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# Litter decomposition at two forest sites in the Italian Alps: a field study

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## A B S T R A C T

We studied in situ litter decomposition at a subalpine (1737 m a.s.l.) and a submontane (570 m) long-term monitoring forest site in the Italian Alps in the period 2000–2003 and 10 years later (2010–2013). Litter bags filled with site-specific (pine needles) and standard (cellulose) litter were exposed in autumn 2000 and 2010 on the soil surface. Cellulose was additionally exposed in litter bags buried at 2 cm soil depth to monitor the effect of soil microclimate. Decomposition rates were calculated after 1, 2, and 3 years of exposition from litter mass loss. Mass loss of litter exposed on soil surface was significantly affected by the site (altitude) and the litter type, while the decade of exposition (2000–2003, 2010–2013) had no significant impact. Litter decomposition was significantly lower at the site at the higher altitude, which was related to the colder climate, and pine needles were decomposed to a significantly lower extent than surface-exposed cellulose. Decomposition of cellulose buried in soil was neither influenced by the decade nor by the site. Cellulose mass loss was higher in soil than on soil surface, which is attributed to the favorable soil microclimate conditions in soil. The better adaptation of the soil population at the submontane site compared to that at the site at the higher altitude for accelerated degradation of easily decomposable compounds (cellulose) was confirmed by a laboratory study.

## INTRODUCTION

Litter decomposition is a key factor in nutrient cycling and thus required for the functioning of the ecosystem. Decomposition is the breakdown of dead organic matter through leaching of soluble compounds, fragmentation by soil fauna and/or abiotic processes, and reduction to CO<sub>2</sub> and inorganic nutrients (mineralization), which can be attributed primarily to the catabolic activity of microorganisms (Gavazov, 2010, and references therein).

The decomposition of plant litter is controlled by three factors: the physical environment (climate), the quantity and quality of the substrate available to decomposers (litter quality), and the character-

istics of the organisms involved in decomposition (Gavazov, 2010, and references therein). The relative contribution of these factors is dependent on the constraining factors in the studied environment (Prescott, 2010). Climatic constraints in soil include temperature, soil moisture, soil insulation by snow cover, and climate-driven shifts in community composition of the decomposers, which in turn influence litter quality (Gavazov, 2010; Duboc et al., 2012, and references therein).

An appropriate method to evaluate decomposition processes in soil are in situ studies. Such studies have been performed to analyze the effect of differences or changes in climate on litter decomposition rates (Schinner, 1982; Murphy et al., 1998; With-

ington and Sanford, 2007). In terrestrial ecosystems, litter bags have been used in a number of studies to determine decomposition rates via mass loss (e.g., Murphy et al., 1998; Sjögersten and Wookey, 2004; Duboc et al., 2012; Purahong et al., 2014). This method enables the determination of the natural decomposition under in situ conditions and is especially useful to compare decomposition rates in different ecosystems and regions in a consistent way (Kurz-Besson et al., 2005). To determine litter decomposition in situ (in the field), site-specific litter or standard litter can be used (Schinner, 1982). Fine root litter has also been used (Solly et al., 2014). Site-specific litter represents the conditions at the site to be investigated and considers litter quality. The use of chemically defined standard litter, such as cellulose, allows for the consistent (standardized) comparison of decomposition rates at different sites. Cellulose is the quantitatively most important natural organic compound; plants consist of 40%–70% celluloses. The ability to degrade cellulose (by the action of cellulases) is widespread among soil microorganisms.

Forests are expected to face significant pressures in the future from climate change. In alpine environments, climate change is expected to cause an upward migration of vegetation zones due to increased surface temperatures. This may alter the composition of vegetation as well as the quantity and quality of plant litter, which in turn affects microbial community composition and functioning (Sjögersten and Wookey, 2004; Djukic et al., 2010). Data from long-term monitoring programs can be used to answer questions on the impacts of climate change on forest ecosystems (Kleemola and Forsius, 2002; Clarke et al., 2011). In a previous study, we monitored as part of the International Cooperative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems (ICP IM) soil microbiological activities and abundances at two forest long-term monitoring sites differing in altitude and vegetation (subalpine, submontane) over a period of 17 years (1993–2010) and demonstrated significant differences between the two sites (Margesin et al., 2014). Over the study period, we noted an increase in mean annual air temperatures by +0.6 and +0.8 °C, respectively, at the two sites.

