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

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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-

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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;

Table 1
Overview of soil physico-chemical parameters at the north- and the south-facing sites (N3 and S8, respectively) and as a function of the different ground covers (branches, grass, moss and litter). Values are means with SD in brackets. Data are expressed on a dry weight basis. Different letters in bold, for each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) with regard to the ground cover type. Due to a high stone content, sampling depth was in some cases reduced to 10 or 12.5 cm.

Soil depth	0–2.5 cm			2.5–5 cm			5–7.5 cm			7.5–10 cm			10–12.5 cm		
Site N3	Branches	Grass	Moss	Branches	Grass	Moss	Branches	Grass	Moss	Branches	Grass	Moss	Branches	Grass	Moss
Moisture (%)	48a (6.4)	50a (13.1)	53a (4.4)	42a (2.8)	55a (2.1)	57a (5.8)	44a (5.8)	42a (7.6)	47a (12)	36a (7.9)	25a (3.6)	36a (8.3)	n.a.	33 (7.1)	n.a.
LOI (%)	83a (4.6)	87a (1.2)	81a (17)	64a (12.8)	77a (18.2)	80a (19)	34a (6.3)	52a (5.8)	83a (10)	22a (2.7)	16a (4.3)	35a (9.2)	n.a.	13 (4.7)	n.a.
pH	5.2a (0.5)	5.4a (0.3)	4.6a (0.1)	4.8a (0.5)	4.7a (0.4)	4.0a (0.2)	4.6a (0.5)	4.3a (0.4)	3.9a (0.2)	4.4a (0.3)	4.4a (0.3)	4.0a (0.4)	n.a.	4.3 (0.6)	n.a.
EC (µS cm^{-1})	160**a** (21)	166**a** (31)	84**b** (22)	74a (11)	67a (22)	60a (19)	45a (14)	50a (13)	55a (18)	29a (7.0)	34a (13)	37a (12)	n.a.	41 (3.0)	n.a.
Total C (%)	47a (1.4)	49a (1.5)	47a (6.7)	33a (6.4)	44a (8.4)	41a (11)	27a (9.4)	49a (1.7)	45a (7.0)	5.8a (1.7)	6.5a (1.8)	4.7a (3.0)	n.a.	18.5 (7.9)	n.a.
Total N (%)	1.7a (0.2)	1.6a (0.1)	1.6a (0.3)	1.4a (0.3)	1.5a (0.5)	1.4a (0.1)	0.9a (0.2)	1.5a (0.4)	1.1a (0.3)	0.4a (0.02)	0.3a (0.05)	0.2a (0.01)	n.a.	0.6 (0.1)	n.a.
NH_4^+ (mg kg^{-1} dw)	145a (35)	123a (37)	116a (20)	118**a** (37)	104**a** (14)	62**b** (16)	49**ab** (17)	35**a** (11)	56**b** (11)	24a (9.0)	15a (4.0)	12a (2.0)	n.a.	26 (9.0)	n.a.
NO_3^- (mg kg^{-1} dw)	64a (12)	44a (13)	142a (35)	39a (8.0)	23a (3.0)	34a (11)	6.0a (2.0)	11a (2.0)	8.0a (2.0)	3.0a (0.3)	0.8a (0.03)	1.0a (0.03)	n.a.	1.3 (0.3)	n.a.
Total P (mg kg^{-1})	542**a** (72)	657**ab** (135)	926**b** (119)	333**a** (96)	734**ab** (152)	1033**b** (338)	534**a** (178)	391**a** (93)	628**b** (110)	266**a** (62)	591**b** (184)	221**a** (21)	n.a.	469 (28)	n.a.
Available P (mg kg^{-1})	291a (43)	216a (42)	358a (17)	105a (55)	193a (91)	139a (47)	66a (23)	87a (28)	55a (19)	13a (2.0)	14a (7.0)	25a (3.0)	n.a.	12 (3.0)	n.a.

Soil depth	0–2.5 cm			2.5–5 cm			5–7.5 cm			7.5–10 cm			10–12.5 cm			12.5–15 cm		
Site S8	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter
Moisture (%)	58a (5.3)	43a (6.5)	50a (8.7)	52a (8.3)	41a (9.5)	35a (8.8)	43a (9.1)	34a (4.7)	26a (8.2)	39a (3.4)	33a (9.7)	19a (4.3)	n.a.	25a (7.3)	18a (4.4)	n.a.	n.a.	19 (4.9)
LOI (%)	79a (10.6)	74a (13)	63a (10)	79**a** (11.3)	29**b** (8.0)	20**b** (19)	72**a** (11)	27**b** (3.5)	20**b** (4.3)	57**a** (5.4)	14**b** (3.2)	14**b** (1.6)	n.a.	14a (1.1)	13a (1.9)	n.a.	n.a.	12 (2.5)
pH	5.3a (0.3)	5.3a (0.1)	5.3a (0.7)	5.6a (0.6)	5.0a (0.4)	5.3a (0.9)	5.2a (0.9)	4.7a (0.6)	5.2a (0.8)	5.0a (0.8)	4.8a (0.5)	5.0a (0.2)	n.a.	5.0a (0.3)	5.2a (0.1)	n.a.	n.a.	5.1 (0.2)
EC (µS cm^{-1})	208**a** (41)	64**b** (16)	62**b** (15)	129**a** (30)	37**b** (9.0)	40**b** (4.0)	105**a** (11)	29**b** (6.8)	35**b** (5.0)	92**a** (21)	24**b** (7.0)	42**b** (11)	n.a.	15a (5.0)	22a (6.0)	n.a.	n.a.	24 (2.0)
Total C (%)	43a (3.6)	39a (7.4)	35a (10)	41**a** (6.6)	15**b** (4.1)	18**b** (7.3)	44**a** (0.2)	14**b** (1.7)	9.1**b** (2.6)	27**a** (7.1)	6.7**b** (2.4)	5.9**b** (0.5)	n.a.	6.0a (1.1)	7.2a (2.1)	n.a.	n.a.	5.4 (1.8)
Total N (%)	1.6a (0.1)	1.2a (0.1)	1.3a (0.5)	1.4a (0.1)	0.7a (0.1)	0.8a (0.06)	1.4**a** (0.1)	0.5**b** (0.2)	0.4**b** (0.01)	1.1**a** (0.2)	0.3**b** (0.06)	0.3**b** (0.01)	n.a.	0.3a (0.01)	0.3a (0.07)	n.a.	n.a.	0.3 (0.08)
NH_4^+ (mg kg^{-1} dw)	160**a** (44)	69**b** (9.0)	60**b** (9.0)	96**a** (14)	29**b** (11)	24**b** (5.0)	77**a** (16)	23**b** (5.0)	17**b** (7.0)	68**a** (18)	12**b** (3.0)	13**b** (0.5)	n.a.	12a (4.0)	14a (5.0)	n.a.	n.a.	9.0 (2.0)
NO_3^- (mg kg^{-1} dw)	154**a** (33)	33**b** (9.0)	32**b** (6.0)	66**a** (17)	15**b** (2.0)	15**b** (3.0)	19**a** (8.0)	3.0**b** (0.9)	3.0**b** (1.0)	26**a** (9.0)	2.0**b** (0.8)	1.0**b** (0.09)	n.a.	1.0a (0.08)	1.0a (0.03)	n.a.	n.a.	0.8 (0.02)
Total P (mg kg^{-1})	687a (189)	663a (199)	592a (144)	537a (190)	635a (176)	582a (40)	496a (95)	321a (84)	407a (72)	566a (103)	344a (34)	549a (149)	n.a.	328a (21)	483a (157)	n.a.	n.a.	456 (167)
Available P (mg kg^{-1})	196a (20)	116a (41)	161a (35)	161**a** (25)	74**ab** (18)	35**b** (9.0)	73**a** (11)	22**b** (6.0)	20**b** (6.0)	65**a** (16)	8.0**b** (0.5)	12**b** (1.5)	n.a.	8.0a (0.5)	8.0a (0.9)	n.a.	n.a.	8.0 (1.5)

LOI: loss on ignition; EC: electrical conductivity; n.a.: not available.

Table 2
Overview of soil potential enzymatic activities at the north- and the south-facing sites (N3 and S8, respectively) and as a function of the different ground covers (branches, grass, moss and litter). Values are means with SD in brackets. Data are expressed as nanomoles of MUF min^{-1} g^{-1} soil dry weight. Different letters in bold, for each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) with regard to the ground cover type. Due to a high stone content, sampling depth was in some cases reduced to 10 or 12.5 cm.

