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## Chemical and microbiological changes in Norway spruce deadwood during the early stage of decomposition as a function of exposure in an alpine setting

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### ABSTRACT

Alpine ecosystems are vulnerable to ever-changing environmental conditions, leading to shifts in vegetation distribution and composition with implications on soil functionality and carbon (C) turnover. Although deadwood represents an important global C stock, scarce information is available on how slope exposure influences the wood-inhabiting microbiota throughout the decomposition process in an Alpine setting. We therefore evaluated the impact of slope exposure (north- vs. south-facing sites) on physicochemical and microbiological properties (microbial abundance based on real-time PCR: fungal 18S rRNA, dinitrogen reductase [*nifH*]; microbial biomass: double strand DNA; and microbial activity: hydrolytic enzyme activities of the main nutrient cycles) of *Picea abies* wood blocks and the underlying soil in a field experiment in the Italian Alps during a three-year period. Overall, a higher abundance of fungi and nitrogen-fixing bacteria was recorded in the soil at the north-facing site where cooler and moister conditions were observed. In contrast, no exposure effects were found for these two microbial groups in the wood blocks, while their abundance increased over time, accompanied by more acidic conditions with progressing decay. The impact of exposure was also enzyme specific and time dependent for both the *P. abies* wood blocks and the underlying soil.

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### Introduction

Mountain Alpine ecosystems have gained increasing attention during the past several years because they are particularly sensitive to ever-changing climatic conditions (Egli et al. 2006, 2009). Indeed, an increase of about 2°C has been recorded in the annual minimum temperature in the European Alps during the twentieth century (Beniston, Diaz, and Bradley 1997). As a consequence, previous studies have reported the occurrence of an upward migration of tree and shrub species in alpine environments owing to rising temperatures (Dullinger, Dirnböck, and Grabherr 2003; Motta and Nola 2001). Furthermore, changes in land use and management also constitute a dominant factor affecting soil biodiversity in alpine ecosystems (Meyer et al. 2013). Altogether, this can ultimately entail changes in the quality and quantity of soil organic matter (SOM), as well as in the vegetation composition and/or activities of soil organisms (Djukic et al. 2010; Myers et al. 2001; Siles

et al. 2017), with implications for both carbon (C) dynamics and soil functionality (A'Bear et al. 2014; Allison et al. 2010; Theurillat and Guisan 2001).

Among the organic C reservoirs, deadwood represents a global C store estimated to be in the range of  $73 \pm 6$  Pg (Pan et al. 2011). This is mainly because of its high lignin content resulting in a slow C turnover rate, making its decomposition dynamics determinant for the soil C balance (Floudas et al. 2012; Moroni et al. 2015). As woody material decays, its physicochemical properties gradually change over time, which can lead to a succession of microbial communities in wood according to their biochemical requirements and nutrient availability (Rajala et al. 2012). Fungi (brown rot, soft rot, and white rot) are considered the main wood decomposers, having different wood-degradation strategies. In particular, white-rot fungi are capable of degrading all components of the wood cell wall (including lignin) by secreting a plethora of extracellular lignocellulolytic enzymes (Kielak et al. 2016; van der Wal et al. 2007).

Evidence also exists of complex fungal-bacterial interactions, both positive and negative, within the deadwood environment as reviewed by Johnston, Boddy, and Weightman (2016), even though the identity and ecology of bacterial communities in decomposing wood remained underexplored compared to fungi. For instance, one assumes that fungi might be able to meet the nitrogen requirements for their vegetative and generative growth through the associations with nitrogen-fixing bacteria. Accordingly, Hoppe et al. (2014) found positive correlations between fungal sporocarps and the richness of *nifH* (dinitrogen reductase) genes in deadwood logs from *Fagus sylvatica* and *Picea abies*. This is also in line with recent findings from Gómez-Brandón et al. (2017), who observed that fungal abundance (qPCR-based) was strongly correlated with *nifH* abundance in the coarse woody debris of *P. abies* at different stages of natural decay.

Several factors were found to act as drivers for the changes in wood-inhabiting microbiota, such as the soil type (Sun et al. 2013); wood physicochemical properties, particularly density; pH; moisture content; and total lignin and cellulose (Hoppe et al. 2016; Purahong et al. 2014). The wood decay stage, with an increase in fungal and bacterial abundance as wood decomposes (Gómez-Brandón et al. 2017), together with the host tree species (Hoppe et al. 2016) are also of importance. In addition, topographic features, particularly the slope exposure, may influence the deadwood decay dynamics in subalpine environments (Fravolini et al. 2016; Petrillo et al. 2015). However, there is still a paucity of information about how slope exposure and climate, in general, affect deadwood-inhabiting microbiota in alpine environments (Gómez-Brandón et al. 2017).

Therefore, we performed a field mesocosm experiment with equally sized wood blocks of *P. abies* to evaluate the effect of exposure (north- vs. south-facing slope) on the abundance of nitrogen-fixing bacteria (*nifH* gene) and fungi assessed by real-time PCR. In addition, several potential enzymatic activities that are involved in the main nutrient cycles were determined during the early stage of decomposition (0–156 weeks) of deadwood in an alpine setting. Furthermore, we assessed which physicochemical parameters were the most important drivers shaping the microbial communities in wood and in the underlying soil.

We hypothesised that: (1) the microbial biomass and activity will be more favored at the south- than at the north-facing slope during *P. abies* wood decomposition in a high alpine setting and that such exposure effects will be time dependent, and (2) the changes in fungal and *nifH* gene abundances as a function of exposure will be more

evident at the end of the monitoring owing to increased nutrient availability as woody decay progresses.

## Material and methods

### Experimental setup

Two study sites were selected at an altitude of 2,400 m above sea level (a.s.l.) in Val di Rabbi (Trentino, Italy) on a north- ( $N_5$ ) and south-facing ( $S_{10}$ ) slope, respectively. We selected natural grasslands close to the border of the potential tree line in order to minimize the influence of human activities and livestock grazing, and to assess how such sites might be affected by the input of wood as trees advance in elevation. Both alpine sites belong to an already well-known climosequence (Bardelli et al. 2017; Egli et al. 2006; Fravolini et al. 2016), and they were located in catchments with acidic paragneiss (Bardelli et al. 2017). The soils were classified as Podzol (north-facing site) and Cambisols (south-facing site; Egli et al. 2006), and are sandy to silty ( $N_5$ : sand 53%, silt 28%, clay 19%;  $S_{10}$ : sand 51%, silt 27%, clay 21%) according to Bardelli et al. (2017). Mean annual precipitation is approximately 1,300 mm, mean annual air temperature is approximately  $-1.0^\circ\text{C}$  (Sboarina and Cescatti 2004), and mean annual soil temperature ranges from  $2.2^\circ\text{C}$  to  $4.5^\circ\text{C}$  (Egli et al. 2016) at north and south exposure, respectively.