It was the objective of this study to assess if and to what extent this climate change has an impact

on litter decomposition. Therefore we monitored in situ decomposition rates at these two long-term monitoring sites in the period 2000–2003 and 10 years later, i.e., in the period 2010–2013. Litter bags filled with site-specific litter (pine needles) and standard litter (cellulose) were exposed in autumn 2000 and 2010 on the soil surface, which, in terms of microbial decomposition, is the most active soil layer. Cellulose was additionally exposed in litter bags buried below the soil surface at 2 cm soil depth to monitor the effect of site-specific soil microclimate conditions. Litter decomposition rates were calculated after 1, 2, and 3 years of exposition from litter mass loss. In addition, we performed a laboratory study to determine the effect of temperature and exposition time on cellulose decomposition on soils from the two sites. The impact of climate conditions on in situ litter decomposition in forest soils has usually been studied in short-term (mainly 1–2 years) investigations (Withington and Sanford, 2014; Duboc et al., 2012; Purahong et al., 2014; Solly et al., 2014); Takeda (1995) monitored litter decomposition in a coniferous forest over a 5-year period; however, no monitoring studies comparing decomposition rates over a period of 10 years are available.

## METHODS

### Description of the Study Sites and Climatic Conditions

The investigated two forest sites IT01 (Ritten/Renon) and IT02 (Montiggl/Monticolo) are two long-term monitoring sites in the Italian Alps and represent two widely distributed and significant forest types in South Tyrol. The detailed description of the two sites including climatic conditions is presented elsewhere (Bonavita et al., 1998; Margesin et al., 2014). Briefly, the subalpine site IT01 is located 7 km north of Bozen/Bolzano below the Rittner Horn at an altitude of 1737 m above sea level (a.s.l.). The soil was classified (FAO) as haplic podsol. The soil had a pH (CaCl<sub>2</sub>) of 3.3; SOM, Corg, and N contents were 33%, 19%, and 0.8%, respectively; the C:N ratio was 24:1. The vegetation consists of coniferous forest close to the timberline, dominated by *Picea abies*, *Pinus cembra*, and *Larix decidua*. The climate is subalpine-continental with a

mean annual temperature of 4 °C and a mean annual rainfall of 1000 mm.

The submontane site IT02 is located 8 km south of Bozen/Bolzano above the Small Lake Montiggl at an altitude of 570 m a.s.l. The soil was classified (FAO) as dystric cambisol. The soil had a pH (CaCl<sub>2</sub>) of 4.1; SOM, Corg, and N contents were 12%, 7%, and 0.3%, respectively; the C:N ratio was 21:1. The vegetation consists of coniferous and deciduous forest, dominated by *P. sylvestris*, *Quercus pubescens*, *Q. robur*, *Fraxinus ornus*, and *Ostrya carpinifolia*. The climate is mild continental with submediterranean influences, with a mean annual temperature of 11 °C and a mean annual rainfall of 900 mm.

## In Situ Study

### *Decomposition of Site-Specific Litter: Pine Needles*

Whole, intact pine needles, a representative litter source at both investigated sites, were collected from the trees of the study sites in August 2000 and August 2010. Litter bags were prepared by placing 1 g of air-dried pine needles evenly into nylon bags (10 × 10 cm) of 1 mm mesh size. The bags were sewed up with nylon thread and the mass (= bag + dry mass of needles before exposition) was determined. Each litter bag was labeled and supplied with a labeled cord for identification. Both in 2000 and in 2010, 30 litter bags with pine needles were prepared for each site.

Exposition started in October 2000 and in October 2010. At each site and in each decade, the 30 litter bags were exposed horizontally onto the soil surface (thereby ensuring a close contact with soil and avoiding air spaces due to plant cover); the distance between the litter bags was 5 m. The cords on the bags were attached to an aluminum pole fixed in the soil. The litter bags were then covered with a thin layer of site-specific litter to simulate litter decomposition under natural conditions, where litter on the soil surface is continuously covered with litterfall during the vegetation period. After 1, 2, and 3 years of exposition, one litter bag was collected from each exposition place (i.e., 10 litter bags were collected per site and year). The bags were transported on ice to the laboratory and processed immediately. Any soil or root particles were removed from the exterior of the bags with a fine brush and the cord was removed. The bags were then air-

dried until constant mass was obtained. Afterward, the bags were emptied and any extraneous material was removed. The bags including the remaining litter were weighed (= bag + dry mass of needles after exposure). Then the litter was removed from the bag and the mass of the empty bag (= dry mass of emptied bag after exposure) was determined. The mass loss of pine needles (%) per year was calculated (Schinner et al., 1996; ICP-IM Manual, 1998). The annual decay constant  $k$  was calculated according to Olson (1963):  $\ln(x_0/x_t) = kt$ , where  $x_0$  is the original litter mass,  $x_t$  is the mass remaining at time  $t$ , and  $t$  is the time in years.