Soil depth	0–2.5 cm			2.5–5 cm			5–7.5 cm			7.5–10 cm			10–12.5 cm		
Site N3	Branches	Grass	Moss	Branches	Grass	Moss	Branches	Grass	Moss	Branches	Grass	Moss	Branches	Grass	Moss
Cellulase	59a (12)	36a (12)	21a (5.1)	12a (6.3)	16a (3.3)	9.0a (3.7)	5.8**a** (2.2)	5.2**a** (4.0)	1.6**b** (0.4)	1.4a (0.7)	2.4a (1.1)	1.2a (0.9)	n.a.	1.1 (0.3)	n.a.
Xylanase	48a (7.2)	37a (2.8)	28a (7.3)	29a (8.8)	36a (13)	22a (4.4)	20a (3.7)	20a (9.3)	9.6a (2.5)	8.2a (3.9)	9.2a (2.0)	5.2a (1.4)	n.a.	5.4 (1.4)	n.a.
α-glucosidase	42a (7.9)	29a (7.0)	29a (7.8)	30a (13.7)	24a (6.1)	24a (1.3)	22a (3.0)	19a (9.0)	12a (4.5)	8.7a (1.8)	16a (7.1)	7.4a (1.4)	n.a.	10 (3.3)	n.a.
β-glucosidase	349a (70)	223a (64)	139a (30.5)	106a (25)	126a (41)	68a (20)	63a (7.0)	59a (10)	24a (9.5)	25a (3.4)	28a (8.8)	15a (5.1)	n.a.	21 (2.6)	n.a.
Acid phosphomonoesterase	520a (134)	516a (194)	641a (93)	626a (188)	733a (182)	554a (125)	366a (41)	533a (95)	198a (52)	210a (69)	432a (98)	160a (40)	n.a.	157 (24)	n.a.
Alkaline phosphomonoesterase	342**a** (88)	286**a** (32)	79**b** (19)	215**a** (69)	84**ab** (16)	50**b** (11)	55**a** (28)	42**a** (14)	18**b** (8.0)	16a (3.2)	29a (8.0)	11a (1.2)	n.a.	20 (9.0)	n.a.
Phosphodiesterase	37a (8.0)	29a (2.9)	16a (0.6)	32a (0.9)	21a (9.0)	16a (2.1)	11a (0.4)	10a (1.9)	4.1a (1.4)	5.1a (0.9)	10a (1.7)	3.7a (0.6)	n.a.	5.3 (0.8)	n.a.
Pyrophosphate-phospodiesterase	35**a** (6.0)	25**a** (4.0)	6.1**b** (1.4)	38**a** (5.0)	6.4**ab** (0.6)	2.9**b** (1.4)	6.0**a** (0.5)	3.1**ab** (0.09)	0.9**b** (0.1)	1.9a (0.3)	3.7a (0.4)	1.0a (0.06)	n.a.	1.9 (0.05)	n.a.
Leucine-aminopeptidase	487**a** (72)	469**a** (143)	177**b** (22)	338**a** (96)	187**ab** (51)	107**b** (25)	121a (8.0)	98a (15)	59a (11)	47a (12)	97a (34)	44a (10)	n.a.	48 (13)	n.a.
Lysine-aminopeptidase	307**a** (35)	340**a** (42)	103**b** (14)	209**a** (55)	115**ab** (32)	65**b** (17)	88a (6.0)	61a (18)	40a (8.0)	32a (8.0)	50a (15)	32a (3.0)	n.a.	30 (13)	n.a.
Arylsulphatase	7.0a (1.6)	6.3a (1.7)	2.7a (0.8)	12a (5.6)	4.9a (0.9)	3.6a (0.8)	10a (0.7)	13a (1.9)	2.3a (1.4)	5.9a (0.05)	6.0a (6.2)	2.0a (0.09)	n.a.	7.1 (1.7)	n.a.

Soil depth	0–2.5 cm			2.5–5 cm			5–7.5 cm			7.5–10 cm			10–12.5 cm			12.5–15 cm		
Site S8	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter	Branches	Grass	Litter
Cellulase	29a (10)	23a (14)	26a (12)	15**a** (2.1)	4.4**b** (1.5)	2.6**b** (0.7)	5.4a (2.7)	1.3a (0.5)	1.6a (0.8)	6.7**a** (3.5)	1.3**b** (0.1)	1.8**b** (0.4)	n.a.	0.6a (0.2)	0.9a (0.1)	n.a.	n.a.	0.9 (0.2)
Xylanase	23a (9.6)	28a (12.3)	21a (10.1)	21a (4.6)	17a (4.9)	14a (5.2)	14a (3.7)	8.9a (2.6)	7.9a (1.4)	18**a** (1.1)	5.9**b** (1.2)	6.7**b** (1.2)	n.a.	4.2a (0.7)	5.0a (1.4)	n.a.	n.a.	4.5 (0.9)
α-glucosidase	18a (3.3)	25a (3.5)	17a (2.7)	18a (3.6)	16a (9.9)	8.3a (1.9)	16a (2.9)	8.5a (1.6)	6.3a (1.8)	14a (5.2)	6.4a (1.5)	5.2a (0.5)	n.a.	3.2a (0.8)	3.6a (0.5)	n.a.	n.a.	2.8 (0.4)
β-glucosidase	140a (41)	141a (35)	126a (35)	129**a** (5.3)	56**b** (14)	37**b** (9.0)	62a (11)	26a (6.5)	25a (5.0)	52a (19)	20a (8.5)	29a (11)	n.a.	9.3a (1.4)	17a (1.6)	n.a.	n.a.	13 (2.7)
Acid phosphomonoesterase	670a (188)	763a (170)	739a (107)	639a (187)	659a (95)	594a (152)	538a (89)	714a (147)	662a (156)	1031a (260)	660a (190)	878a (157)	n.a.	264a (25)	805a (217)	n.a.	n.a.	647 (85)
Alkaline phosphomonoesterase	135a (12)	168a (29)	137a (18)	183**a** (22)	62**b** (17)	35**b** (6.1)	177**a** (29)	27**b** (1.0)	15**b** (1.8)	106**a** (27)	29**b** (8.0)	13**b** (2.5)	n.a.	18a (1.5)	12a (1.1)	n.a.	n.a.	14 (0.8)
Phosphodiesterase	29a (9.5)	35a (12)	29a (5.1)	26a (3.6)	19a (1.1)	10a (1.0)	33**a** (1.2)	11**b** (3.0)	7.0**b** (0.9)	29**a** (7.5)	10**b** (3.0)	6.2**b** (0.5)	n.a.	8.2a (1.4)	6.7a (1.3)	n.a.	n.a.	5.5 (1.4)
Pyrophosphate-phospodiesterase	28a (3.6)	40a (15)	47a (12)	49a (17)	12a (6.0)	11a (0.7)	44**a** (8.0)	6.4**b** (0.9)	6.4**b** (1.0)	26**a** (2.0)	8.2**b** (0.8)	8.3**b** (0.09)	n.a.	5.8a (0.6)	7.2a (0.03)	n.a.	n.a.	5.6 (1.2)
Leucine-aminopeptidase	200a (23)	226a (65)	124a (31)	205**a** (41)	106**ab** (20)	52**b** (4.0)	187**a** (28)	58**b** (9.0)	31**b** (10)	152**a** (34)	50**b** (14)	31**b** (5.0)	n.a.	39a (4.0)	27a (2.0)	n.a.	n.a.	25 (8.0)
Lysine-aminopeptidase	133a (22)	143a (41)	80a (23)	126**a** (18)	59**b** (13)	29**b** (9.0)	105**a** (22)	37**b** (6.0)	17**b** (0.7)	96**a** (15)	33**b** (11)	18**b** (1.5)	n.a.	22a (2.0)	13a (1.0)	n.a.	n.a.	12 (1.5)
Arylsulphatase	2.4a (0.1)	3.0a (0.8)	3.3a (0.9)	5.6a (0.7)	4.9a (0.7)	2.2a (0.3)	14a (0.6)	7.5a (1.4)	3.8a (1.2)	8.0a (1.7)	8.0a (1.9)	6.7a (3:4)	n.a.	9.3a (1.3)	6.1a (1.9)	n.a.	n.a.	4.7 (0.5)

n.a.: not available.

Table 3
Overview of the microbial biomass index determined by dsDNA along with the yields of intracellular (iDNA) and extracellular DNA (eDNA) and the eDNA/iDNA ratio at the north- and the south-facing sites (N3 and S8, respectively) and as a function of the different ground covers (branches, grass, moss and litter). Values are means with SD in brackets. Data are expressed on a dry weight basis. Different letters in bold, for each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) with regard to the ground cover type. Due to a high stone content, sampling depth was in some cases reduced to 10 or 12.5 cm.