A field experiment using open mesocosms was set up at both study sites with the purpose of monitoring the early stage of *P. abies* (L.) Karst deadwood decomposition as a function of slope exposure and time in wood blocks and the topsoil layer (0–5 cm) that is in intimate contact with the wood blocks. Mesocosms (PVC tubes, 10.2 cm and 25 cm diameter and height, respectively) were installed into the natural soil in summer 2012, prior to the addition of the wood blocks of *P. abies* at a distance of greater than 0.5 m from the adjacent mesocosms, leaving at the surface a border of about 1 cm. From the mesocosm set-up (August 2012) to the placement of the wood blocks (June 2013), one year passed in order to permit normal conditions to be restored and, as such, the decay monitoring study would be performed under undisturbed conditions. Considering that the size and geometry of deadwood may have a strong influence on the decay processes (van der Wal et al. 2007), equal-sized (5 cm  $\times$  5 cm  $\times$  2 cm) wood blocks of *P. abies* were directly placed on the soil surface in each of the mesocosm tubes. Three replicate mesocosms for each time point were installed in each of the study sites. The wood blocks and the topsoil layer (0–5 cm) were collected

(using lab gloves) in July 2014 (t1; 52 weeks), in July 2015 (t2; 104 weeks), and in July 2016 (t3; 156 weeks), resulting in a total of eighteen samples for each substrate (two sites  $\times$  three times  $\times$  three replicates), with three wood blocks kept as controls (t0). Prior to the placement of the wood blocks into the mesocosms, five soil subsamples (t0) were collected in the surrounding area of the mesocosm. All samples were kept in cooling boxes and transported to the laboratory. The soil samples were sieved (<2 mm), the wood blocks were air dried at room temperature, cut milled (4 mm; Retsch mill), and aliquoted into 50-ml sterile conical centrifuge tubes. The samples were then stored at 4°C and -20°C for physicochemical and (micro)biological analyses, respectively.

### Wood and soil physicochemical analyses

The fresh and dry weight of the wood blocks were determined to assess the wood mass as a function of progressing decay as described in Fravolini et al. (2016) and Petrillo et al. (2016). Wood (1 g fresh weight [fw]) and soil (5 g, fw) samples were oven dried (105°C) for 24 h to determine their dry weight. The volatile solid (VS) concentration was determined from the weight loss following ignition in a muffle furnace (Carbolite, CWF 1000) at 550°C for 5 h. Total C and nitrogen contents of the oven-dried samples were analyzed using a CN analyzer (TruSpec CHN; LECO, Michigan, USA). Electrical conductivity (EC) and pH were determined in water extracts (1:10 and 1:20, w/v for soil and wood, respectively) using a conductivity Meter LF 330 (WTW, Weilheim, Germany) and a pH meter Metrohm 744, respectively. Inorganic nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ) was measured in 0.0125 M  $\text{CaCl}_2$  soil extracts (Kandeler 1993a, 1993b). Both the total and available P contents in soil samples were determined according to the ascorbic acid method (Kuo 1996).

### Potential enzyme activities

A heteromolecular exchange procedure, using a 4 percent solution of lysozyme as desorbant and a bead-beating agent (Fornasier and Margon 2007), was performed to assess the following hydrolases in wood (0.1 g, fw) and soil (0.2 g, fw) samples—(1) *C-cycle*:  $\beta$ -glucosidase (*gluc*), xylosidase (*xylo*), and cellulase (*cell*); (2) *N-cycle*: chitinase (*chit*) and leucine-aminopeptidase (*leu*); and (3) *P-cycle*: alkaline and acid phosphomonoesterases (*alkP* and *acP*). In order to perform the enzymatic multiple assay for wood extracts, some modifications were required as described by Gómez-Brandón et al. (2017). All measurements were performed in duplicate for each field replicate, and the activities were expressed as nanomoles of 4-methyl-umbelliferyl (MUF)  $\text{min}^{-1} \text{g}^{-1}$  dry (wood/soil).

## Molecular analyses

### Wood and soil microbial biomass index (dsDNA)

Direct extraction of total wood (0.1 g, fw) and soil (0.2 g, fw) DNA followed by PicoGreen-based quantification of crude (not purified) double-stranded DNA (dsDNA) was performed to estimate wood and soil microbial biomass (Fornasier et al. 2014). Some modifications were required to determine the dsDNA content in wood as described in Gómez-Brandón et al. (2017).

### DNA extraction

The whole-community DNA was extracted from wood (0.1 g, fw) and soil samples (0.2 g, fw) and was purified using a commercial kit (FastDNA Kit for Soil, MP-Biomedicals) as described in Ascher et al. (2009) for further downstream analyses. For wood samples, one ceramic sphere (Lysing Matrix E, MP, Biomedicals) was added to the lysing tubes so as to guarantee accurate cell disruption of the woody tissue. DNA extracts were qualitatively and quantitatively characterized as described in Bardelli et al. (2017).

### Quantitative real-time PCR

Quantitative real-time PCR (qPCR) was performed to determine the 18S rRNA gene copy number of fungi, the abundance of the functional gene *nifH* in the wood and the soil samples. qPCR was carried out using a Rotorgene 6000 Real Time Thermal Cycler (Corbett Research, Sydney, Australia) in combination with the Rotorgene Series Software 1.7. To build the standard curves we used as templates the purified PCR products of known concentrations of the following pure cultures: *Fusarium solani* (DSMZ 10696), fungi, and *Azospirillum irakense* (DSMZ 11586), *nifH* gene. The primer pairs were FF390/FR1 (fungi; Prévost-Bouré et al. 2011) and *nifHf/nifHr* (*nifH* gene; Töwe et al. 2010). Stock concentration (gene copies  $\mu\text{L}^{-1}$ ) was determined via PicoGreen measurement and was freshly prepared for each run. Tenfold dilutions ranging from  $10^9$  to  $10^2$  copies  $\mu\text{L}^{-1}$  were used for the standard curve construction. Quantitative PCR was performed in 20- $\mu\text{L}$  volumes. Each reaction mix contained 1X Sensimix™ SYBR® Hi-rox (Bioline, USA), forward and reverse primers (200 nM each primer), 0.4 mg  $\text{mL}^{-1}$  BSA, distilled water (RNase/DNase free, Gibco™, UK), and 2  $\mu\text{L}$  of either 1:10 diluted DNA extract or tenfold diluted standard DNA. All the standards and samples were run in duplicate. After an initial denaturation at 95°C for 10 min, thermal cycling comprised forty cycles of 15 s at 95°C, 30 s at 50°C, 30 s at 72°C for fungi, and 45 s at 95°C, 45 s at 55°C, 45 s at 72°C for *nifH* gene. To check for product specificity

and potential primer dimer formation, runs were completed with a melting analysis starting from 65°C to 95°C with temperature increments of 0.25°C (0.5°C for *nifH* gene) and a transition rate of 5 s. The purity of the amplicons was checked by the presence of a single band of the expected length on a 1 percent agarose gel stained with the DNA stain Midori Green (Nippon Genetics, Germany) and visualized by UV transillumination (Vilber Lourmat Deutschland GmbH).