### *Decomposition of Standard Litter: Cellulose*

Litter bags were prepared by placing air-dried alpha-cellulose sheets (Macheri-Nagl; Quality 2668 Schleicher & Schüll). Four cellulose sheets (30 × 50 mm, 1 mm thick) were placed evenly into nylon bags (10 × 10 cm, 1 mm mesh size). The bags were sewed up with nylon thread and the mass (bag + dry mass of cellulose before exposition) was determined. Each litter bag was labeled and supplied with a labeled cord for identification. Both in 2000 and in 2010, 60 litter bags were prepared per site.

Exposition started in October 2000 and in October 2010. At each site, 30 litter bags were exposed horizontally onto the soil surface (thereby ensuring a close contact with soil and avoiding air spaces due to plant cover) with a distance of ~5 m between the litter bags. The litter bags were then covered with a thin layer of site-specific litter. The other 30 litter bags were buried ~2 cm below the soil surface at a 15° angle as described (ICP-IM Manual, 1998). Burying of litter bags gives reproducible results due to more constant microclimate conditions compared to litter exposed on the soil surface (Schinner, 1982). By comparing cellulose decomposition on the soil surface and buried in soil, we were able to monitor the effect of site-specific microclimatic conditions, with desiccation playing a major role.

After 1, 2, and 3 years of exposition, one litter bag was collected from each exposition place (i.e., 10 litter bags exposed on the soil surface and 10 litter bags exposed at 2 cm soil depth were collected per site and year). The bags were transported to the laboratory and treated as described for the litter bags with pine needles.

## Laboratory Study on Decomposition of Cellulose

Soil samples were collected at the time of exposition of litter bags (October 2000, October 2010) from each of the 10 exposition places per site and composite soil samples per site were produced. Water content was adjusted to ~50% of the maximal water holding capacity. Per composite sample, 35–40 g soil fresh mass was weighed into glass petri dishes. A preweighed air-dried cellulose sheet (30 × 50 mm, 1 mm thick; used as standard litter in the litter bags) was exposed on the surface of each soil sample. Petri dishes were covered with a glass lid. In total, 24 petri dishes were prepared per sampling site; 12 of the 24 petri dishes were incubated at 10 °C and 20 °C, respectively. Water loss was compensated regularly by the addition of water. After 1, 2, 4, and 6 months, three petri dishes (= replicates) per incubation temperature were collected. Cellulose sheets were removed, cleaned from soil residues, and air-dried. Cellulose decomposition (%) was calculated from the mass loss obtained after incubation.

## Statistical Data Analysis

Statistical calculations were done by using Statistica, version 9.0. Normal distribution was evaluated by the Kolmogorov-Smirnov test. Factorial ANOVA was applied to determine whether the site, the exposition decade and/or the litter type, and in case of cellulose the exposition place (on soil surface / in soil) had a significant ( $P < 0.05$ ) effect on litter mass loss and on the decay constant  $k$ . ANOVA was also used to test whether exposition time (1, 2, and 3 years of exposition) had a significant ( $P < 0.05$ ) effect on litter decomposition.

## RESULTS

### Climatic Conditions

The subalpine site IT01 was characterized by lower annual air temperatures and higher annual precipitation than the submontane site IT02. The mean annual air temperature ranged from 4.4 to 4.8 °C and from 3.7 to 5.8 °C during the two exposition periods 2000–2003 and 2010–2013, respectively, at site IT01. Values at site IT02 were

higher by ~6 °C (11.1–11.7 °C and 10.4–11.8 °C). At both sites, annual air temperatures (mean, minimum, and maximum) and precipitation recorded in the first decade were not significantly different ( $P < 0.05$ ) from the values recorded 10 years later (data not shown). The same result was obtained when monthly climate data were analyzed: at both sites, mean monthly air temperature or precipitation in the two exposition decades were not significantly different ( $P < 0.05$ ). Monthly air temperatures showed that the site IT01 at the higher altitude was exposed in both decades to temperatures of or below 0 °C between December and March (one-third of the year), while site IT02 was never exposed to subzero temperatures (Fig. 1). Monthly precipitation was only in the first monitoring period at both sites much higher in November than in other months of the year (Fig. 1), however, a significant effect between monthly precipitation at the two sites and in the two decades was not detected. The detailed analysis of precipitation and air temperature data in each exposition year showed at both sites twofold higher precipitation in August 2010 (248 mm and 188 mm at sites IT01 and IT02, respectively) compared to August 2000.