Soil depth	0–2.5 cm		2.5–5 cm			5–7.5 cm		7.5–10 cm			10–12.5 cm			12.5–15 cm		
Site N3	Branch	Grass	Moss	Branch	Grass	Moss	Branch	Grass	Moss	Branch	Grass	Moss	Branch	Grass	Moss	Branch	Grass	Moss
dsDNA yields (μg g^{-1} soil)	452a (79)	354a (98)	308a (31)	349a (145)	346a (45)	282a (15)	281a (76)	276a (67)	120a (32)	153a (84)	119a (55)	87a (15)	n.a.	84 (12)	n.a.	n.a	n.a	n.a
iDNA yields (μg g^{-1} soil)	197a (41)	97a (20)	69a (24)	91a (10)	83a (22)	48a (7.0)	71a (15)	83a (28)	60a (17)	31**a** (0.3)	73**b** (2.0)	29**a** (15)	n.a.	25 (2.0)	n.a.	n.a	n.a	n.a
eDNA yields (μg g^{-1} soil)	17a (5.0)	45a (10)	36a (5.0)	27a (15)	55a (15)	27a (4.0)	18a (3.0)	27a (6.0)	15a (3.0)	12a (7.0)	13a (7.0)	9.0a (3.0)	n.a.	24 (4.0)	n.a.	n.a	n.a	n.a
eDNA/iDNA ratio	0.13a (0.02)	0.46a (0.18)	0.56a (0.19)	0.38a (0.06)	0.65a (0.15)	0.60a (0.19)	0.32a (0.15)	0.45a (0.11)	0.23a (0.04)	0.38a (0.13)	0.18a (0.06)	0.34a (0.16)	n.a.	0.96 (0.08)	n.a.	n.a	n.a	n.a
Site S8	Branch	Grass	Litter	Branch	Grass	Litter	Branch	Grass	Litter	Branch	Grass	Litter	Branch	Grass	Litter	Branch	Grass	Litter
dsDNA yields (μg g^{-1} soil)	237a (40)	241a (73)	247a (63)	325a (164)	272a (85)	155a (45)	322**a** (68)	150**b** (41)	91**b** (47)	390**a** (97)	160**b** (39)	65**b** (15)	n.a.	82a (24)	51a (9.0)	n.a.	n.a.	41 (9.0)
iDNA yields (μg g^{-1} soil)	83a (14)	92a (30)	119a (5.0)	97a (32)	68a (21)	29a (8.0)	84a (26)	55a (16)	57a (19)	99a (32)	61a (18)	58a (14)	n.a.	34a (6.0)	53a (15)	n.a.	n.a.	28 (10)
eDNA yields (μg g^{-1} soil)	12a (5.0)	9.0a (1.0)	12a (4.0)	13a (7.0)	17a (8.0)	9.0a (2.0)	12a (3.0)	15a (5.0)	8.0a (2.0)	27a (3.0)	13a (6.0)	7.0a (1.0)	n.a.	7.0a (2.0)	7.0a (0.6)	n.a.	n.a.	6.0 (0.2)
eDNA/iDNA ratio	0.15a (0.05)	0.11a (0.05)	0.10a (0.04)	0.13a (0.07)	0.23a (0.09)	0.29a (0.07)	0.16a (0.05)	0.31a (0.08)	0.17a (0.09)	0.25a (0.09)	0.23a (0.10)	0.13a (0.05)	n.a.	0.21a (0.02)	0.18a (0.09)	n.a	n.a	0.28 (0.11)

n.a.: not available.

Methanobacterium formicicum (DSMZ 1535) − archaea; and *Fusarium solani*- fungi. The primer pairs used were: 1055f/1392r (bacteria; Ferris et al., 1996); FF390/FR1 (fungi; Prévost-Bouré et al., 2011); and Parch 519f/Arc915r (archaea; Coolen et al., 2004). Stock concentration [gene copies μL^{-1}] was determined via PicoGreen measurement and freshly prepared for the standard curve construction with ten-fold dilutions ranging from 10^9 to 10^2 copies μL^{-1}. All standards and samples were run in duplicate following the cycling conditions shown in Bardelli et al. (2017). To check for product specificity and potential primer dimer formation, runs were completed with a melting analysis starting from 60 °C to 95 °C with temperature increments of 0.25 °C and a transition rate of 5 s. The purity of the amplified products was also checked by the presence of a single band of the expected length on a 1% agarose gel stained with the DNA stain Midori Green (Nippon Genetics, Germany) and visualised by UV-transillumination (Vilber Lourmat Deutschland GmbH).

2.7. Humus forms and soil fauna

2.7.1. Humus forms

The designation of the humus forms and organic horizons followed Zanella et al. (2011). The mineral horizon designation was done according to the Guidelines for Soil Description (FAO, 2006) and the German soil classification system (Ad-hoc-AG Boden, 2005).

2.7.2. Microannelid sample treatment

Microannelid extraction from soil samples was performed over 48 h by a wet-funnel technique without heating (Dunger and Fiedler, 1989; ISO 23611-3, 2007). The extracted animals were counted and then identified alive according to the key of Schmelz and Collado (2010). Knowledge-based acidity indicator values and humus form preferences according to Graefe and Schmelz (1999) are referred to. The following acidity indicator groups are distinguished: 'indicators of strong acidity' (species with indicator values 1–3), 'indicators of moderate acidity' (species with indicator values 4–6) and 'indicators of slight acidity' (species with indicator value 7). During preliminary investigations in the precedent year, microannelid assemblages were found that did not match the visible humus form. In order to disentangle this inconsistency a sampling design was chosen that allowed physico-chemical measurements like pH in the direct vicinity of the microannelid assemblage.

2.8. Statistical analyses

One-factor ANOVA was carried out for each soil depth separately to evaluate the effects of *ground cover* on the soil physico-chemical and microbiological parameters at both north-and south-facing sites. Significant differences ($p < 0.05$) were further analysed by paired comparisons with the Tukey HSD test. The vertical gradient of each variable was tested by repeated measurements ANOVA (ANOVAR) considering the sequentially sampled soil horizons in 2.5-cm intervals. The normality and the variance homogeneity of the data were tested prior to ANOVA by using the Kolmogorov-Smirnov and Levenés tests, respectively. Before analysis, data were log- or square root-transformed to meet the assumptions for ANOVA (when it was required). The software package Statistica 9 (StatSoft, USA) was used to perform these analyses.

Statistical analyses for microannelid parameters were performed using the software package Systat 13.1 (Systat Software inc., USA). The normality and the variance homogeneity of the data were tested prior to ANOVA or Two-sample *t*-test. Before analysis, microannelid abundance data were square root-transformed to meet the assumptions for ANOVA (Post-Hoc: Tukey HSD-test) or Two-sample *t*-test. A nonparametric Mann-Whitney U-Test was carried out to evaluate the effects of exposure and ground cover on the dominance of microannelid acidity indicator groups as well as on the thickness of organic horizons (L, OF, OH), as transformations (ln, log, square-root) did not produce satisfac-

Table 4
Abundance of bacteria, fungi and archaea determined by quantitative real-time PCR in both the intra- and extracellular DNA fractions at the north- and the south-facing sites (N3 and S8, respectively) and as a function of the different ground covers (branches, grass, moss and litter). Values are means with SD in brackets. Data are expressed as gene copy number per g soil on a dry weight basis. Different letters in bold, for each of the soil depths, indicate significant differences ($p < 0.05$; ANOVA followed by Tukey post-hoc test) with regard to the ground cover type.