### Statistical analyses

A factorial analysis of variance (ANOVA) was done to evaluate the effect of exposure (north vs. south exposure) and time (t0, t1, t2, t3) on wood and soil physicochemical and microbiological parameters. Normality and variance homogeneity of the data were tested prior to ANOVA using the Shapiro-Wilks and Levene tests, respectively. Prior to analysis, data were log- or square root-transformed to meet the assumptions for ANOVA (when it was necessary). Significant differences ( $p \leq 0.05$ ) were analyzed by paired comparisons with the Tukey HSD test. A nonparametric test (Kruskal-Wallis test) was performed when the data did not meet the normality condition. Associations between the potential enzymatic activities and the main chemical and microbiological variables were explored by Pearson's correlation. These

analyses were performed using Statistica 9 (StatSoft, USA). Nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distance was performed using R 3.1.2 (open source software) to map the wood and soil physicochemical parameters to the shifts in microbial biomass (dsDNA), microbial abundance (qPCR), and hydrolytic enzyme activities as a function of slope exposure and time. The lengths of the arrows indicate the direction of maximum correlation of the physicochemical parameters, and the significance level was assessed with a permutation test implemented in the envfit function of vegan library (Oksanen et al. 2008).

### Results

An overview of the wood physicochemical and (micro) biological parameters as a function of exposure (north- vs. south-facing slope) and time (0–156 weeks) is given in Tables 1 and 3 and those for the soil in Tables 2 and 3. The statistical results are shown in Table 4.

#### Wood physicochemical parameters

Exposure had a significant impact on the wood moisture content, with higher values at the north- than at the south-facing slope (Tables 1 and 4). Moreover, a sevenfold increase in moisture levels

**Table 1.** Physicochemical properties of the wood samples collected in August 2013 (t0), July 2014 (t1; 52 weeks), July 2015 (t2; 104 weeks), and July 2016 (t3; 156 weeks) in the *in-field* mesocosm experiment at north- and south-facing sites (N<sub>5</sub> and S<sub>10</sub>, respectively) located at the same elevation (2,400 m a.s.l.). Values are means ( $n = 3$ ) with standard deviations in brackets. Data are expressed on a dry weight (dw) basis.

Sites	Time	Moisture (%)	Volatile Solids (%)	Wood Mass (g)	pH	Electrical Conductivity ( $\mu\text{S cm}^{-1}$ )	Total C (%)
N <sub>5</sub>	t0	8.2 (2.0)	99.2 (0.8)	22.5 (0.0)	5.5 (0.1)	51.7 (6.5)	49.0 (0.3)
	t1	54.2 (2.8)	99.0 (0.5)	21.1 (1.1)	5.4 (0.1)	9.8 (1.4)	50.7 (1.3)
	t2	29.4 (7.9)	99.8 (0.2)	24.3 (2.0)	5.3 (0.1)	32.4 (8.7)	50.5 (1.0)
	t3	39.1 (17.1)	99.6 (0.2)	16.8 (6.2)	5.1 (0.1)	28.5 (6.9)	51.2 (0.9)
S <sub>10</sub>	t1	56.1 (4.8)	99.0 (0.2)	23.1 (0.9)	5.2 (0.1)	19.1 (10.1)	55.0 (8.0)
	t2	21.1 (7.9)	99.9 (0.2)	19.2 (0.8)	5.1 (0.1)	29.4 (10.2)	51.3 (0.9)
	t3	17.6 (4.5)	99.5 (0.1)	20.4 (3.1)	4.9 (0.1)	77.3 (21.6)	50.4 (0.5)

**Table 2.** Physicochemical properties of the soil samples collected in August 2013 (t0), July 2014 (t1; 52 weeks), July 2015 (t2; 104 weeks), and July 2016 (t3; 156 weeks) in the *in-field* mesocosm experiment at north- and south-facing sites (N<sub>5</sub> and S<sub>10</sub>, respectively) located at the same elevation (2,400 m a.s.l.). Values are means ( $n = 3$ ) with the standard deviations in brackets. Data are expressed on a dry weight (dw) basis.

Sites	Time	Moisture (%)	Volatile Solids (%)	pH	Electrical Conductivity ( $\mu\text{S cm}^{-1}$ )	Total C (%)	Total N (%)	NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> dw)	NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> dw)	Total P (mg kg <sup>-1</sup> dw)	Available P (mg kg <sup>-1</sup> dw)
N <sub>5</sub>	t0	54.9 (0.4)	41.1 (1.0)	5.5 (0.1)	15.8 (0.8)	23.5 (1.4)	1.4 (0.1)	4.3 (0.6)	10.2 (3.1)	802.9 (346.3)	4.0 (0.8)
	t1	52.5 (4.0)	40.6 (8.1)	5.0 (0.2)	19.2 (4.1)	20.7 (3.7)	1.1 (0.2)	54.6 (8.0)	15.6 (2.3)	821.6 (552.1)	9.3 (3.1)
	t2	40.1 (6.7)	43.0 (5.1)	5.0 (0.1)	38.9 (4.2)	23.5 (2.0)	1.3 (0.1)	52.0 (15.6)	6.1 (1.9)	891.6 (111.9)	33.0 (7.0)
	t3	51.5 (4.3)	41.8 (7.5)	5.2 (0.01)	39.3 (14.1)	21.9 (5.2)	1.2 (0.3)	117.7 (55.1)	5.6 (2.2)	870.6 (319.6)	25.3 (14.1)
S <sub>10</sub>	t0	37.5 (4.4)	22.7 (12.0)	5.3 (0.01)	29.4 (0.3)	17.0 (1.4)	1.0 (0.01)	3.3 (0.8)	14.9 (8.9)	931.9 (128.2)	21.3 (8.4)
	t1	49.1 (3.2)	33.0 (4.1)	5.5 (0.1)	26.9 (3.1)	16.3 (2.6)	1.1 (0.2)	97.2 (10.5)	10.4 (1.6)	611.2 (482.9)	46.1 (8.6)
	t2	30.2 (10.2)	26.2 (1.0)	5.3 (0.1)	37.7 (4.5)	13.5 (2.0)	0.9 (0.1)	62.4 (25.7)	6.0 (2.8)	722.9 (312.6)	61.6 (3.1)
	t3	35.4 (1.9)	26.1 (1.7)	5.3 (0.1)	60.5 (10.5)	12.0 (1.5)	0.8 (0.2)	84.3 (16.0)	9.1 (1.3)	450.8 (264.0)	30.0 (3.5)

**Table 3.** Microbiological properties of the wood and soil samples collected in August 2013 (t0), July 2014 (t1; 52 weeks), July 2015 (t2; 104 weeks), and July 2016 (t3; 156 weeks) in the *in-field* mesocosm experiment at north- and south-facing sites (N<sub>5</sub> and S<sub>10</sub>, respectively) located at the same elevation (2,400 m a.s.l.). Values are means ( $n = 3$ ) with the standard deviations in brackets. Data are expressed on a dry weight (dw) basis.