### In Situ Litter Mass Loss on Soil Surface

Exposition time (1–3 years) had a highly significant ( $P < 0.001$ ) effect on litter decomposition (Table 1, Fig. 2). In both decades, mass loss was at both sites—independently of the litter type (i.e., pine needles or cellulose)—significantly lower after 1 year of exposition than after 2 and 3 years. Significant differences between mass losses after 2 and 3 years were only detected for cellulose exposed on the soil surface at site IT01 in the period 2010–2013. In fact, cellulose decomposition was delayed in the first year. The detailed analysis of precipitation and air temperature data in each exposition year showed at both sites twofold higher precipitation in August 2010 (248 mm and 188 mm at sites IT01 and IT02, respectively) compared to August 2000. Although the relation between precipitation in August 2000 and 2010 was almost equal (precipitation was 1.3–1.4 fold higher in 2000 compared to 2010), at both sites we hypothesize that the substantially higher precipitation in August 2010 has contributed to conditions



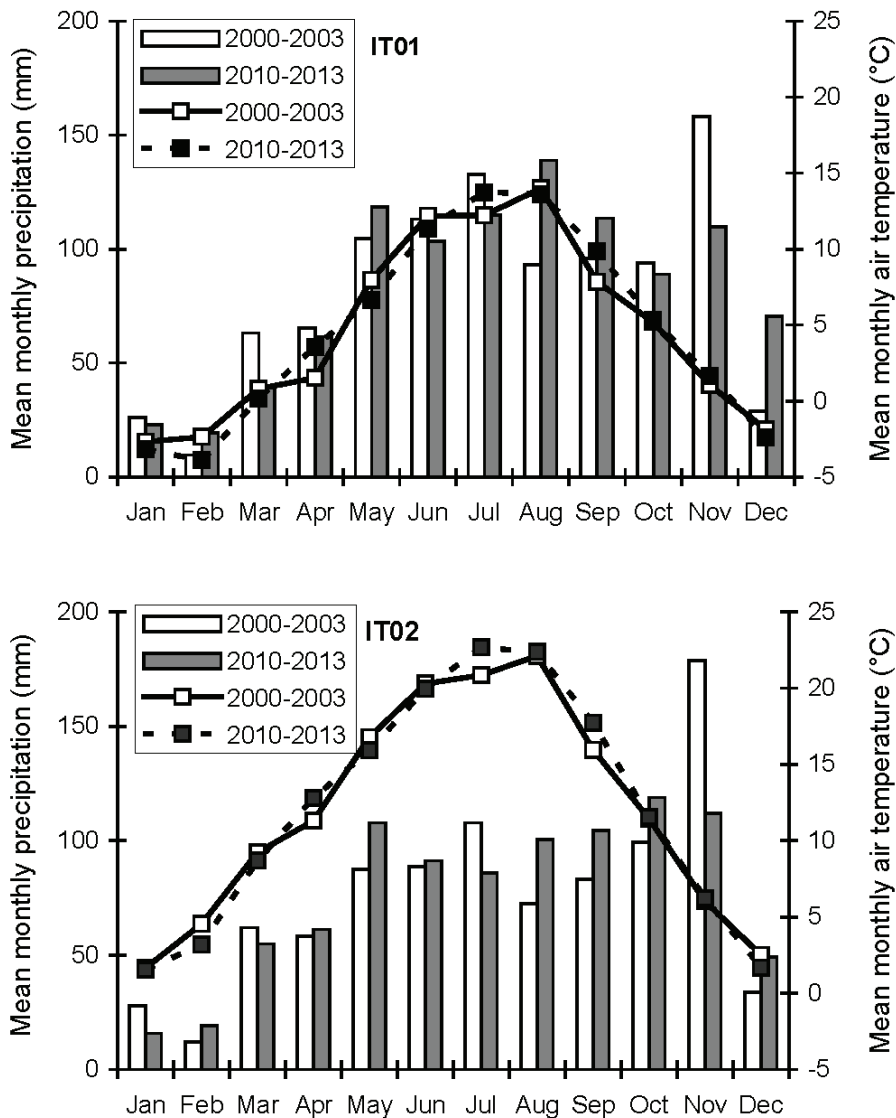


Figure 1. Monthly air temperature (□ 2000–2003; ■ 2010–2013) and monthly precipitation (white bars 2000–2003, dark bars 2010–2013) during the two exposition periods at the two investigated sites.

unfavorable (inhibiting) for cellulose decomposition at the site at the higher altitude, such as stagnant moisture. At site IT02 this effect was compensated by higher air temperatures. The fact that pine needle decomposition was not affected can be explained by the lower water absorption of this litter type due to the water-repellent wax layer on the epidermis, compared to the hygroscopic nature of cellulose.

Factorial ANOVA taking into account the three independent factors site (altitude), litter type, and exposition decade on mass loss of litter exposed on soil surface (i.e., without cellulose buried in soil, since cellulose decomposition in soil cannot be directly compared with litter decomposition on soil surface) demonstrated that overall mass loss was affected by the site (decomposition was significantly

higher at the site at the lower altitude) and the litter type (decomposition of pine needles was significantly lower than that of cellulose); however, neither the decade nor any of the possible interactions were significant (Table 2). The separate analysis of the data obtained in each decade revealed a highly significant effect of litter type on mass loss in both decades, while a site-specific effect was only recognized for the second decade, but not for the first decade, most likely due to the delayed cellulose decomposition at site IT01 in the first year of the second decade (Fig. 2). The interaction of the two factors site and litter type was in both decades not significant. With regard to the litter type, the site affected mass loss of litter exposed on the soil surface, while the exposition decade had no effect. How-

TABLE 1

Effect of exposition time (1–3 years) on litter mass loss on soil surface during the two monitoring periods at the two study sites, as determined by ANOVA.

