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# UNIVERSITÀ DEGLI STUDI DI TORINO

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# Winter-Sprint transition induces changes in nutrients and microbial biomass in mid-alpine forest soils

Michele Freppaz<sup>ab</sup>, Daniel Said-Pullicino<sup>a</sup>, Gianluca Filippa<sup>b</sup>, Fabienne Curtaz<sup>ab</sup>, Luisella Celi<sup>a</sup>, Ermanno Zanini<sup>ab</sup>

<sup>a</sup> Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco, Italy.

<sup>b</sup> NATRISK - Research Centre on Natural Risks in Mountain and Hilly Environments, University of Torino, Italy.

## Abstract

Active microbial biomass beneath seasonal snowpacks may influence biogeochemical processes in mountain soils. Here we show that soil microbial biomass in temperate forest ecosystems reaches its annual peak under snow, and declines rapidly as snow melts in the spring. This decline was associated with changes in temperature regime and resource availability. However, in contrast to alpine ecosystems at higher elevations, fungal/bacterial ratios, evaluated by amino sugar analysis, were lower in snow-covered winter soils and increased during snowmelt. We associated this shift to a faster decline in bacterial with respect to fungal biomass during snowmelt.

**Keywords:** Snow-covered alpine soils; seasonal variation; resource availability; microbial biomass dynamics; amino sugars.

Seasonally snow-covered ecosystems play a critical role in global carbon (C) and nitrogen (N) biogeochemical cycles. The development of a persistent snow cover in mountain forest soils which decouples soil from atmospheric temperatures may represent a key factor influencing the dynamics of the microbial biomass and related seasonal variations in C and N cycling (Brooks et al., 1998; Freppaz et al., 2008; Viglietti et al., 2013). These ecosystems are especially susceptible to climate change as small changes in temperature or precipitation may result in large variations in the amount and timing of snow cover (Barnett et al., 2005; Edwards et al., 2007). This could have important implications on organic matter (OM) turnover and nutrient cycling which in turn affect C source/sink functions and plant nutrition (Haei et al., 2013). The objectives of our study were to investigate temporal patterns in fungal and bacterial microbial biomass in seasonally snow-covered mountain forest soils, and to link these dynamics to the variations in available C and N under different forest stands.

The study was conducted in three forest sites (Navette, Secae and Valcona) located in the Ligurian mid-alpine area (Ormea, NW Italy, ~30 km North from the Mediterranean sea), under a maritime snow climate, at elevations of 1609, 1312 and 1321 m a.s.l. respectively. Tree vegetation is dominated by Silver fir (*Abies alba* Mill.), beech (*Fagus sylvatica* L.) and Scots pine (*Pinus sylvestris* L.), respectively, while the soils are classified as Typic Udorthent, fine-loamy, mixed, active, nonacid, frigid (Navette), Typic Udorthent, loamy-skeletal, mixed, frigid (Secae) and Ultic Hapludalf, clayey-skeletal, mixed, frigid (Valcona) (Soil Survey Staff, 2010). Annual precipitation during the experimental period (2010/11) accumulated to 1282 mm and the mean annual air temperature was +5.4°C. In 2010, snowpack accumulation started in the middle of November reaching a maximum of 216 cm by the middle of March 2011, while snowmelt lasted throughout April 2011 (Fig. 1a). At each of the experimental sites, triplicate soil samples (200 cm<sup>3</sup> core) were collected from the A horizon (0–10 cm

depth) on the 21<sup>st</sup> October 2010 and 26<sup>th</sup> January, 8<sup>th</sup> March, 5<sup>th</sup> May and 13<sup>th</sup> July 2011. Within 24 h from collection, fresh samples were homogenized by sieving at 2 mm, and analysed for microbial biomass C and N, inorganic N and water-extractable organic carbon (WEOC) and nitrogen (WEON). Fungal and bacterial microbial biomass were also evaluated by analysing for amino sugars as described by Zhang and Amelung (1996). These biomarkers are important constituents of the cell wall of bacteria and fungi and can thus be used as an indicator for the origin of different microbial C and N residues in soils (Amelung, 2001; Glaser et al., 2004). To test for difference between sampling dates and sites we used a linear mixed effect model (Bates et al., 2014), incorporating two fixed effects (sampling date and site) and two random effects (site and replicate) to account for autocorrelation data. Significant differences between sampling dates and sites were then identified at  $p < 0.05$  by the Tukey Honest significant difference test. Pearson correlation analyses were run with soil C and N forms.