	Sites	Time	Microbial Biomass Index ( $\mu\text{g dsDNA g}^{-1}$ )	Fungi (gene copy number $\text{g}^{-1}$ )	<i>nifH</i> Gene (gene copy number $\text{g}^{-1}$ )
<b>Wood</b>	N <sub>5</sub>	t0	1.4 (0.2)	$3.12 \times 10^9$ ( $1.67 \times 10^9$ )	$8.77 \times 10^6$ ( $1.37 \times 10^6$ )
		t1	9.0 (0.9)	$1.66 \times 10^9$ ( $3.87 \times 10^8$ )	$1.46 \times 10^7$ ( $3.57 \times 10^6$ )
		t2	10.5 (4.3)	$3.20 \times 10^9$ ( $1.20 \times 10^9$ )	$3.75 \times 10^7$ ( $2.44 \times 10^7$ )
	S <sub>10</sub>	t3	11.6 (4.5)	$3.83 \times 10^8$ ( $1.88 \times 10^8$ )	$2.32 \times 10^7$ ( $9.73 \times 10^6$ )
		t1	12.6 (4.3)	$9.24 \times 10^8$ ( $3.30 \times 10^8$ )	$1.51 \times 10^7$ ( $1.90 \times 10^6$ )
		t2	30.0 (9.9)	$2.98 \times 10^9$ ( $4.24 \times 10^8$ )	$2.61 \times 10^7$ ( $6.58 \times 10^6$ )
<b>Soil</b>	N <sub>5</sub>	t3	26.5 (17.2)	$2.26 \times 10^9$ ( $1.29 \times 10^9$ )	$4.06 \times 10^7$ ( $3.15 \times 10^7$ )
		t0	120.7 (11.3)	$1.96 \times 10^9$ ( $2.91 \times 10^8$ )	$1.47 \times 10^8$ ( $1.91 \times 10^7$ )
		t1	124.2 (5.4)	$1.06 \times 10^9$ ( $2.40 \times 10^8$ )	$1.74 \times 10^8$ ( $7.97 \times 10^7$ )
	S <sub>10</sub>	t2	128.4 (4.2)	$1.88 \times 10^9$ ( $5.39 \times 10^8$ )	$1.10 \times 10^8$ ( $3.13 \times 10^7$ )
		t3	140.1 (19.5)	$1.45 \times 10^9$ ( $2.06 \times 10^8$ )	$1.74 \times 10^8$ ( $3.91 \times 10^7$ )
		t0	127.3 (12.4)	$4.38 \times 10^8$ ( $2.72 \times 10^8$ )	$3.57 \times 10^7$ ( $5.29 \times 10^6$ )
		t1	128.1 (15.8)	$5.83 \times 10^8$ ( $2.83 \times 10^8$ )	$3.90 \times 10^7$ ( $7.68 \times 10^6$ )
		t2	102.1 (3.7)	$5.99 \times 10^8$ ( $2.56 \times 10^8$ )	$5.69 \times 10^7$ ( $1.75 \times 10^7$ )
		t3	123.7 (18.1)	$5.47 \times 10^8$ ( $7.39 \times 10^8$ )	$5.04 \times 10^7$ ( $2.21 \times 10^7$ )

**Table 4.** Factorial ANOVA of the soil and wood physicochemical and microbiological parameters as a function of slope exposure and time at the north- and south-facing sites (N<sub>5</sub> and S<sub>10</sub>, respectively) located at the same elevation (2,400 m a.s.l.).

	Exposure		Time		Exposure $\times$ Time	
	F	p	F	p	F	p
<b>Soil</b>						
Moisture	<b>30.34</b>	***	<b>9.60</b>	***	2.31	ns
Volatile solids	<b>32.67</b>	***	0.63	ns	0.88	ns
pH	<b>12.43</b>	**	<b>6.51</b>	**	<b>9.47</b>	***
EC	<b>20.38</b>	***	<b>23.59</b>	***	<b>3.27</b>	*
Total C	<b>46.17</b>	***	1.40	ns	1.44	ns
Total N	<b>17.71</b>	***	1.87	ns	1.99	ns
NH <sub>4</sub> <sup>+</sup>	0.05	ns	<b>41.68</b>	***	3.12	ns
NO <sub>3</sub> <sup>-</sup>	0.32	ns	<b>6.26</b>	**	1.77	ns
Total P	1.41	ns	0.43	ns	0.64	ns
Available P	<b>54.24</b>	***	<b>22.83</b>	***	<b>5.53</b>	**
dsDNA	2.38	ns	1.76	ns	2.34	ns
Fungi	<b>42.21</b>	***	1.43	ns	1.99	ns
<i>nifH</i>	<b>86.20</b>	***	0.54	ns	1.95	ns
<i>gluc</i>	<b>13.77</b>	**	1.32	ns	<b>8.28</b>	**
<i>xylo</i>	<b>15.34</b>	**	0.11	ns	<b>5.99</b>	**
<i>cell</i>	3.45	ns	1.89	ns	<b>7.18</b>	**
<i>chit</i>	<b>13.64</b>	**	0.80	ns	<b>5.72</b>	**
<i>leu</i>	<b>4.70</b>	*	<b>12.24</b>	***	<b>17.08</b>	***
<i>acP</i>	<b>85.00</b>	***	1.70	ns	<b>4.71</b>	*
<i>alkP</i>	0.04	ns	<b>13.50</b>	***	<b>21.05</b>	***
<b>Wood</b>						
Moisture	<b>4.93</b>	*	<b>44.27</b>	***	2.31	ns
Volatile solids	0.04	ns	<b>12.16</b>	**	na	na
Mass	0.01	ns	2.75	ns	na	na
pH	<b>14.20</b>	**	<b>36.57</b>	***	1.87	ns
EC	<b>9.04</b>	**	<b>26.39</b>	***	<b>4.54</b>	*
Total C	0.01	ns	<b>9.60</b>	*	na	na
dsDNA	<b>12.13</b>	**	<b>21.49</b>	***	2.64	ns
Fungi	0.27	ns	<b>5.64</b>	**	1.71	ns
<i>nifH</i>	0.13	ns	<b>11.15</b>	***	0.65	ns
<i>gluc</i>	2.30	ns	<b>18.15</b>	***	1.54	ns
<i>xylo</i>	1.09	ns	<b>44.17</b>	***	1.47	ns
<i>cell</i>	1.08	ns	<b>19.92</b>	***	0.42	ns
<i>chit</i>	<b>12.96</b>	**	<b>45.92</b>	***	<b>9.60</b>	***
<i>leu</i>	<b>16.67</b>	***	<b>43.52</b>	***	<b>8.56</b>	**
<i>acP</i>	0.03	ns	<b>19.75</b>	***	0.82	ns
<i>alkP</i>	<b>9.07</b>	**	<b>35.74</b>	***	<b>5.99</b>	**