Decade	Litter type	Site	Litter mass loss (%)					
			1 year		2 years		3 years	
2000–2003	Pine needles	IT01	36.3	a	61.8	b	55.7	b
		IT02	40.5	a	65.2	b	75.5	b
2010–2013	Pine needles	IT01	30.9	a	52.3	b	56.5	b
		IT02	35.3	a	64.4	b	66.2	b
2000–2003	Cellulose	IT01	60.5	a	92.4	b	89.8	b
		IT02	68.5	a	83.9	ab	99.0	b
2010–2013	Cellulose	IT01	36.6	a	65.9	b	97.1	c
		IT02	74.5	a	96.3	b	97.4	b
2000–2003	Cellulose in soil	IT01	75.6	a	95.3	b	99.4	b
		IT02	83.2	a	96.8	b	96.1	b
2010–2013	Cellulose in soil	IT01	81.1	a	99.1	b	100.0	b
		IT02	83.5	a	98.6	b	98.4	b

Data represent mean values ( $n = 10$ ). Different letters (a, b, c) in a row indicate statistically significant differences (LSD,  $P < 0.05$ ) between years.

ever, a significant influence of the decade became visible at the site IT01 at the higher altitude for litter exposed on the soil surface (IT01 < IT02). The interaction decade/litter type was not significant.

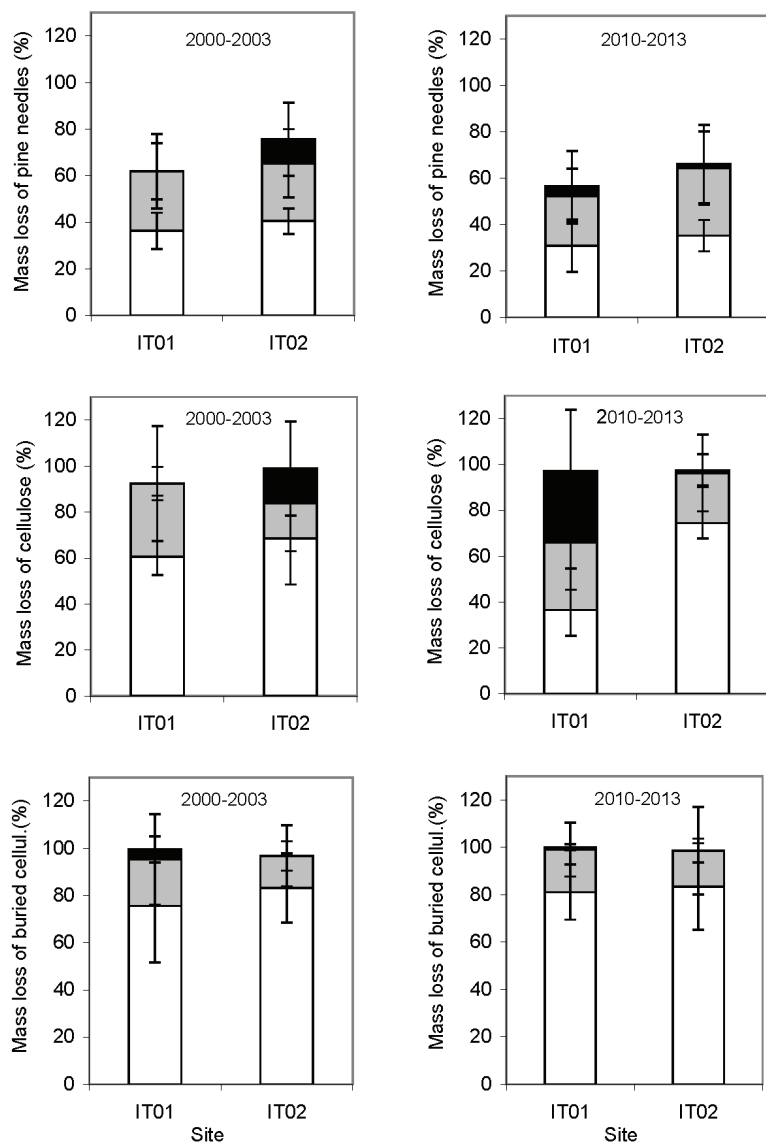
The decay constant  $k$  (Olson, 1963) provided an estimate for the decomposition rate at each site in each decade by integrating variations during the 3-year exposition time in each decade and showed the development of the litter decomposition processes (Table 3). Overall data analysis of litter exposed on soil showed a significant effect of the site and the litter type and of all possible interactions on  $k$ ; the decade alone, however, was not of significance (Table 4). In each decade, the effect of litter type (on soil surface) and the interaction litter type/site were significant.

