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Physico-chemical and microbiological evidence of exposure effects on *Picea abies* – Coarse woody debris at different stages of decay

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ABSTRACT

Although slope aspect determines the amount of solar irradiation, with implications on the functioning of forest ecosystems, little is known yet about how this affects the aboveground deadwood decomposition dynamics. Therefore, we set up a climosequence case study to evaluate the impact of slope exposure (north- *vs.* south-facing sites) on the physico-chemical and microbiological properties of *Picea abies* coarse woody debris (CWD) at different stages of natural decay (decay classes, DCls 1–5) in an Italian Alpine setting. Variations in bacterial, fungal and archaeal abundances were assessed by real-time PCR in the extra- and intracellular DNA fractions (eDNA *vs.* iDNA) of the total deadwood DNA pool. Physico-chemical wood properties (macro- and micronutrients; lignin and cellulose content; 3D structure via X-ray microtomography) were also performed along with the determination of key enzymatic activities involved in the main nutrient cycles. Overall, higher microbial abundances were registered in *Picea abies* CWD samples at the cooler, more acidic and moister north-facing site, which are favourable conditions especially for fungal wood decomposers. This thermal signal (N > S) was more evident for the advanced decay stages (DCls 4 and 5), being wood pH the most determinant factor for discriminating between both slopes. We also found that the impact of exposure was enzyme-specific and strongly dependent on the decay class, except for those enzymes involved in the P cycle. In addition, the eDNA/iDNA ratio provided a simple yet powerful index of microbial activity in terms of exposure, with lower values at the north-facing slope indicative of a higher microbial activity. This is in line with the more pronounced physical wood damage detected at this slope by the X-ray microtomography. A higher microbial activity at the cooler north-facing site rather seems surprising – a circumstance that probably is not due to temperature itself but due to increased moisture availability at this slope.

1. Introduction

Deadwood is an important structural and functional component of forest ecosystems, acting as a temporal store of plant nutrients and water, and providing shelter and nutrition to various organisms, primarily fungi and saproxylic insects (Floudas et al., 2012; Harmon et al., 1986; Zuo et al., 2016). Moreover, deadwood represents a global carbon store estimated to be in the range of 73 ± 6 Pg (Pan et al., 2011), making its decomposition dynamics a determinant of the soil carbon balance and forest productivity (Bradford et al., 2014). However, as pointed out by Lombardi et al. (2013), in European cool to temperate and Mediterranean forest ecosystems deadwood is still poorly described and there is a paucity of information on its contribution to soil carbon and nutrient pools and to long-term forest sustainability.

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Microorganisms, particularly fungi, are key determinants of lignocellulose decomposition thanks to the secretion of a battery of oxidoreductases and hydrolases (Purahong et al., 2016) and, therefore, of deadwood decomposition in forest ecosystems (Fischer et al., 2012). Through their presence as spores in the atmosphere or even as mycelial filaments in the surrounding soil, fungi are always in the vicinity of living, dying and dead wood (Hoppe et al., 2015a). It has also been shown that wood-inhabiting fungi may benefit from wood-colonising bacteria if they metabolise toxic intermediates and/or provide fungi with limiting nutrients like iron or nitrogen via nitrogen-fixation (de Boer and Van der Wal, 2008; Hoppe et al., 2014; Valášková et al., 2009). It is known that N availability in deadwood is highly restricted, with a C to N ratio ranging from 350 to 800, which suggests that wood-inhabiting fungi may benefit from associations with N-fixing bacteria to fulfil their N requirements for vegetative and generative growth (Hoppe et al., 2014). On the other hand, wood-inhabiting bacteria may potentially confer negative effects to fungi, as they compete for easily degradable substrates that are necessary for fungal colonization and degradation activities (Folman et al., 2008). Although the direct contribution of bacteria to wood decomposition rate and dynamics seems to be smaller than that exerted by fungi, bacterial communities might play a more important role in deadwood turnover than previously thought and ultimately, in nutrient cycling in forest ecosystems (Hervé et al., 2016; Hoppe et al., 2014, 2015b; Johnston et al., 2016).

The so-called five-decay class system has commonly been used to describe the wood decay progression in the field (Harmon et al., 2013; Hunter, 1990; Lombardi et al., 2013; Petrillo et al., 2015, 2016). As woody material decays, its physical and chemical quality gradually changes and consequently a microbial community succession takes place as species are replaced by those more suited to the substrate (Rajala et al., 2012). In this regard, a climosequence approach may provide insight into deadwood decay dynamics in response to thermal conditions represented by different altitudes and exposure (Fravolini et al., 2016; Petrillo et al., 2015, 2016).