pH (pH H<sub>2</sub>O), EC (electrical conductivity), NH<sub>4</sub><sup>+</sup> (ammonium content), NO<sub>3</sub><sup>-</sup> (nitrate content), dsDNA (double-stranded DNA; soil and wood microbial biomass index), fungi (18S rRNA gene copy number), *nifH* (bacterial *nifH* gene copy number), *gluc* ( $\beta$  glucosidase), *xylo* (xylosidase), *cell* (cellulase), *chit* (chitinase), *leu* (leucine-aminopeptidase), *acP* (acid phosphomonoesterase), *alkP* (alkaline phosphomonoesterase).

na (not available); ns (not significant); \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

was recorded after fifty-two weeks compared to the beginning of the experiment at both slopes, followed by a decrease until the end of the monitoring period

(156 weeks). The wood VS concentration was not significantly influenced by the slope exposure, and neither exposure nor time significantly affected the

wood mass (Tables 1 and 4). In addition, the lowest pH value was measured at the end of the monitoring period (156 weeks) at both the north- and the south-facing slopes (Tables 1 and 4). The exposure effect on the EC of the wood was only evident after 156 weeks, with higher values (three times higher) at the south- than at the north-facing slope. A significant decrease in EC was found after fifty-two weeks (three to five times lower than at t<sub>0</sub>), followed by an increase until the end of the monitoring at both slopes (Tables 1 and 4). Exposure did not have a significant impact on the wood total C content (Tables 1 and 4). The nitrogen content in the wood blocks was below the detection limit irrespective of the exposure and time.

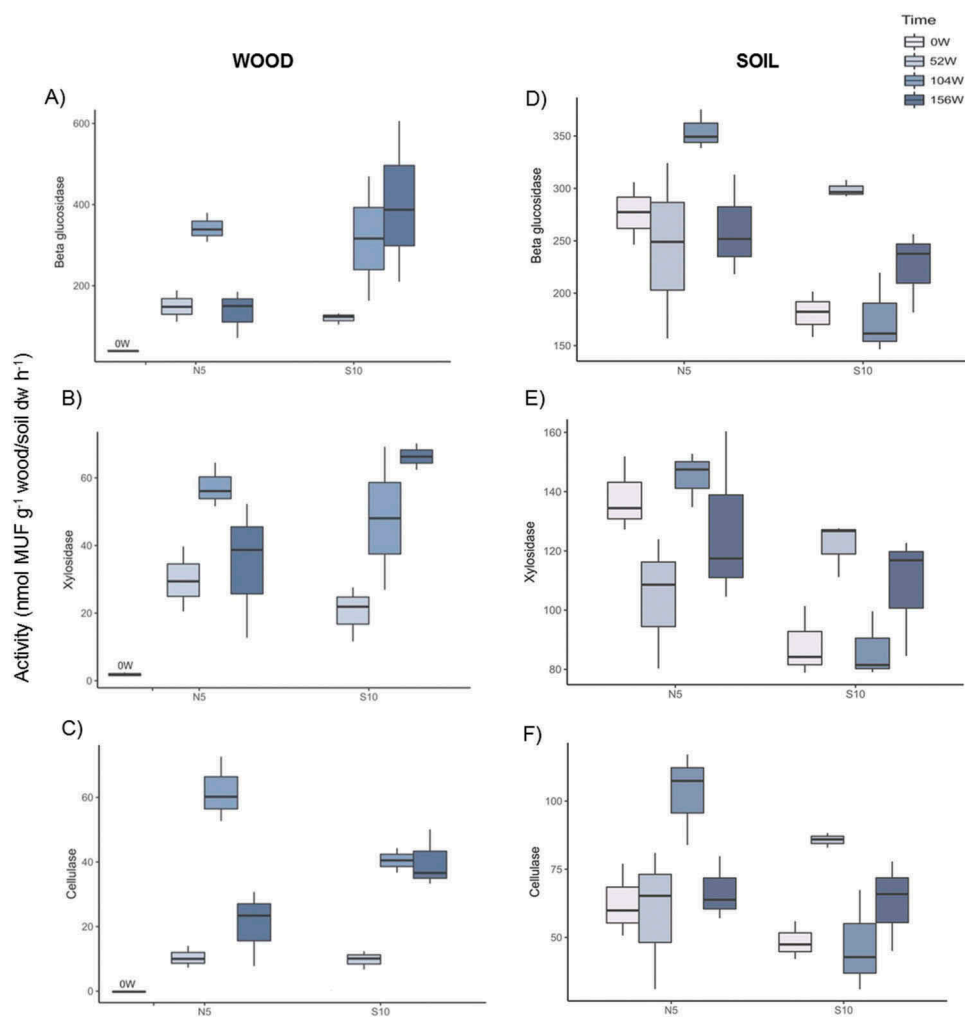
### Soil physicochemical parameters

Soil moisture levels were significantly higher at the north- than at the south-facing slope, and the lowest values were found after 104 weeks at both slopes (Tables 2 and 4). The VS concentration was almost twice as high at the north- than at the south-facing site, whereas no significant changes were found over time (Tables 2 and 4). Slope exposure had a significant impact on the soil pH after fifty-two weeks, with an average value of 5.0 at the north- and 5.5 at the south-facing site (Tables 2 and 4). A significant decrease in soil pH was recorded after fifty-two weeks at the north-facing slope, but it remained stable afterward until the end of the monitoring period. No significant differences in soil pH with time were observed at the south-facing slope (Tables 2 and 4). The soil EC at the south-facing site was twice as high as at the north-facing site at both the beginning and the end of the monitoring period (156 weeks), while no exposure effects were found at the intermediate sampling times. Additionally, the highest EC value was recorded after 156 weeks at both slopes (Tables 2 and 4). In general, the north-facing slope showed higher total C and nitrogen contents compared to the south-facing slope, regardless of the duration of the experiment (Tables 2 and 4). There was no significant exposure effect on the NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> contents. Both parameters, however, significantly varied over time. Indeed, a very distinct increase in NH<sub>4</sub><sup>+</sup> was measured after fifty-two weeks at both slopes, followed by a continuous increase (two times) at the end of the monitoring period at the north-facing slope (Tables 2 and 4). Concerning the NO<sub>3</sub><sup>-</sup> content,