### In Situ Cellulose Mass Loss on Soil Surface and at 2 cm Soil Depth

In contrast to cellulose exposed on the soil surface, the decomposition of cellulose buried in soil was neither influenced by the decade nor by the site

or their interaction (Table 5). The comparable (not significantly different) mass loss at 2 cm soil depth at the two sites points to comparable microclimate conditions at the two sites. When comparing the mass loss of cellulose exposed on the soil surface with that of buried cellulose, no significant difference ( $P < 0.05$ ) was found at site IT02, regardless of the decade, or at site IT01 in the first decade (detailed data not shown). The apparently higher mass loss of cellulose buried in soil compared to that exposed on soil at site IT01 in the second decade is attributed to the delayed cellulose mass loss on soil in the first year of the second decade (Fig. 2).

Analysis of the determining factors for cellulose decomposition on soil surface and in soil demonstrated that the place of exposition (on/in soil) significantly affected mass loss, this was also confirmed with data obtained in each decade and at each site. Overall mass loss was higher in soil than on soil. The site was of significance for mass loss of cellulose exposed on soil but had no influence on mass loss of cellulose buried in soil (Table 5).



**Figure 2.** (top) Decomposition of pine needles, (middle) cellulose on soil surface, and (bottom) cellulose buried in 2 cm soil depth at the sites IT01 and IT02 in the first (white part of the columns), second (gray part of the columns), and third (black part of the columns) year of exposition. (left) Exposition period 2000–2003; (right) exposition period 2010–2013. Data show mean values and standard deviation of 10 replicate litter bags exposed per year and site.

## Laboratory Study on Decomposition of Cellulose

The laboratory study on cellulose decomposition on soil surface at 10 and 20 °C demonstrated the important role of temperature and thus of climatic conditions (Fig. 3). Independent of the site, cellulose decomposition was significantly higher at 20 °C compared to 10 °C; nonetheless soil from site IT02 had a significantly higher decomposition potential than soil from site IT01. The study also showed the time course of cellulose utilization by soil microorganisms. After 6 months at 10 °C, cellulose mass loss was low on soil from site IT01 ( $2\% \pm 0.2\%$ ), but significantly higher on soil from site IT02 ( $26\% \pm 8\%$ ). In contrast,  $14\% \pm 7\%$  and  $84\% \pm 6\%$  mass loss were

found on soil from site IT02 and IT01, respectively, after 6 months at 20 °C (Fig. 3).

## DISCUSSION

In this study, we examined the effect of environmental site conditions (defined by altitude) and litter type (site-specific vs. standard litter) over 3 years in the period 2000–2003 and 10 years later on litter decomposition at two distinctly different forest sites in the Italian Alps. The sites differed considerably in their climatic conditions and nutrient contents (Bonavita et al., 1998; Margesin et al., 2014). The site IT01 at a higher elevation was characterized by significantly colder climatic conditions compared to the submontane site IT02. Soil from site IT01



**TABLE 2**

**ANOVA analysis applied on mass loss of litter (pine needles, cellulose) exposed on soil surface at the two sites during the two monitoring periods.**

Litter mass loss	Factor						Interaction							
	Site (S)		Decade (D)		Litter type (L)		S × D		S × L		D × L		S × D × L	
Overall	16.807	*	3.030	NS	100.983	***	3.361	NS	0.545	NS	0.010	NS	3.676	NS
Decade 1 (2000–2003)	2.644	NS			50.935	***			0.716	NS				
Decade 2 (2010–2013)	17.108	***			50.083	***			3.428	NS				
Pine needles (P)	8.168	**	2.455	NS			0.005	NS						
Cellulose (C)	8.944	**	1.026	NS			5.375	*						
Site IT01			5.463	*	37.0792	***					1.409	NS		
Site IT02			0.005	NS	70.014	***					2.463	NS		

Data shown are F values of three-factorial ANOVA and levels of significance: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , NS not significant.

contained an almost threefold higher amount of SOM and was more acidic (pH 3.3) than soil from site IT02 (pH 4.1), the C:N ratios, however, were similar (Margesin et al., 2014).