Soil depth	0–2.5 cm			2.5–5 cm			5–7.5 cm		
Site N3	Branch	Grass	Moss	Branch	Grass	Moss	Branch	Grass	Moss
Bacteria iDNA	1.3×10^{8}**b** (6.8×10^{7})	1.3×10^{9}**a** (3.1×10^{7})	1.6×10^{9}**a** (2.1×10^{7})	4.9×10^{8}a (5.4×10^{7})	9.6×10^{8}a (5.9×10^{7})	3.6×10^{8}a (2.8×10^{7})	3.3×10^{8}a (3.1×10^{7})	1.2×10^{9}a (7.3×10^{8})	1.3×10^{9}a (7.7×10^{7})
Bacteria eDNA	9.2×10^{6}**b** (3.4×10^{5})	1.7×10^{8}**a** (9.7×10^{6})	2.4×10^{8}**a** (9.7×10^{7})	2.0×10^{8}a (7.7×10^{7})	1.1×10^{8}a (3.6×10^{6})	1.1×10^{8}a (7.5×10^{6})	3.8×10^{7}a (1.6×10^{6})	9.6×10^{7}a (3.0×10^{6})	2.8×10^{7}a (1.9×10^{6})
Fungi iDNA	3.2×10^{7}**b** (3.5×10^{6})	2.1×10^{8}**a** (8.3×10^{6})	3.6×10^{8}**a** (1.6×10^{7})	1.6×10^{8}a (1.7×10^{7})	2.7×10^{8}a (2.1×10^{6})	1.9×10^{8}a (1.5×10^{7})	1.8×10^{8}a (2.2×10^{6})	1.7×10^{8}a (6.5×10^{6})	1.4×10^{8}a (9.1×10^{6})
Fungi eDNA	1.3×10^{6}**b** (6.6×10^{4})	3.3×10^{7}**a** (8.7×10^{5})	4.3×10^{7}**a** (3.8×10^{5})	1.0×10^{7}a (9.8×10^{5})	3.4×10^{7}a (4.4×10^{5})	6.5×10^{7}a (3.5×10^{6})	4.1×10^{7}a (9.5×10^{5})	9.7×10^{6}a (4.1×10^{5})	4.6×10^{6}a (1.5×10^{4})
Archaea iDNA	5.7×10^{7}**b** (4.2×10^{5})	7.8×10^{7}**b** (5.1×10^{6})	2.6×10^{8}**a** (5.8×10^{6})	1.2×10^{8}a (7.5×10^{6})	8.7×10^{7}a (4.6×10^{5})	6.8×10^{7}a (2.8×10^{6})	1.0×10^{8}a (9.4×10^{6})	1.1×10^{8}a (3.3×10^{7})	2.7×10^{8}a (8.0×10^{6})
Archaea eDNA	4.5×10^{5}a (3.9×10^{3})	8.9×10^{5}a (4.1×10^{4})	1.1×10^{6}a (6.5×10^{3})	8.2×10^{5}a (1.3×10^{3})	7.5×10^{5}a (2.0×10^{4})	8.7×10^{5}a (1.3×10^{4})	1.4×10^{6}a (1.1×10^{3})	4.5×10^{5}a (1.1×10^{4})	1.0×10^{5}a (8.2×10^{3})
Site S8	Branch	Grass	Litter	Branch	Grass	Litter	Branch	Grass	Litter
Bacteria iDNA	3.9×10^{8}a (1.2×10^{7})	1.3×10^{8}a (8.0×10^{6})	2.3×10^{8}a (7.7×10^{6})	2.3×10^{8}a (1.2×10^{6})	4.5×10^{8}a (3.8×10^{6})	1.4×10^{8}a (9.9×10^{6})	3.6×10^{8}a (2.7×10^{6})	3.7×10^{8}a (2.5×10^{7})	1.5×10^{8}a (9.8×10^{6})
Bacteria eDNA	1.4×10^{7}a (8.9×10^{5})	7.9×10^{6}a (1.5×10^{5})	1.1×10^{7}a (9.7×10^{4})	1.1×10^{7}a (6.5×10^{5})	2.7×10^{7}a (1.3×10^{5})	9.1×10^{6}a (9.7×10^{4})	6.1×10^{6}a (4.6×10^{4})	2.3×10^{7}a (1.6×10^{5})	5.3×10^{6}a (1.6×10^{4})
Fungi iDNA	6.5×10^{7}a (1.5×10^{5})	3.5×10^{7}a (6.6×10^{5})	3.3×10^{7}a (8.7×10^{5})	2.8×10^{7}a (9.5×10^{5})	6.8×10^{7}a (2.5×10^{5})	2.7×10^{7}a (1.5×10^{7})	4.1×10^{7}a (4.0×10^{6})	4.9×10^{7}a (8.5×10^{5})	4.7×10^{7}a (3.4×10^{6})
Fungi eDNA	3.9×10^{6}a (2.2×10^{5})	1.8×10^{6}a (9.3×10^{4})	1.3×10^{6}a (6.4×10^{4})	1.2×10^{6}a (8.5×10^{4})	2.6×10^{6}a (1.4×10^{3})	1.7×10^{6}a (2.0×10^{4})	5.0×10^{5}a (3.7×10^{3})	3.4×10^{6}a (2.3×10^{4})	2.4×10^{6}a (2.5×10^{4})
Archaea iDNA	7.4×10^{7}a (3.5×10^{5})	3.8×10^{7}a (1.7×10^{5})	3.3×10^{7}a (8.3×10^{5})	5.3×10^{7}a (1.4×10^{6})	8.4×10^{7}a (5.4×10^{6})	2.3×10^{7}a (9.5×10^{5})	6.0×10^{7}a (1.4×10^{6})	6.8×10^{7}a (2.0×10^{6})	3.4×10^{7}a (7.7×10^{4})
Archaea eDNA	1.7×10^{5}a (7.9×10^{3})	4.9×10^{5}a (2.3×10^{4})	5.4×10^{5}a (6.9×10^{2})	8.8×10^{5}a (1.1×10^{2})	7.3×10^{5}a (4.8×10^{3})	1.7×10^{6}a (4.8×10^{3})	1.6×10^{5}a (8.9×10^{4})	1.1×10^{6}a (5.3×10^{3})	9.0×10^{5}a (9.5×10^{3})

Soil depth	7.5–10 cm			10–12.5 cm			12.5–15 cm		
Site N3	Branch	Grass	Moss	Branch	Grass	Moss	Branch	Grass	Moss
Bacteria iDNA	2.3×10^{8}a (3.1×10^{7})	1.1×10^{9}a (1.3×10^{8})	7.3×10^{8}a (6.3×10^{6})	n.a.	7.2×10^{8} (2.5×10^{7})	n.a.	n.a.	n.a.	n.a.
Bacteria eDNA	3.7×10^{7}a (9.2×10^{5})	5.4×10^{7}a (3.6×10^{6})	1.7×10^{7}a (1.3×10^{6})	n.a.	3.9×10^{7} (8.5×10^{6})	n.a.	n.a.	n.a.	n.a.
Fungi iDNA	5.2×10^{7}a (4.8×10^{5})	1.4×10^{8}a (9.2×10^{6})	7.6×10^{7}a (6.2×10^{5})	n.a.	4.0×10^{7} (8.0×10^{5})	n.a.	n.a.	n.a.	n.a.
Fungi eDNA	2.1×10^{6}a (6.2×10^{4})	5.7×10^{6}a (4.3×10^{4})	2.4×10^{6}a (2.0×10^{4})	n.a.	9.6×10^{6} (1.1×10^{5})	n.a.	n.a.	n.a.	n.a.
Archaea iDNA	4.5×10^{7}a (7.7×10^{5})	8.9×10^{7}a (8.3×10^{5})	8.4×10^{7}a (7.9×10^{5})	n.a.	5.3×10^{7} (4.0×10^{5})	n.a.	n.a.	n.a.	n.a.
Archaea eDNA	1.4×10^{6}a (3.0×10^{4})	7.4×10^{5}a (9.5×10^{3})	5.4×10^{5}a (4.9×10^{3})	n.a.	2.2×10^{6} (8.2×10^{3})	n.a.	n.a.	n.a.	n.a.
Site S8	Branch	Grass	Litter	Branch	Grass	Litter	Branch	Grass	Litter
Bacteria iDNA	3.2×10^{8}a (2.9×10^{6})	2.1×10^{8}a (1.2×10^{6})	1.8×10^{8}a (7.6×10^{6})	n.a.	1.5×10^{8}a (5.1×10^{6})	6.5×10^{7}a (7.9×10^{5})	n.a.	n.a.	2.8×10^{7} (1.5×10^{5})
Bacteria eDNA	1.6×10^{7}a (7.1×10^{5})	1.3×10^{7}a (7.7×10^{5})	7.0×10^{6}a (3.9×10^{5})	n.a.	6.4×10^{6}a (5.1×10^{4})	4.6×10^{6}a (2.3×10^{4})	n.a.	n.a.	2.8×10^{6} (1.1×10^{4})
Fungi iDNA	2.5×10^{7}a (1.3×10^{6})	2.7×10^{7}a (3.0×10^{5})	2.3×10^{7}a (9.8×10^{4})	n.a.	1.7×10^{7}a (7.0×10^{5})	1.3×10^{7}a (5.7×10^{4})	n.a.	n.a.	2.4×10^{7} (7.3×10^{4})
Fungi eDNA	1.3×10^{6}a	1.3×10^{6}a (3.9×10^{3})	2.7×10^{6}a	n.a.	2.0×10^{6}a (1.5×10^{3})	4.3×10^{6}a (4.2×10^{3})	n.a.	n.a.	1.6×10^{6}