the lowest values were generally detected after 104 and 156 weeks at both north- and south-facing slopes (Tables 2 and 4). The total P content was influenced by neither slope exposure nor time (Tables 2 and 4). Overall, higher available P levels were observed at the south- than at the north-facing slope irrespective of time, except for the last sampling point (no exposure effect). Moreover, the highest available P levels were recorded after 104 weeks at both slopes, followed by a significant decrease after 156 weeks at the south-facing slope (Tables 2 and 4).

### Wood (micro)biological parameters

Wood microbial biomass assessed as double-stranded DNA yield (dsDNA) was as much as three times higher at the south- than at the north-facing slope irrespective of time (Tables 3 and 4). Moreover, a significant increase in the dsDNA content was found over time at both slopes (Table 3). Fungal and *nifH* gene abundances in wood were not significantly affected by the slope exposure; however, significant differences were recorded in both microbial groups over time (Tables 3 and 4). The highest *nifH* abundance was recorded after 104 and 156 weeks at the north- and the south-facing slopes, respectively. A reduction in the fungal abundance was registered after fifty-two weeks at both slopes, followed by an increase after 104 weeks, reaching similar values to those observed in the initial wood blocks (Table 3).  $\beta$ -glucosidase, xylosidase, and cellulase activities did not vary significantly in terms of exposure (Table 4); however, a sharp increase (approximately sixteen times higher for  $\beta$ -glucosidase and sixtyfold higher for xylosidase and cellulase activities) was observed after 104 weeks compared to the beginning of the experiment at both slopes (Figure 1A–C). Afterward, a decrease in these three enzyme activities was recorded after 156 weeks at the north-facing slope. The exposure effect on the chitinase, leucine-aminopeptidase, and alkaline phosphomonoesterase activities was time dependent (Figure 2A–C, Table 4). Higher activities (between four and six times higher) were recorded at the south- than at the north-facing slope after 104 and 156 weeks, while no exposure effect was found after fifty-two weeks (Figure 2A–C). Moreover, the highest activity of the three aforementioned enzymes was registered at the end of the monitoring



**Figure 1.** Potential activities of  $\beta$ -glucosidase (A, D), xylosidase (B, E), and cellulase (C, F) of the wood and soil samples collected in August 2013 (0 weeks), July 2014 (52 weeks), July 2015 (104 weeks), and July 2016 (156 weeks) at the north- and south-facing sites ( $N_5$  and  $S_{10}$ , respectively). Values are means ( $n = 3$ ) with standard deviation.

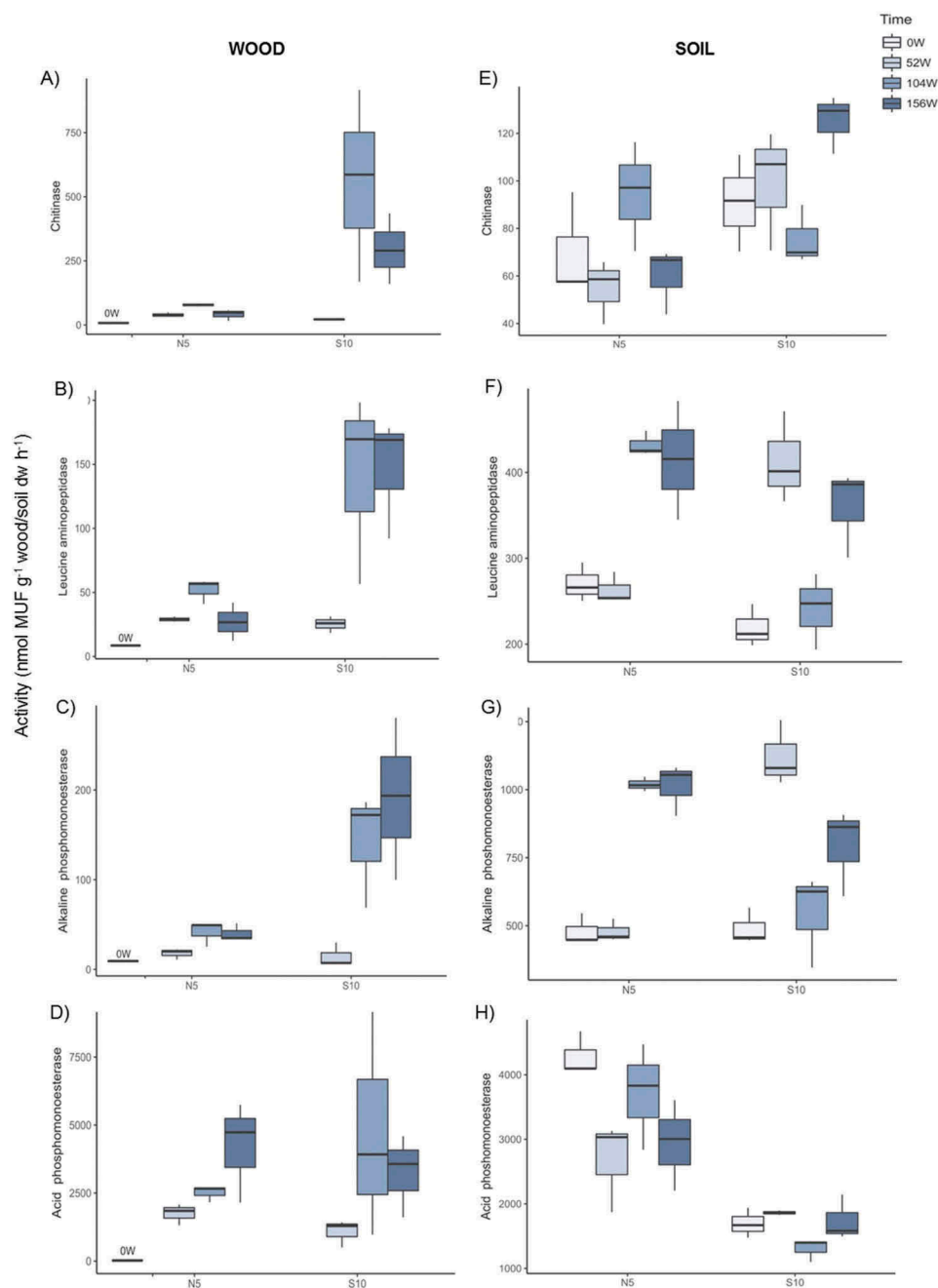
at both slopes. Although the acid phosphomonoesterase activity was not significantly influenced by slope exposure (Table 4), a pronounced increase was found over time compared to the beginning of the monitoring period (Figure 2D).

