from each other in a total area of 25 × 25 m. For the chemical and microbiological analyses five soil samples were randomly taken in each plot with a sampling depth of 15 cm wherever possible, using a corer device (ø 5 cm). Due to a high stone content, sampling depth was in some cases reduced to 10 or 12.5 cm. Samples were divided using a knife into sub-samples of 2.5 cm depth intervals starting at the top of the organic layer (2.5 cm depth correspond to ~50 cm^3). For the soil fauna and description of the humus profile one soil sample was collected from each plot in the immediate vicinity of the sample taken for the pH measurement by using the corer device as described above. The humus profile description was performed directly in the field using the open soil corer. Afterwards, soil samples for microbiological and physico-chemical analyses were kept in cooling boxes and transferred to the laboratory. They were sieved (< 2 mm), carefully separated from root fragments and stones, and stored at 4 °C for physico-chemical and biological analyses and at −20 °C for molecular analyses, respectively. Soil samples for microannelid determination were kept in plastic bags, transported to the laboratory at ambient temperature and stored at 10 ± 2 °C until extraction.

2.2. Physico-chemical analyses

Soil samples were oven-dried (105 °C) for at least 24 h to determine their dry weight. The volatile solids (VS) content was determined by loss on ignition (LOI) in a muffle furnace (Carbolite, CWF 1000) at 550 °C for 5 h. Total C and N contents were analysed in dried samples, using a CN analyzer (TruSpec CHN; LECO, Michigan, U.S.A.). Electrical conductivity (EC) and pH were determined in soil:water extracts (1:10, w/v) by using a conductivity Meter LF 330 WTW (Weilheim, Germany) and a pH Meter Metrohm 744, respectively. Inorganic nitrogen (NH_4^+ and NO_3^-) was determined in 0.0125 M $CaCl_2$ extracts, as described by Kandeler (1993a, 1993b). Total P was determined by H_2SO_4-H_2O_2-HF digestion as described by Bowman (1988). Available P was assessed following the Bray and Kurtz method based on NH_4F extraction recommended for acid soils (Bray and Kurtz, 1945). Both the total and available P concentrations were determined according to the ascorbic acid method as described by Kuo (1996).

2.3. Potential enzymatic activities

A heteromolecular exchange procedure as described by Fornasier and Margon (2007) by using a 4% solution of lysozyme as desorbant and bead-beating agent was used for the assessment of the following hydrolases: i) C-cycle: cellulase (*cell*); xylanase (*xyl*); α- and β-glucosidases (*alfagluc* and *betagluc*); ii) P-cycle: acid and alkaline phosphomonoesterase (*acP* and *alkP*); phosphodiesterase (*bisP*); pyrophosphate-phosphodiesterase (*piroP*); iii) N-cycle: leucine- and lysine-aminopeptidase (*leu* and *lys*); iv) S-cycle: arylsulfatase (*aryS*);. All the measurements were performed in duplicate for each field replicate and the activities were expressed as nanomoles of 4-methyl-umbelliferyl (MUF) min^{-1} g^{-1} dry soil.

2.4. Microbial biomass assessed as double-stranded DNA (dsDNA)

Direct extraction of total soil DNA (tDNA) followed by PicoGreen-based quantification of *crude* (not purified) double-stranded DNA (*ds*DNA) was performed to estimate soil microbial biomass (Fornasier et al., 2014).

2.5. Sequential DNA extraction (eDNA vs. iDNA)

The sequential extraction of the extracellular (eDNA) and intracellular fraction (iDNA) of the soil metagenome was performed according to Ascher et al. (2009a) by applying a combined mechanical-chemical cell lysis using the Fast DNA Kit for soil and FastPrep instrument (MP Biomedicals). DNA extracts were purified using the GeneClean® procedure (MP Biomedicals) and quantitatively and qualitatively characterised by PicoGreen based fluorometry (*ds*DNA; Qubit, LifeTechnologies), µL-spectrophotometry (PicoDrop) and agarose-gel electrophoresis (Ascher et al., 2012).

2.6. Quantitative real-time PCR

Quantitative real-time PCR (qPCR) analysis was chosen to determine the 16S rRNA gene copy number of bacteria and archaea, and the 18S rRNA gene copy number of fungi from both the intracellular and extracellular fractions of the total soil DNA pool. Real-time PCR was conducted using the 1X Sensimix™ SYBR® Hi-rox (Bioline, USA) and performed in a Rotor-Gene™ 6000 (Corbett Research, Sydney, Australia) in 20-µl volumes. Each standard reaction mix contained 1X Sensimix™ SYBR® Hi-rox (Bioline, USA), forward and reverse primers (200 nM each primer), 0.4 mg mL^{-1} BSA, distilled water (RNase/DNase free, Gibco™, UK) and 2 µL of 1:10 diluted DNA-extracts, and ten-fold diluted standard DNA. To build the standards we used purified PCR products of known concentrations of the following pure cultures as template: *Nitrosomonas europaea* (DSMZ 21879) − bacteria;

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