(continued on next page)

Table 4 *(continued)*

Soil depth	7.5–10 cm			10–12.5 cm			12.5–15 cm		
Site N3	Branch	Grass	Moss	Branch	Grass	Moss	Branch	Grass	Moss
	(4.4×10^3)		(5.7×10^4)						(1.3×10^5)
Archaea iDNA	5.0×10^7a	6.0×10^7a (2.2×10^6)	2.4×10^7a	n.a.	3.9×10^7a (3.5×10^5)	1.9×10^7a (8.8×10^4)	n.a.	n.a.	6.7×10^6
	(3.8×10^5)		(2.1×10^3)						(2.6×10^6)
Archaea eDNA	6.4×10^5a	3.5×10^5a (8.3×10^3)	2.4×10^6a	n.a.	8.5×10^5a (5.0×10^2)	2.2×10^6a (8.8×10^4)	n.a.	n.a.	2.2×10^6
	(6.8×10^4)		(2.6×10^3)						(1.7×10^5)

tory results. Data for the total thickness of the humus layer were normally distributed, thus a *t*-test was used, checking for differences between N3 and S8. Pearson correlations to evaluate the potential use of eDNA as a nutrient source for microannelids were also assessed with the Systat 13.1 software program.

3. Results

3.1. Soil physico-chemical and microbiological parameters

An overview of the soil physico-chemical and microbiological parameters of the topsoil (0–15 cm: 2.5 cm intervals) at the north- and the south-facing sites (N3 and S8) under the different ground covers is given in Tables 1–4. For each of the soil depths, those parameters that led to significant differences among the ground cover types according to one-factor ANOVA are reported below.

In the uppermost soil layer (0–2.5 cm) at N3 the electrical conductivity was 2-fold lower in the moss plots than in the branch and grass plots (ANOVA $F_{2,6} = 5.3$, $p = 0.047$). Likewise, leucine- and lysine-aminopeptidase potential activities were also around 3-times lower in the moss plots than in the other two ground covers (*leu*: ANOVA $F_{2,6} = 9.5$, $p = 0.014$; *lys*: ANOVA $F_{2,6} = 9.4$, $p = 0.014$). The same trend was recorded for the alkaline phosphomonoesterase activity (*alkP*: ANOVA $F_{2,6} = 5.5$, $p = 0.044$). Furthermore, the highest total P content was recorded in the moss plots (ANOVA $F_{2,6} = 8.5$, $p = 0.018$). For both iDNA and eDNA fractions the bacterial and fungal abundance assessed by qPCR were significantly higher in the moss plots followed by grass and branch plots (*bacteria*, iDNA: ANOVA $F_{2,6} = 18.4$, $p = 0.003$; eDNA: ANOVA $F_{2,6} = 12.1$, $p = 0.008$; *fungi*, iDNA: ANOVA $F_{2,6} = 41.1$, $p = 0.0003$; eDNA: ANOVA $F_{2,6} = 15.8$, $p = 0.004$). In addition, the archaeal abundance in the iDNA fraction was around 3-times higher in the moss plots than in those covered with grass and branches (ANOVA $F_{2,6} = 5.7$, $p = 0.04$).

In the soil layers ranging from 2.5 to 7.5 cm at N3 the highest total P content was also found in the moss plots (ANOVA $F_{2,6} = 6.1$, $p = 0.035$; ANOVA $F_{2,6} = 82.8$, $p = 0.00004$, respectively). Moreover, for the 2.5–5 cm soil layer the lowest alkaline phosphomonoesterase, leucine- and lysine-aminopeptidase activities were registered in the moss plots (*alkP*: ANOVA $F_{2,6} = 5.4$, $p = 0.045$; *leu*: ANOVA $F_{2,6} = 5.6$, $p = 0.042$; *lys*: ANOVA $F_{2,6} = 6.4$, $p = 0.032$). Likewise, in the 5–7.5 cm layer the alkaline phosphomonoestarase, together with pyrophosphate-phosphodiesterase and cellulase activities were between 3 and 6 times lower in the moss plots than in the other ground cover types (*alkP*: ANOVA $F_{2,6} = 7.3$, $p = 0.024$; *piroP:* ANOVA $F_{2,6} = 11.2$, $p = 0.009$; *cell*: ANOVA $F_{2,6} = 5.4$, $p = 0.045$). In contrast to the top 7.5 cm, in the 7.5–10 cm layer total P was 2-fold higher in the grass plots compared to moss and branch plots (ANOVA $F_{2,6} = 14.8$, $p = 0.005$).

In the top 2.5 cm at S8 the branch ground cover had a higher EC level (3-fold higher) compared to grass and litter plots (ANOVA $F_{2,6} = 15.8$, $p = 0.004$). The same trend was observed for inorganic N (NH_4^+: ANOVA $F_{2,6} = 10.4$, $p = 0.011$; NO_3^-: ANOVA $F_{2,6} = 28.8$, $p = 0.0008$). Accordingly, in the subsequent layers from 2.5 to 10 cm, the branch plots showed the highest EC, loss on ignition, total C and N, as well as the highest inorganic N and available P content. Moreover, certain enzyme activities involved in the C cycle (*betagluc*: ANOVA $F_{2,6} = 5.6$, $p = 0.042$; and *cell*: ANOVA $F_{2,6} = 8.3$, $p = 0.019$), together with those related to the N- and P-cycles (*leu*: ANOVA $F_{2,6} = 8.7$, $p = 0.017$; *lys*: ANOVA $F_{2,6} = 11.52$, $p = 0.009$; *alkP*: ANOVA $F_{2,6} = 10.2$, $p = 0.012$) were also significantly higher (between 2 and 5 times) in the branch plots compared to the grass and litter ones in the 2.5-5-cm soil layer. These three latter enzyme activities followed the same trend with respect to the ground covers in the 5–7.5-cm and 7.5-10-cm layers. Additionally, the highest phosphodiesterase and pyrophosphate-phosphodiesterase activities were registered in the branch plots in the 5–7.5- cm soil layer (*bisP*: ANOVA $F_{2,6} = 17.4$, $p = 0.003$; *piroP*:

ANOVA $F_{2,6} = 5.9$, $p = 0.038$). The branch plots also showed the highest xylanase and phosphodiesterase activities in the 7.5-10-cm layer (*xyl*: ANOVA $F_{2,6} = 5.2$, $p = 0.04$; *bisP*: ANOVA $F_{2,6} = 22.6$, $p = 0.002$). Accordingly, soil microbial biomass assessed as dsDNA content was also significantly higher in the branch plots than in the grass and litter ones for the soil layers ranging from 5 to 10 cm (ANOVA $F_{2,6} = 12.9$, $p = 0.006$; ANOVA $F_{2,6} = 7.4$, $p = 0.023$ for 5–7.5 and 7.5–10-cm layers, respectively).