Litter decomposition is primarily attributed to microbial activity (Gavazov, 2010). Soil microfauna also contributes to litter decomposition, especially at warm and humid sites compared to subalpine

**TABLE 3**

**Mean decay values (*k*) for litter (pine needles, cellulose) exposed at two sites for 3 years in two monitoring periods**

Litter type	Decade	Site	<i>k</i> value per year mean ( $n = 30$ )	Confidence limits (LS means)	
				–95.000%	95.000%
Pine needles	2000–2003	IT01	0.351	0.047	0.655
		IT02	1.254	0.950	1.558
	2010–2013	IT01	0.422	0.117	0.726
		IT02	1.494	1.190	1.799
Cellulose on soil	2000–2003	IT01	2.915	2.629	3.200
		IT02	0.462	0.176	0.747
	2010–2013	IT01	1.969	1.684	2.255
		IT02	0.547	0.262	0.832
Cellulose in soil	2000–2003	IT01	2.162	1.790	2.535
		IT02	2.917	2.545	3.290
	2010–2013	IT01	1.631	1.259	2.004
		IT02	1.832	1.460	2.204

Table 4

**ANOVA analysis applied on the decay constant (*k*) of litter (pine needles, cellulose) exposed for 3 years on soil surface at the two sites during the two monitoring periods.**

<i>k</i> value	Factor					Interaction								
	Site (S)		Decade (D)		Litter type (L)	S × D		S × L	D × L	S × D × L				
Overall	20.338	***	1.698	NS	31.793	***	8.125	**	192.958	***	7.725	**	4.178	*
Decade 1 (2000–2003)	22.268	***			29.073	***			104.272	***				
Decade 2 (2010–2013)	1.747	NS			5.189	*			89.557	***				

Data shown are *F* values of three-factorial ANOVA and levels of significance: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , NS not significant.

forest (Gonzalez and Seastedt, 2000, 2001; Wang et al., 2009). The contribution of soil fauna has been shown to vary with altitude, especially with regard to systematic variation (Wang et al., 2009).

During both monitoring periods, litter decomposition was at both forest sites, independently of the litter type, significantly higher after 1 year of exposition than after 2 and 3 years. The slowing down of litter decomposition with time has been shown in several decomposition experiments (Sun et al., 2012) and can be explained by the higher

substrate availability during the first year compared to the following years.

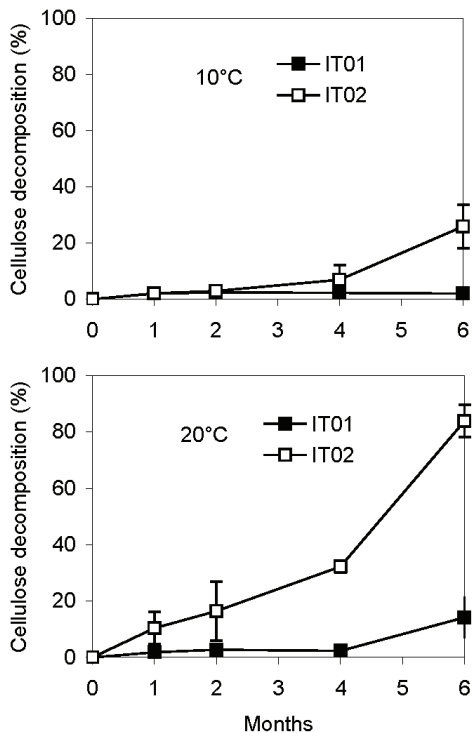
With regard to litter type exposed on the soil surface, mass loss of standard litter (cellulose) was at both sites significantly higher (by up to 40%) than mass loss of site-specific litter (pine needles). The faster decomposition of cellulose compared to pine needles can be attributed to the better biodegradability of cellulose in comparison to pine needles, which contain a high amount of compounds difficult to degrade, such as lignins, waxes, resins, and

Table 5

**ANOVA analysis applied on mass loss of cellulose exposed on soil surface and in soil (2 cm depth) at the two sites during the two monitoring periods.**

Litter mass loss	Factor					Interaction								
	Site (S)		Decade (D)		Exposition (E) on soil / in soil	S × D		S × E	D × E	S × D × E				
Overall	7.912	**	0.164	NS	23.996	***	3.371	NS	5.779	*	1.856	NS	4.876	*
Decade 1 (2000–2003)	0.479	NS			6.279	*			0.019	NS				
Decade 2 (2010–2013)	10.759	**			19.516	***			10.589	**				
Cellulose on soil	8.944	**	1.026	NS			5.375	*						
Cellulose in soil	0.175	NS	0.959	NS			0.145	NS						
Site IT01			1.930	NS	20.507	***			4.903	*				
Site IT02			1.465	NS	4.447	*			0.511	NS				

Data shown are *F* values of three-factorial ANOVA and levels of significance: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , NS not significant.



**Figure 3. Effect of incubation time and temperature (top, 10 °C; bottom, 20 °C) on decomposition of cellulose (mass loss) on soil from sites IT01 and IT02. Data show mean values and standard deviation of 3 replicates.**

tannins. The measure of cellulose decomposition can be taken as an index of SOM decomposition, because fresh organic material is composed primarily of cellulose, lignin, and hemicelluloses (Gestel et al., 2003; Withington and Sanford, 2007).