### Soil (micro)biological properties

Soil microbial biomass (dsDNA) was influenced by neither slope exposure nor time (Tables 3 and 4). Nonetheless, slope exposure had a significant impact on soil fungal and *nifH* gene abundances (Table 4), being approximately three times higher at the north- than at the south-facing slope irrespective of the time (Table 3). Overall, the  $\beta$ -glucosidase, xylosidase, and cellulase activities were two times higher at the north- than at the south-facing slope after 104 weeks (Figure 1D–F), whereas no exposure effect was

observed for the other sampling times, except for the xylosidase activity at the beginning of the monitoring period ( $N > S$ ; Figure 1E). However, no significant differences over time were recorded for these enzyme activities (Figure 1D–F, Table 4), except for the  $\beta$ -glucosidase at the south-facing site (52 > 104 weeks; two times higher). Chitinase activity significantly varied in terms of exposure only after 156 weeks ( $S > N$ ; Figure 2E, Table 4), while no significant differences were detected over time irrespective of the slope exposure (Table 4). The exposure effect on the leucine-aminopeptidase and alkaline phosphomonoesterase activities was also time dependent (Table 4). A higher activity was recorded at the south- than at the north-facing slope after fifty-two weeks, whereas the opposite trend ( $N > S$ ) was found after 104 weeks (Figure 2F and G). Furthermore, both enzymes showed higher activities at the last two sampling times (104 and



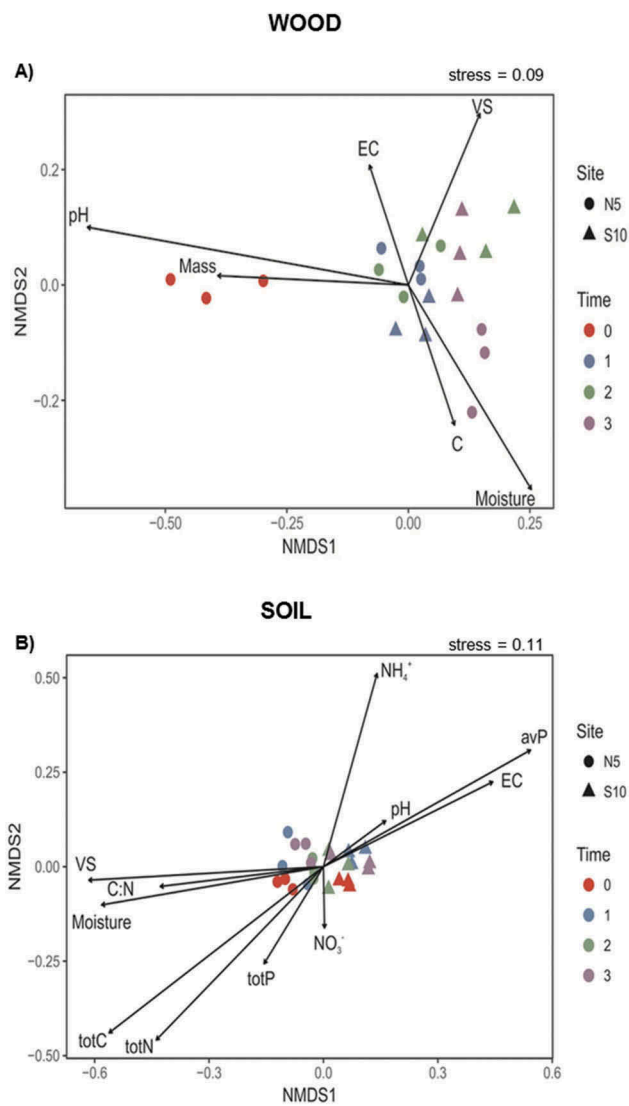


**Figure 2.** Potential activities of chitinase (A,E), leucine aminopeptidase (B,F), alkaline phosphomonoesterase (C, G), and acid phosphomonoesterase (D, H) of the wood and soil samples collected in August 2013 (0 weeks), July 2014 (52 weeks), July 2015 (104 weeks), and July 2016 (156 weeks) at the north- and south-facing sites (N<sub>5</sub> and S<sub>10</sub>, respectively). Values are means ( $n = 3$ ) with standard deviation.

156 weeks) at the north-facing site, while a higher activity was recorded after fifty-two weeks at the south-facing site. Nevertheless, acid phosphomonoesterase activity was significantly higher at the north- than at the south-facing slope regardless of the sampling time (Figure 2H, Table 4).

### **Nonmetric multidimensional scaling (NMDS) analysis**

Overall, the *P. abies* wood blocks collected at the beginning of the monitoring (t<sub>0</sub>) clustered in the negative side of the first ordination axis (Figure 3A), indicating that they represent a specific microhabitat. All the other



**Figure 3.** Nonmetric multidimensional scaling (NMDS) ordination was performed to map the physicochemical parameters to the shifts in wood (A) and soil (B) microbiological properties (microbial abundances and enzyme activities) as a function of time (t0 = control, August 2013; t1 = after 52 weeks, July 2014; t2 = after 104 weeks, July 2015; t3 = after 156 weeks, July 2016) and slope exposure (north-facing slope = point symbol; south-facing slope = triangle symbol).

wood samples were located on the positive side of the first axis (Figure 3A), with wood pH ( $R^2 = 0.45$ ,  $p \leq 0.01$ ) being the major determinant for this differentiation. Additionally, along the second axis a separation as a function of exposure was mainly observed for the wood samples collected at the end of the monitoring period (i.e., 156 weeks; t3). Wood moisture appeared to be the most determinant factor for this differentiation ( $R^2 = 0.31$ ,  $p = 0.06$ ). Furthermore, a clear separation in terms of slope exposure was recorded for the soil samples collected at the north- and south-facing slopes (negative and positive sides,

respectively) along the first ordination axis (Figure 3B). The major physicochemical parameters responsible for this differentiation were total C ( $R^2 = 0.51$ ,  $p \leq 0.001$ ), total nitrogen ( $R^2 = 0.40$ ,  $p \leq 0.01$ ), and available P ( $R^2 = 0.40$ ,  $p \leq 0.01$ ), followed by VS ( $R^2 = 0.38$ ,  $p \leq 0.05$ ) and soil moisture ( $R^2 = 0.35$ ,  $p \leq 0.05$ ).