The vertical gradient (0–15 cm) of each parameter was also evaluated for each of the ground cover types at N3 and S8 (Tables 1–4). A reduction in moisture content with soil depth was only recorded for the litter ground cover, being around 2-fold lower in the last three soil layers (from 7.5 to 15 cm) than in the uppermost 2.5-cm soil fraction (ANOVAR $F_{5,20} = 26.6$, $p < 0.0001$). A reduced loss on ignition as a function of soil depth was detected for both the branch and the grass plots (ANOVAR $F_{3,12} = 18.5$, $p = 0.02$; ANOVAR $F_{4,16} = 66.2$, $p < 0.0001$). Likewise, the uppermost layer from the moss and litter ground covers showed a higher loss on ignition (3–4 times higher) compared to the deeper layers (*moss*: ANOVAR $F_{3,12} = 30.1$, $p < 0.0001$; *litter*: ANOVAR $F_{5,20} = 40.02$, $p < 0.0001$). At N3 soil pH was significantly higher in the top 2.5 cm regardless of the ground cover (*branches*: ANOVAR $F_{3,12} = 3.8$, $p = 0.04$; *grass*: ANOVAR $F_{4,16} = 5.9$, $p = 0.004$; *moss*: ANOVAR $F_{3,12} = 12.8$, $p = 0.0004$). A significant reduction in EC with depth was recorded for all of the ground covers at N3 and S8 (*branches*: ANOVAR $F_{3,12} = 24.9$, $p < 0.0001$; *grass*: ANOVAR $F_{4,16} = 16.1$, $p < 0.0001$; *moss*: ANOVAR $F_{3,12} = 7.9$, $p = 0.003$; *litter*: ANOVAR $F_{5,20} = 11.4$, $p < 0.0001$). For the branch ground cover the lowest total C content was found in the deepest layer ranging from 7.5 to 10 cm, being 8 and 1.5-fold lower than in the top 2.5 cm at N3 and S8, respectively (ANOVAR $F_{3,12} = 23.1$, $p < 0.0001$). A similar trend with increasing depth was found for total N (ANOVAR $F_{3,12} = 24.6$, $p = 0.009$) and inorganic N (NH_4^+: ANOVAR $F_{3,12} = 14.07$, $p = 0.003$ and NO_3^-: ANOVAR $F_{3,12} = 19.5$, $p < 0.0001$). The above-mentioned parameters were also significantly reduced with depth in the moss and litter plots. The same occurred in the grass plots, even though at N3 there was an increase in the deepest layer (10–12.5 cm) in comparison with the 7.5-10-cm soil layer. Total P significantly varied with soil depth only for the moss plots where its content was between 2 and 5 times lower in the 7.5-10-cm layer than in the other soil layers (ANOVAR $F_{3,12} = 8.1$, $p = 0.003$). Overall, a decrease in available P with increasing soil depth was detected for all the ground covers at both study sites (*branches*: ANOVAR $F_{3,12} = 26.4$, $p < 0.0001$; *grass*: ANOVAR $F_{4,16} = 15.8$, $p < 0.0001$; *moss*: ANOVAR $F_{3,12} = 15.3$, $p = 0.0002$; *litter*: ANOVAR $F_{5,20} = 44.6$, $p < 0.0001$).

The branch plots at N3 showed the highest cellulase, xylanase and β-glucosidase activities in the uppermost 2.5-cm soil layer (*cell*: ANOVAR $F_{3,12} = 10.5$, $p = 0.001$; *xyl*: ANOVAR $F_{3,12} = 19.1$, $p < 0.0001$; *betagluc*: ANOVAR $F_{3,12} = 14.3$, p = 0.008). The same trend as a function of depth was observed for the alkaline phosphomonoesterase (ANOVAR $F_{3,12} = 5.2$, $p = 0.01$), and the N-related enzyme activities (*leu*: ANOVAR $F_{3,12} = 5.5$, $p = 0.013$; *lys*: ANOVAR $F_{3,12} = 7.1$, $p = 0.005$). These changes with depth were, however, less evident in the branch plots located at S8. For the grass ground cover all the enzymes related to the C cycle (*cell*: ANOVAR $F_{4,16} = 13.1$, $p < 0.0001$; *xyl*: ANOVAR $F_{4,16} = 23.4$, $p < 0.0001$; *alfagluc*: ANOVAR $F_{4,16} = 10.4$, $p = 0.0002$; *betagluc*: ANOVAR $F_{4,16} = 23.8$, $p < 0.0001$) and those involved in the P cycle (*acP*: ANOVAR $F_{4,16} = 3.3$, $p = 0.04$; *alkP*: ANOVAR $F_{4,16} = 13.4$, $p < 0.0001$; *bisP*: ANOVAR $F_{4,16} = 16.1$, $p < 0.0001$; *piroP*: ANOVAR $F_{4,16} = 13.9$, $p < 0.0001$) were significantly higher in the first top 2.5 cm at both slopes. This trend was also found for leucine- and lysine-aminopeptidase activities at N3 (*leu*: ANOVAR $F_{4,16} = 21.5$, $p < 0.0001$; *lys*: ANOVAR $F_{4,16} = 11.4$, $p = 0.0001$). Likewise, the highest enzyme activities in moss and litter plots (except for arylsulphatase activity) were also recorded in the uppermost soil layer.

The eDNA yields, and consequently the eDNA/iDNA ratio, were significantly lower in the 7.5-10-cm soil layer than in the uppermost layer in the grass plots at N3 (*eDNA*: ANOVAR $F_{4,16} = 5.6$, $p = 0.005$; *ratio*: ANOVAR $F_{4,16} = 3.5$, $p = 0.03$). Moreover, there was a significant decrease in the *ds*DNA content with increasing depth in both grass and moss plots (ANOVAR $F_{4,16} = 5.7$, $p = 0.005$; ANOVAR $F_{3,12} = 67.9$, $p < 0.0001$, respectively). The lowest iDNA yields were also recorded in the deepest layer (7.5–10 cm) of the moss plots (ANOVAR $F_{3,12} = 4.8$, $p = 0.02$). The same occurred for the abundance of the different microbial groups assessed by qPCR in the extracellular (*bacteria*: ANOVAR $F_{3,12} = 36.7$, $p < 0.0001$; *fungi*: ANOVAR $F_{3,12} = 8.9$, $p = 0.02$; *archaea*: ANOVAR $F_{3,12} = 5.2$, $p = 0.015$) and intracellular DNA fractions (*bacteria*: ANOVAR $F_{3,12} = 6.1$, $p = 0.009$; *fungi*: ANOVAR $F_{3,12} = 4.9$, $p = 0.01$; *archaea*: ANOVAR $F_{3,12} = 8.0$, $p = 0.003$). In the litter plots the highest *ds*DNA, iDNA and eDNA yields were also found in the top 2.5 cm (*dsDNA*: ANOVAR $F_{5,20} = 44.6$, $p < 0.0001$; *eDNA*: ANOVAR $F_{5,20} = 6.1$, $p = 0.002$; *iDNA*: ANOVAR $F_{5,20} = 11.2$, $p < 0.0001$), being around 2-fold higher than in the other soil layers.

3.2. Soil mesofauna (microannelids) and humus forms

The list of microannelid species extracted from soil samples at N3 and S8 plots is compiled in Table 5. All species belong to the family Enchytraeidae. Species are arranged according to their allocation to acidity indicator groups. These coincide, in general, with the preferred occurrence of the species in Moder or Mull humus profiles.

Total microannelid abundance was significantly affected by slope exposure, being higher at N3 ($p = 0.001$, SQR transformation, *t*-test). This was mainly due to a considerably higher number of strong acidity indicators, while the number of indicators of moderate as well as slight acidity was similar at N3 and S8 (Fig. 1). The comparison of plots with the same exposure but different ground covers revealed no significant difference in total abundance. In terms of dominance (relative abundance) indicators of strong acidity reached an average of > 70% at N3, whereas at S8 indicators of slight acidity amounted to > 50% at each ground-cover type (Table 5). The dominance of indicators of strong acidity was thus significantly higher at N3 ($p = 0.001$), while the dominance of indicators of moderate and slight acidity was significantly higher at S8 ($p = 0.041$ and $p = 0.009$, respectively, *U* test).

The average thickness of the organic layers OL + OF + OH amounted to 8 cm at N3 and to 5 cm at S8 (Fig. 2). Most discriminating between both sites was the thickness of the OH-horizon, being significantly ($p = 0.001$) thicker north-facing (5.0 cm) than south-facing (1.9 cm). The comparison of the morphological humus profile with the vertical distribution of microannelids revealed that

> 80% of the animals at N3 lived in the organic horizons above the mineral soil, whereas at S8 the animals were almost evenly distributed between the organic layer and the A-horizon. Total microannelid abundance showed no relation to soil depth in the uppermost 10 cm neither at N3 nor at S8. However, by the distinction of indicator groups the tendency to higher proportions of indicators of slight acidity in the upper layers became visible (Fig. 2). This was in agreement with the pH values that tended to decrease with increasing soil depth. For each single sample, the relation between the occurrence of acidity indicators and the pH value showed a recurring pattern: in layers with a pH < 5 indicators of strong acidity were by far predominant, whereas indicators of slight acidity prevailed above that threshold. This was found in spite of the fact that the humus profile in all the three field replicates includes a thick OH-horizon, which characterises the humus form as Moder (Fig. S1). This finding is partly blurred when pH as well as dominance of indicator groups is displayed as mean values of several replicates (comp. Fig. 2 with Figs. S1–S3).

Regarding the relation between microbial DNA and microannelids, the eDNA yields were positively correlated with the abundances of microannelids at the N3 grass plot (total enchytraeids: $p = 0.003$;

Table 5
Microannelid species extracted from soil samples at N3 (north-facing) and S8 (south-facing) at different ground covers (G = grass, B = branches, M = moss, L = litter) and their ecological classification with respect to soil acidity and humus form preference. n = 3 for each ground-cover type. sd: standard deviation.