Mean air temperatures during the snow-covered winter months fluctuated between  $-11.4$  and  $+8.0^{\circ}\text{C}$ , while daily mean soil temperatures in all sites were rather constant with mean values of  $+0.5$ ,  $+1.1$  and  $+1.2^{\circ}\text{C}$  for Valcona, Secae and Navette, respectively (Fig. 1). Although it is generally assumed that microbial activity is related to temperature (Pietikäinen et al., 2005), long-term soil warming may actually reduce biomass and activity of the microbial community (Rousk et al., 2012). Recent studies have shown that soil conditions under alpine snow packs can be very favourable for microbial growth and activity (Brooks et al., 2011) with important impacts on element cycling and annual fluxes. In all sites, greater content of microbial biomass C was observed in January and March with respect to the other sampling dates (Fig. 2a), as reported for alpine tundra (Lipson et al., 2000) and temperate beech forest sites (Kaiser et al., 2011). Moreover, seasonal variations were greater than differences that could be attributed to the different vegetation cover (Björk et al., 2008). Accordingly, mixed effect models for these two variables showed F values that were 1 to 2 order of magnitude greater for factor time than for factor site, indicating a much higher variance explained by seasonal variation compared to vegetation cover.

Microbial biomass in forest soils is not only controlled by temperature and moisture status, but also by the supply of nutrients and labile organic C required to sustain microbial growth. In fact, relatively high biomass C content observed during the winter months, coincided with highest concentrations of inorganic N ( $r = 0.314$ ;  $p < 0.05$ ) and labile WEOC ( $r = 0.496$ ;  $p < 0.05$ ) and WEON ( $r = 0.441$ ;  $p < 0.05$ ) pools (Fig. 2b and c). Snow-covered soil temperature and moisture regimes may strongly influence nutrient release and labile C supply through their effects on litter decomposition as well as nutrient and dissolved organic C fluxes (Celi et al., 2010; Brooks et al., 2011). During the snow-covered winter months, the suppression of nitrification and denitrification at low temperatures (Frolking et al., 1998), together with limited plant uptake could have contributed to increasing  $\text{NH}_4^+$  concentrations (Fig. 2b). Similarly, slower rates of mineralization during litter degradation could have resulted in a net accumulation of labile WEOC and N pools (Fig. 2c). This was most evident at the Navette site that also showed lowest mean winter soil temperatures. Similarly, Koranda et al. (2013) found that the winter community in a temperate beech forest soil showed a higher capacity for degrading complex C substrates (cellulose, plant cell walls) but a lower utilization of labile C sources than the summer community. As temperatures rise, the microbial biomass and growing vegetation compete for available N that, together with significant nutrient leaching during snowmelt, were probably responsible for the decrease in inorganic N contents with time (Fig. 2b). Moreover, the rapid consumption of labile OM with warmer temperatures may result in a decrease in C availability that coincides with the increase in microbial C demand causing a steep decline in biomass (Brooks et al., 1998; Lipson et al., 2000).

Variations in total amino sugars reflected the changes in microbial biomass with greatest biomarker contents during the winter months (Fig. 3). We assume that the observed seasonal variations in amino sugars were due to fresh biomass in winter and accelerated microbial turnover during the warmer months. We generally observed a higher abundance of fungal biomarkers (GluN) during the winter that decreased during snowmelt ( $p < 0.05$ ). This is consistent with other studies that report a higher predominance of fungi during the snow-covered winter months, more adapted to cold temperatures and capable of utilizing available complex substrates to a larger extent than the summer community (Lipson, 2007; Björk et al., 2008; Buckeridge et al., 2013). Nonetheless, the lower glucosamine-to-muramic acid ratio (GluN/MurA) generally observed in January (Fig. 3) suggested that there is an active bacterial population at this time, in line with the increase in  $\text{NH}_4^+$  contents related to heterotrophic activity (Lipson D.A., 2007). In all sites, all amino sugar biomarkers decreased during snowmelt ( $p < 0.05$ ) while GluN/MurA increased ( $p < 0.01$ ) suggesting a more rapid turnover of bacterial with respect to fungal biomass. This is in contrast with the decrease in the fungal/bacterial ratio during snowmelt observed in alpine ecosystems at higher elevations (Lipson et al., 2002; Schadt et al., 2003; Björk et al., 2008; Drake et al., 2013). The different seasonal pattern of microbial biomass change with respect to ecosystems at higher elevation was probably due to a very dynamic bacterial biomass linked to changing substrate availabilities. This could have also involved a shift from Gram-positive to Gram-negative bacteria (Björk et al., 2008), possibly leading to a significant decrease in bacterial amino sugar biomarkers (Amelung, 2001). The predominance of fungi over bacteria in alpine snow-covered soils may thus depend on environmental drivers, community interactions and resource availability that vary with elevation.