The accelerated decomposition of cellulose buried in soil compared to that exposed on soil surface in both decades and at both sites points to the important impact of soil microclimate. A well-balanced relation between moisture and temperature is crucial for soil biological activities (Murphy et al., 1998; Sjögersten and Wookey, 2004). Litter exposed on soil surface is not protected against weather conditions and thus subjected to climate fluctuations (temperature, moisture), including desiccation, which may inhibit decomposition processes. Litter buried in soil is subjected to lower temperatures, but its degradation is favored by more constant soil microclimate conditions (temperature, moisture, better protection from surface-related desiccation) and a better contact with soil microorganisms (Schinner et al., 1996), which results in

accelerated decomposition. The decomposition of buried substrates is primarily controlled by water availability (Withington and Sanford, 2007). In soils in the forest-alpine tundra ecotone, cellulose decomposition was found to increase gradually with depth; decomposition of buried cellulose in forest soils varied between approximately 40% and 80%–90%, depending on the mountain region (Withington and Sanford, 2007). Our data indicate that the effects of stimulating/inhibiting cellulose decomposition on soil surface and in soil were balanced at both sites, despite significantly different air climate conditions.

The considerably different climate (air temperature, precipitation) prevailing at the two sites due to different altitudes affected litter decomposition rates. A long-term monitoring study (1993–2010) on microbiological properties of soils from the two sites investigated in this study demonstrated generally significantly lower microbial activity (respiration, enzymes) and abundance of culturable bacteria and fungi at the site IT01 at the higher altitude than at the submontane site IT02 (Margesin et al., 2014). In the present study, pine needles were decomposed at both sites approximately at a similar rate during the first 2 years (IT01: 31%–36% and 52%–62% after 1 and 2 years, respectively; IT02: 35%–41% and 64%–65% after 1 and 2 years, respectively, in the two exposition decades). Nonetheless, this high decomposition of pine needles at the site IT01 is remarkable since this site is subjected to substantially colder climate conditions than site IT02. Hence, we conclude that soil microorganisms at site IT01 are well adapted to the degradation of compounds present in pine needles (lignins, waxes, resins, tannins). This could also be due to the fact that this type of litter—although representative for both investigated sites—is represented to a higher extent at site IT01 than at site IT02, and consequently soil microorganisms are forced to adapt their metabolism to compounds that are contained in this litter substrate. Additionally, alpine soil microbial communities are better adapted to the cold than microorganisms at lower elevations and are also highly resistant to freeze-thaw events (Lipson, 2007; Lipson et al., 2009; Margesin et al., 2009; Margesin, 2011).

Cellulose was generally faster decomposed at the site IT02 at the lower altitude (75% and 96% after 1

and 2 years) than at site IT01 (37% and 66% after 1 and 2 years, respectively). These data show that soil microorganisms at this site are better adapted for the accelerated decomposition of easily degradable compounds, such as cellulose, than microorganisms at site IT01, which is seen as a result of the more favorable climatic conditions. Results of the laboratory study on the effect of temperature and incubation time on cellulose decomposition confirmed this hypothesis and demonstrated significantly higher cellulose decomposition at site IT02 than at site IT01, as well as the influence of temperature.

In conclusion, our data demonstrate the significant impact of site-specific conditions (altitude) on the decomposition of site-specific litter and on standard litter exposed on soil surface at two forest sites. The comparison of the two litter types showed that pine needles were decomposed to a lower extent than cellulose, at both sites and in both monitoring periods. We also showed the important impact of soil microclimate, which resulted in higher decomposition of cellulose in soil than on soil surface. The monitoring of overall litter mass loss in 2000–2003, followed by a second monitoring 10 years later, clearly revealed that climate-related differences between the two decades were not detectable in terms of decomposition. This shows the importance of long-term monitoring research to determine the impact of (changing) environmental conditions on decomposition processes in forest soils. An increase in the mean annual air temperature (+0.6 °C and +0.8 °C) over the period 1993–2010 was noted at the two studied sites (Margesin et al., 2014). A 34-year climatic series (1977–2011) on site IT02 even showed an increase in mean annual air temperature over time from 10 to 11.4 °C (S. Minerbi, personal communication). The results obtained in our study demonstrate that litter mass loss was not significantly influenced by this climate change. It is important to consider climatic conditions and biological activities over long-term periods, on one hand, but we also showed the importance of taking into account short-term climate effects. The analysis of climate regime in the period 2005–2009 over the alpine area and northern Italy demonstrated the repeated occurrence of seasonal deviations (such as very cold winters, warm winters, rainy winters) over the last decade (Bertini et al., 2011).

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