		N3G	N3B	N3M	S8G	S8B	S8L	Acidity indicator group	Humus form preference
Bryodrilus ehlersi		12	10	–	–	–	–	strong	Moder
Cognettia sphagnetorum		103	114	116	6	26	6	strong	Moder
Euenchytraeus bisetosus		–	2	4	–	–	–	strong	Moder
Marionina clavata		58	161	30	–	–	–	strong	Moder
Mesenchytraeus pelicensis		–	4	1	2	–	–	strong	Moder
Enchytraeus norvegicus		1	–	–	14	–	6	moderate	Intermed
Enchytronia parva		10	1	–	8	5	17	moderate	Intermed
Mesenchytraeus glandulosus		7	2	11	–	5	–	moderate	Intermed
Achaeta sp. (dzwi)[1]		–	–	–	–	–	1	slight	Mull
Buchholzia appendiculata		19	55	–	1	–	21	slight	Mull
Fridericia bisetosa		–	–	–	–	2	3	slight	Mull
Fridericia bulboides		4	9	–	17	3	5	slight	Mull
Fridericia connata		–	–	–	–	36	1	slight	Mull
Fridericia stephensoni		–	–	–	3	–	–	slight	Mull
Fridericia waldenstroemi		–	–	–	–	1	–	slight	Mull
Fridericia sp. juv.[2]		3	1	–	7	3	17	slight	Mull
Hemifridericia parva		18	24	–	–	–	–	slight	Mull
Henlea perpusilla		7	10	–	4	2	3	slight	Mull
Total of extracted animals		242	393	162	62	83	80		
Number of species		11	12	5	9	9	10		
Abundance (Ind.m^{-2})	mean	41081	66718	27502	10525	14091	13581		
	sd	*21130*	*34508*	*7345*	*4894*	*10442*	*2984*		
Indicators of strong acidity		72%	74%	93%	13%	31%	7%		
Indicators of moderate acidity		7%	1%	7%	35%	12%	29%		
Indicators of slight acidity		21%	25%	0%	52%	57%	64%		

[1] Species not yet formally described.
[2] Juvenile specimens not determinable to species level.

R = 0.727; and *Cognettia sphagnetorum*: $p = 0.026$; R = 0.591). Likewise, positive correlations were found between eDNA yields and the total enchytraeid abundances at the N3 moss plot ($p = 0.024$; R = 0.642); even though in this case enchytraeid abundance was also correlated with iDNA and total dsDNA (data not shown).

4. Discussion

4.1. Changes in soil physico-chemical and microbiological parameters as a function of ground cover and slope exposure

Understanding how the ground cover type affects the composition and activity of soil microbial communities enables a better comprehension of forest ecosystem functioning and sustainability (Uroz et al., 2016). In particular, at the N-facing slope the moss ground cover was

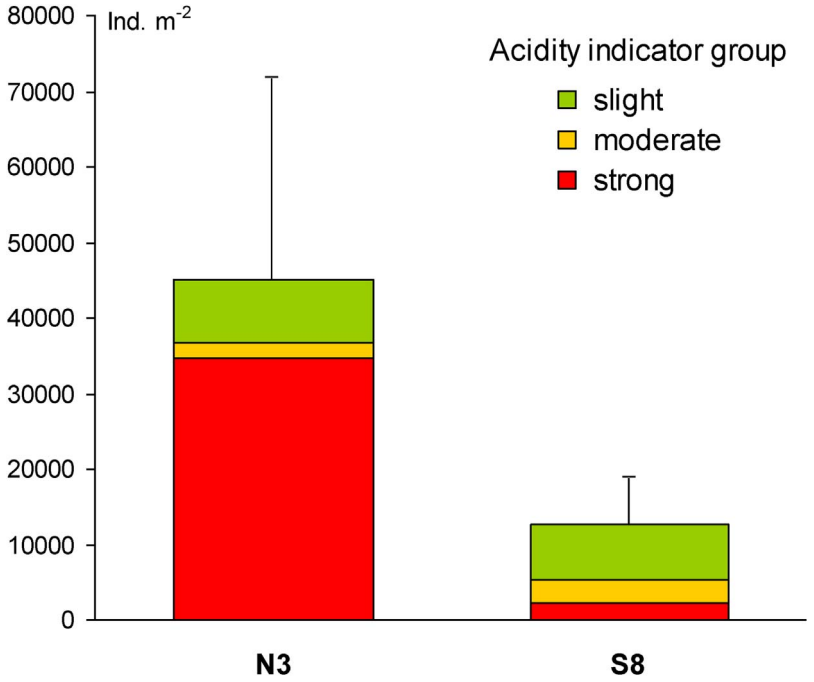

Fig. 1. Total microannelid abundance (mean) at the two study sites N3 and S8. Microannelid species are divided into three acidity indicator groups: indicators of slight, moderate and strong acidity. Error bar: standard deviation.

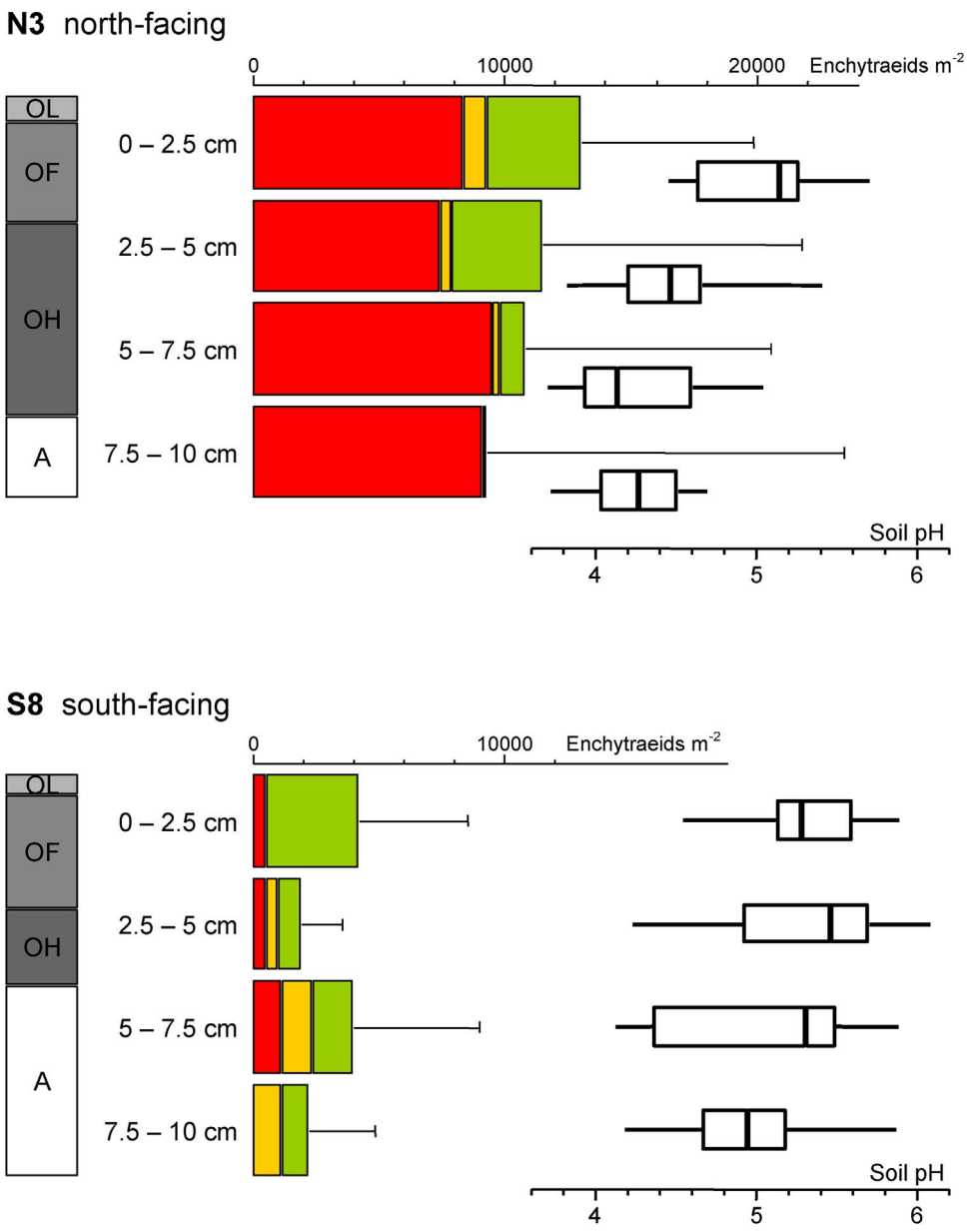

Fig. 2. Comparison between the morphological humus profile (column), the vertical distribution of microannelids (bars) and the pH (box-plots) at study sites N3 and S8. Thickness of organic horizons and abundance of microannelids are arithmetic means (all parameters n = 9). Microannelid species are summarized according to acidity indicator groups (green: slight acidity; yellow: moderate acidity; red: strong acidity). Designation of humus horizons according to Zanella et al. (2011).

characterised by a lower alkaline phosphomonoesterase activity in comparison with both grass and branch ground covers and irrespective of the soil depth. This could be due to the fact that the highest total P content was also recorded in the moss plots. Indeed, an increase in P-acquiring enzyme activities would be expected in case of P deficiency (Fraser et al., 2015). This enzyme activity is responsible for the mineralisation of organic P into phosphate by hydrolysing phosphoric (mono) ester bonds under alkaline conditions. Although plants exude phosphomonoesterases, especially under P deficiency, the majority of phosphatase enzymes in soil probably originate from microorganisms (Nannipieri et al., 2012).