In conclusion, our study suggests that soil microbial activity and dynamics during the snow-covered winter months, strongly influence C and N cycling across different pedo-environments, and may have important implications on nutrient availability during the warmer growing season. Independently from the differences in vegetation cover (broadleaves vs conifers) the soil microbial biomass and nutrient dynamics showed a common pattern under this maritime snow climate. In these temperate forests we observed a shift in the soil microbial biomass with a rapid turnover in bacterial biomass during spring snowmelt, a pattern which seems different from that observed in sites at higher elevation. Since this activity is sensitive to changes in snow cover, winter climate change may play a crucial role in the nutrient cycling in forest ecosystems.

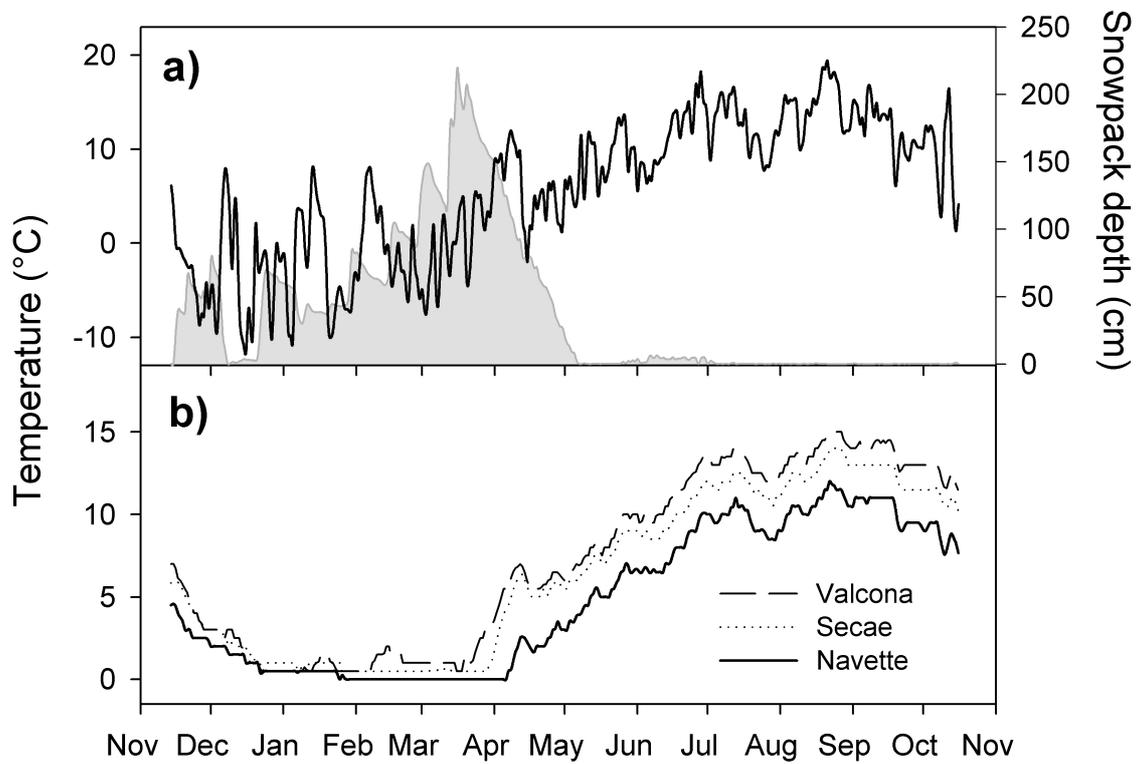
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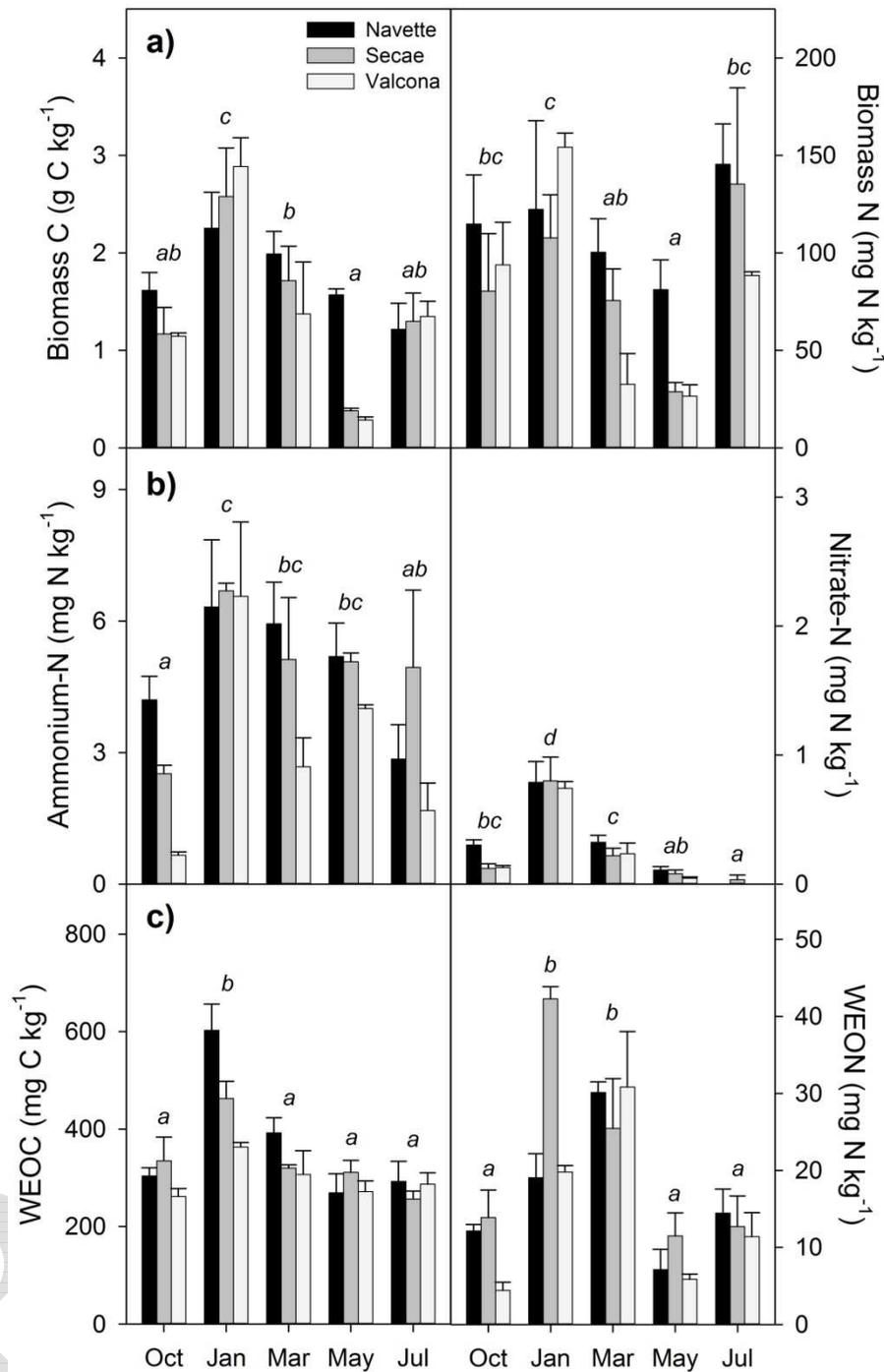
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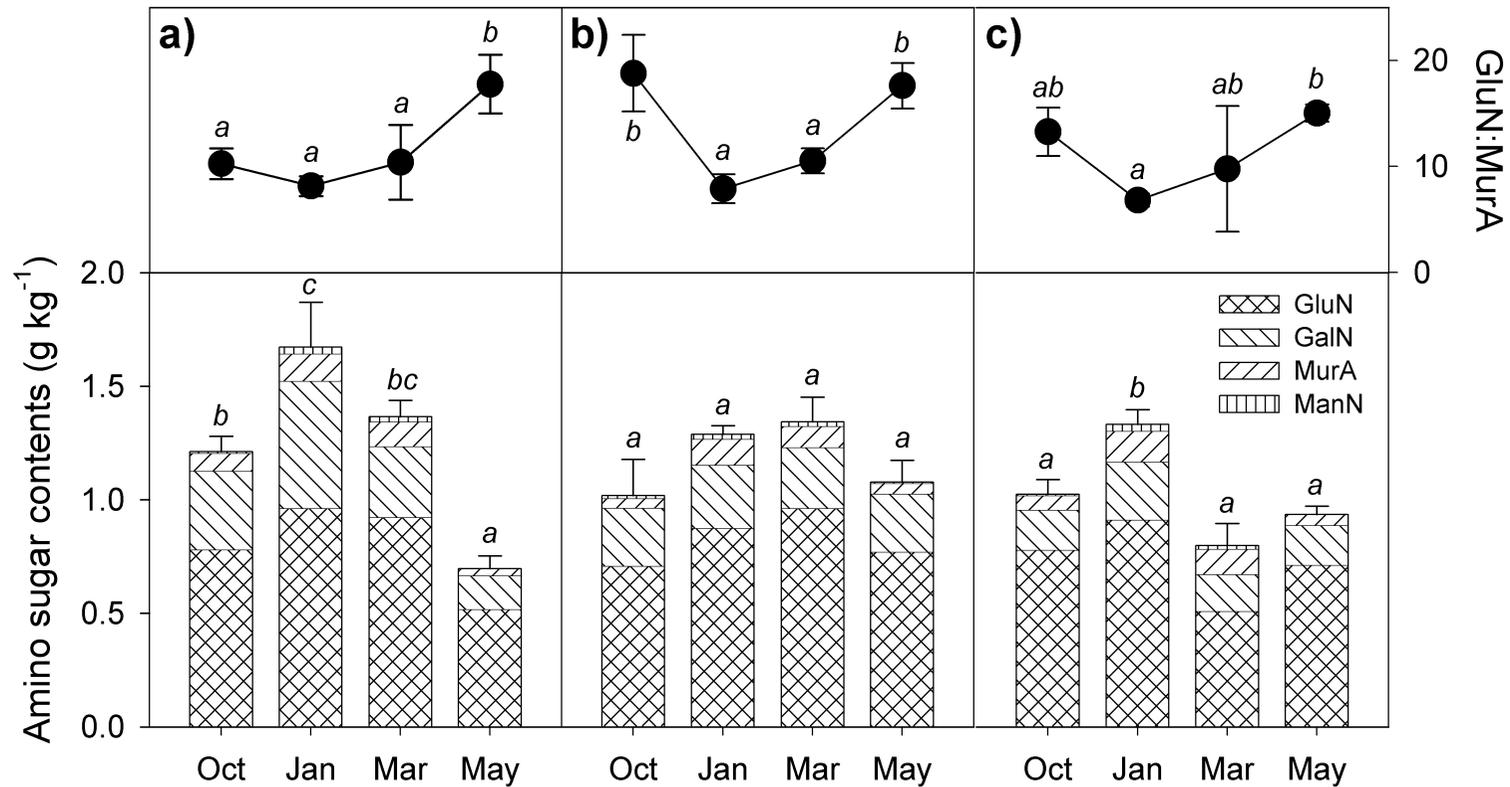
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**Fig. 1.** Variations in (a) daily average air temperatures, snowpack depth (shaded area), and (b) daily average soil temperature measured at each of the three sites over the experimental period.



**Fig. 2.** Seasonal variations in (a) microbial biomass C and N, (b) inorganic N, and (c) water extractable organic C and N. Error bars represent the standard error. Different letters indicate significant differences ( $p < 0.05$ ) between sampling dates, as determined by the Tukey HSD test.



**Fig. 3.** Seasonal variations in amino sugar contents (bars) and glucosamine-to-muramic acid ratio (points) in soils from (a) Navette, (b) Secae, and (c) Valcona. Error bars represent the standard error. Different letters indicate significant differences ( $p < 0.05$ ) between sampling dates, as determined by the Tukey HSD test. GluN, glucosamine; GalN, galactosamine; ManN, mannosamine; MurA, muramic acid.