The aim of the present study was to evaluate the shifts in the wood-inhabiting microbiota of *Picea abies* (L.) Karst at different stages of decay at two subalpine sites located at north and south exposure in the Italian Alps (Val di Rabbi, Trentino) to account for different thermal conditions. Variations in bacterial, fungal and archaeal abundances with exposure were assessed in the extra- and intracellular DNA fractions (eDNA *vs.* iDNA) of the total deadwood DNA pool and related to specific physico-chemical properties of wood (i.e., pH, macro-micronutrients; lignin and cellulose content); as well as to the potential activities of key enzymes involved in the main nutrient cycles. In addition, we were interested in better characterising the structural organisation of the deadwood because it may distinctly affect the decay processes (Mayo et al., 2010; Sedighi Gilani et al., 2014).

We hypothesised that: (1) warmer climatic conditions at south exposure will favour the deadwood decomposition in terms of microbial biomass and activity; (2) the exposure-effects on deadwood physico-chemical and microbiological properties will be more evident for the advanced decay stages; (3) the eDNA/iDNA ratio will provide a simple yet powerful index of microbial activity (the lower the ratio, the higher the activity) as a function of exposure and progressing wood decay.

2. Material and methods

2.1. Study sites and sampling strategy

The investigation area was located in Val di Rabbi (Trentino) in the south Alpine belt in northern Italy. The two study sites were located at an altitude of 1930 and 1995 m a.s.l. at north (N) and south (S)-facing slopes, respectively (Egli et al., 2006). The main characteristics of the N-facing slope were: aspect 20°N; slope 12°; mean annual air temperature (MAAT) 1.4 °C; mean annual precipitation (MAP) 1180 mm yr^{-1}; while the S-facing slope was characterised by: aspect 160°N; slope 25°; MAAT 4.4 °C; MAP 1180 mm yr^{-1} (Petrillo et al., 2015). Both study sites were on acidic paragneiss or morainic parent material consisting of paragneiss (Egli et al., 2006; Petrillo et al., 2015).

Coarse woody debris (CWD) samples from decaying Norway spruce (*Picea abies* (L.) Karst) were collected in August 2013 as described by Petrillo et al. (2015). Norway spruce constitutes the dominant tree species in these study sites together with the co-occurring European larch (*Larix decidua* Mill.) (Petrillo et al., 2015, 2016). CWD samples were classified *in-situ* using the five decay class system based on visual, geometric and tactile features according to Hunter (1990): (1) hard wood, penetrable with a knife to only a few mm, bark and twigs (diameter < 1 cm) intact; (2) rather hard wood, penetrable with a knife to less than 1 cm, bark and twigs begin to shed away, branches (diameter 1–4 cm) intact; (3) distinctly softened wood, penetrable with a knife to approximately 1–4 cm, bark and branches partially lost, original log circumference intact; (4) considerably decayed wood, penetrable with a knife to approximately 5–10 cm, bark lost in most places, original log circumference begins to disintegrate; (5) wood that disintegrates either to a very soft crumbly texture or is flaky and fragile, penetrable with a knife to more than 10 cm, original log circumference barely recognizable or not discernable.

Briefly, at each site five replicates for each decay class were collected, resulting in a total of 50 samples (2 study sites × 5 decay classes × 5 field replicates). Each field replicate was a composite sample deriving from 5 sub-samples that were pooled together in the field. The CWD volume assessment of each decay class was assessed as shown by Petrillo et al. (2015). All CWD samples were placed in a coolbox until they were taken to the laboratory, where before analyses they were pulverized using a cutting-mill (Fritsch-Pulverisette; 4 mm). After each sample, drill bits were cleaned by air-brushing and rinsed with distilled water between samples. All drill dust samples from each decay class were placed in a single sterilised vinyl bag and stored at 4 °C and −20 °C for physico-chemical and (micro)biological characterisation, respectively. For X-ray microtomography analyses, untreated (not cut-milled) wood samples were stored at −20 °C (as described in Section 2.7).

2.2. Physico-chemical analyses

Cut-milled CWD samples (5 g, fresh weight) were placed into a Petri dish and oven-dried (105 °C) for at least 24 h in order to determine the dry mass of the different decay classes. The volatile solids (VS) content was determined from the mass loss following ignition in a muffle furnace (Carbolite, CWF 1000) at 550 °C for 5 h. Electrical conductivity (EC) and pH were determined in wood:water extracts (1:5, w/v) by using a conductivity Meter LF 330 WTW (Weilheim, Germany) and a pH Meter Metrohm 744, respectively. Ammonium content was measured in 0.0125 M $CaCl_2$ extracts as described by Kandeler (1993). Total C and N were determined using a CN analyser (Vario Macro CN, Elementar, Hanau, Germany– combustion analysis). The concentration of P, K, Ca, Mg, Fe and Mn was assessed by using ICP-OES (Optima 8300, Perkin Elmer, Waltham, USA) after acid digestion of 0.5 g of powdered wood with 4 mL of HNO_3 in a closed vessel (UltraWAVE Milestone, Shelton, CT, USA – maximum temperature 230 °C). The α-cellulose content was assessed following the protocol of Leavitt and Danzer (1993) and Boettger et al. (2007). The Klason lignin, which is insoluble in strong acid, was determined according to Dence and Lin