In our study, the three representative ground covers at N3 (branch, grass and moss) significantly differed in terms of microbial abundance only in the first top 2.5 cm, where the highest abundance for the three microbial domains was registered in the moss plots regardless of the DNA fraction. Despite this fact the moss ground cover showed the lowest value of both leucine- and lysine-aminopeptidase activities in the top 5 cm, even though it has been reported that bacteria play an important role in the production of leucine-aminopeptidases (Burke et al., 2011). Sinsabaugh et al. (2008) also found a higher leucine-aminopeptidase activity with increasing soil pH levels (pH 4–8.5). Although the three predominant ground covers were generally acidic at N3, a slightly lower pH was recorded in the moss plots.

Nevertheless, at the S-facing slope most of the enzymes related to C and N cycles showed a higher potential activity in the branch plots within the 10-cm soil depth. Soil moisture and soil organic matter (SOM) content have been shown as major determinants of the level and activity of soil enzymes (Makoi and Ndakidemi, 2008; Šturšová and Baldrian, 2011). Indeed, in our study SOM determined by loss on ignition, along with the total C and the inorganic N content (NH_4^+ and NO_3^-) were also higher under the branch ground cover at S8. Accordingly, soil microbial biomass assessed as *ds*DNA yields followed the same trend as SOM within the 5–10-cm soil depth. On the other hand, the fact that at S8 alkaline phosphomonoesterase and phospho-diesterase activities showed a higher activity in the branch plots, which were characterised by a higher content of available P, might indicate

that the soil microbial community in these plots has a P demand greater than in the other ground covers. Consequently, a higher metabolic effort via secretion of these enzymes is made to access organic P.

Overall, most of the enzymatic activities decreased with increasing soil depth at both slope exposures. This occurred in concert with a decline in microbial biomass as well as in inorganic N, total C and N content. In fact, the availability of substrate for enzymatic breakdown decreases with depth, which increases the probability of a spatial disconnection of enzyme and substrate (Holden and Fierer, 2005). In addition, in deeper layers SOM can be bound to minerals or occluded in aggregates therefore limiting the access for microorganisms (Schnecker et al., 2015).

The eDNA/iDNA ratio was calculated to obtain i) an index of microbial activity as a function of the ground cover, and ii) information about the movement (vertical distribution) of DNA along the topsoil profile (0–15 cm). Lower eDNA/iDNA ratios could be indicative of a higher microbial activity and be related to an increase in iDNA yields (higher potentially living microbial biomass under favorable conditions, i.e., in the upper layers of the topsoil), and/or to a higher eDNA degradation. Contrarily, an increase in this ratio indicative of lower microbial activity might be related to a lower degradation of eDNA and/or to an accumulation of eDNA in deeper soil layers as a result of leaching (Poté et al., 2003; Ceccherini et al., 2007, 2009). In our case, only the grass plots at the north-facing slope showed a significantly higher eDNA/iDNA ratio in the 10–12.5 cm topsoil layer. Due to its vertical distribution along the topsoil there is also the possibility for eDNA to reach a microbial cell far from the donor cell (structural/functional flexibility). This fact together with the possibility of eDNA to persist in soil (Nielsen et al., 2007; Agnelli et al., 2007) has to be considered within the context of genetic exchange via natural transformation with evolutionary implications (Ascher et al., 2009a, 2009b; Pietramellara et al., 2009).

4.2. Changes in enchytraeid community as a function of ground cover and slope exposure

Exposure and, in general, climate seems to have a significant influence on the thickness of the organic layer being thicker at the north-facing slope as shown in Ascher et al. (2012). This is related to a striking difference between the north-facing and south-facing site concerning the partitioning of total C between the organic layers (OL + OF + OH) and the mineral soil (A, E) in the uppermost 10 cm of the humus profile. However, there is only a gradual difference in terms of humus form classification, as at every sample point at both sites an OH horizon was present, which characterises the humus form as Moder according to the European (Zanella et al., 2011) as well as to the German classification system (Ad-hoc-AG Boden, 2005). The south-facing grass and litter plots included an only shallow OH of 0.5 cm thickness. Such a profile may be classified as Mullartiger Moder (German system) or Hemimoder (Zanella et al., 2011). This relates well to the much lower dominance of Moder-preferring species at S8 compared to N3.

Certainly, the presence of Mull-preferring microannelid species in a Moder is a rather unexpected phenomenon. One could argue the preference classes were based on observations from central Europe and cannot be applied to other geographic regions. However, the phenomenon is consistent with the measured pH values in the soil. Species with preference for mull are at the same time indicators of slight acidity (Table 5), occurring mainly in soils above pH(water) 5.0 as known from investigations at soil monitoring sites in Germany (Graefe and Beylich, 2003). The change of dominance of indicator species along the pH-gradient is less a gradual shift than a switch into another community that often occurs between the exchanger and the aluminium buffer range (Graefe and Beylich, 2006). This is most obvious when pH and the occurrence of species are compared at the microscale of single plots.

What are the reasons for the observed discontinuities in the humus profile? First of all, it has to be considered that the studied sites are located at relatively steep slopes (N3: 29°; S8: 33°), where erosion and accumulation processes cannot be excluded. Deadwood branches often accumulate uphill close to tree trunks catching there also other material transported under the influence of gravity and water. Some results (i.e., higher moisture content, microannelid density increasing with depth) can be explained by such slope related accumulation processes. Melt-water run-off during snowmelt may be another key factor. Snow in Alpine regions can accumulate eolian dust containing carbonates and other weatherable minerals. This leads to the release of base cations during the melting period and contributes to the relative high base saturation in organic layers in high mountains (Küfmann, 2003). The interaction of melt-water and soil during surface and subsurface run-off modifies both the chemistry of the percolate and the pH of the forest floor (Seip, 1980; Stottlemyer and Toczydlowski, 1996). Moreover, melt-water and/or eolian dust as potential carrier of eDNA (Wackernagel, 2006; Pietramellara et al., 2009) may have impact on the discontinuities in the humus profile and the correlated discontinuity in the mesofauna, as the observed site-specific correlations between microbial DNA and microannelids indicate. These site-specific correlations indicate that eDNA (N3 grass plots) and microbes (DNA in general, independent of its presence as extracellular or intracellular DNA; N3 moss plots) could act as a potential nutrient source for the mesofauna. Indeed, DNA can be present in the gut of the mesofauna as a part of the undigested soil and therefore be indirectly used to satisfy their nutrient demands.

It turns out that the thermal effect due to exposure determines large-scale differences in morphological, chemical and biological properties of the topsoil (Ascher et al., 2012). The small scale heterogeneity of the humus profile properties seems to be, however, more influenced by slope related processes such as microerosion, accumulation and melt-water run-off than by the type of ground cover (grass, moss, branches, litter).

5. Conclusions

The enzyme type-specific reactions to ground cover and slope exposure indicate the importance of performing multiple enzyme assays in order to avoid misinterpretation of nutrient cycles. Moreover, testing the soils in 2.5-cm increments to a depth of up to 15 cm revealed depth gradients on the soil chemical properties (e.g. the NH_4^+ content) but also the enzymes and micro- (e.g. eDNA) and macrobiological properties (enchytraeid community). Microannelids appeared to be sensitive, accurate and reliable biological indicators in the forested subalpine soils. Indicators of slight acidity clearly dominate on south-facing soils. It furthermore seems that eDNA might act as a nutrient source for the mesofauna. All in all, our findings encourage the use of the discriminatory assessment of both DNA fractions (eDNA *vs.* iDNA) as a sort of 'low cost alternative' for a generic screening of microbial communities and their response to changing environmental conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2017.05.032.

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