## Discussion

In line with previous studies performed in the Trentino area (Egli et al. 2006, 2009; Fravolini et al. 2016; Bardelli et al. 2017), the soils located at the north-facing slope were moister and richer in total C and nitrogen contents compared to those at the south-facing slope. Along a climosequence, Fravolini et al. (2016) observed faster decay rates of the *P. abies* wood blocks at the north- than at the south-facing slopes up to an elevation of 1,800 m a.s.l., probably because of the higher soil moisture and clay content along with a lower soil pH. These are favorable conditions, especially for fungi, which can better develop on north-facing sites. Nevertheless, we did not observe distinct changes in the *P. abies* wood-block mass during the three-year observation period. In general, this fits well with the findings of Fravolini et al. (2016) and Petrillo et al. (2016), who measured very slow and sometimes almost barely measurable decay rates of *P. abies* in alpine environments with wood-mass decay rates often between 0.018 and 0.040  $y^{-1}$ . These authors explained the low decay rates to be the result of climatic conditions and the extremely slow decomposition rate of lignin.

In this study, the higher soil moisture and OM levels recorded at the northern slope were accompanied by higher soil fungal and *nifH* gene abundances in comparison with the southern slope. Bardelli et al. (2017) did not observe an exposure effect on soil fungal communities (qPCR-based) up to an altitude of 1,800 m a.s.l. in the same study area. These discrepancies in terms of exposure might be related to the higher proportion of grassland and a colder climate registered at 2,400 m a.s.l. compared to the other study sites surveyed by Bardelli et al. (2017). Indeed, complex interactions between local-scale factors, such as soil properties and vegetation composition, are known to largely affect soil microbial communities and their spatial distribution in mountain environments (Ascher et al. 2012; Regan et al. 2017; Siles et al. 2017).

In contrast to soil, exposure did not have a significant impact on the two previously mentioned microbial groups (fungi and *nifH*) in the *P. abies* wood

blocks. This is in disagreement with our second hypothesis. However, a general increase in their microbial abundance was observed over time. This phenomenon could be ascribed to the release of nutrients with progressing wood decay, which provide a source of nutrients for new colonizing microorganisms—microbial succession (Hoppe et al. 2015). Moreover, more acidic conditions (lower pH values) were detected in the *P. abies* wood blocks at the end of the monitoring period, which might lead to favorable conditions for fungal growth and their activity. In accordance with Baldrian et al. (2016) and Gómez-Brandón et al. (2017), we also found that pH was one of the most influential driving factors shaping wood microbial abundance and activity during the early stages of decomposition. Hoppe et al. (2015) reported, however, that variations in the abundances of bacterial phyla (using pyrosequencing analysis) are, rather, determined by a combination of several wood properties (i.e., C and nitrogen contents and wood moisture) than by single parameters such as pH alone.

Although previous studies pointed to an existing association between fungi and diazotrophic bacteria in wood (Gómez-Brandón et al. 2017; Hoppe et al. 2014; Johnston, Boddy, and Weightman 2016), we did not find a significant interaction between these two microbial groups in terms of abundance. This might be because of the fact that we focused on the early stages of decomposition of Norway spruce deadwood. Indeed, Hoppe et al. (2014) found a stronger positive correlation between fungal fructification and *nifH* diversity during the intermediate stage of decay of *P. abies* and *Fagus sylvatica* logs. Another plausible explanation could be related to the moisture content, because it is known to be one of the limiting factors of the biological nitrogen fixation (Rinne et al. 2017). In fact, a decrease in wood moisture level was found at the end of the monitoring period in our study.

Furthermore, enzyme-specific exposure effects were observed for the *P. abies* wood blocks as previously shown by Gómez-Brandón et al. (2017). On the one hand, the C-related enzyme activities were not affected in terms of exposure, while, on the other hand, those activities involved in the N cycle (leucine-aminopeptidase and chitinase) and P cycle (alkaline phosphomonoesterase) showed higher activities at the southern slope. This thermal signal ( $S > N$ ) was time dependent (i.e., after 104 and 156 weeks), partially corroborating our first hypothesis. This is in line with the fact that the wood samples at the south-facing slope were characterized by a higher microbial biomass (based on dsDNA).

Accordingly, the dsDNA yields were positively correlated with most of the previously mentioned enzymes (data not shown) at the end of the monitoring period, probably because of more suitable conditions (i.e., higher nutrient availability) for microbial growth as wood decay progresses (Gonzalez-Polo, Fernandez-Souto, and Austin 2013; Pastorelli et al. 2017; Petrillo et al. 2015, 2016). In fact, the increase in fungal abundance after 104 weeks was also accompanied by an increase in the C-related enzyme activities in the *P. abies* wood blocks, suggesting the higher activity of the wood-inhabiting fungi at this stage of decay.

As occurred with wood, the exposure effect on soil enzyme activities related to C and N cycles was also time dependent. Nonetheless, soil microbial biomass (dsDNA) did not vary in terms of exposure, which is in agreement with Bardelli et al. (2017). These authors, however, found higher levels of microbial biomass on northern slopes by using the substrate-induced respiration approach. The different effect of exposure on soil microbial biomass could be interpreted in light of the so-called method-result effect (Nannipieri et al. 2003, 2017). Furthermore, we observed higher soil acid phosphomonoesterase activity at the northern slope over time probably because of the lower soil P availability at this slope than in the comparable south-facing one. Indeed, an increase in P-acquiring enzyme activities is expected under conditions of soil P deficiency (Fraser et al. 2015).

## Conclusion

Our field mesocosm experiment enabled us to observe how different thermal and moisture conditions (due to exposure effects) affected wood- and soil-inhabiting microbiota in terms of their abundance and activity in the studied alpine setting. A higher abundance of fungi and nitrogen-fixing bacteria was recorded in the topsoil layer at the north-facing site, characterized by cooler and moister conditions. In the *P. abies* wood blocks, however, the abundance of these two microbial groups did not respond to exposure, while an increase in their abundance was observed with progressing wood decay along with more acidic conditions. This points to wood pH as a crucial driving factor of the deadwood microbiota during the early decay stages. The impact of exposure was also enzyme specific and time dependent for both the *P. abies* wood blocks and the underlying soil. Altogether, this indicates the importance of using multiple methods that include a broad array of enzymes to

avoid potential misinterpretation of effects on specific nutrient cycles (Nannipieri et al. 2012) caused by environmental changes in alpine ecosystems.

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