Integrating chemical, biological and soil fauna variables during beech leaf litter decay: A partial least squares approach for a comprehensive view of the decomposition process

Integrating chemical, biological and soil fauna variables during beech leaf litter decay: A partial least squares approach for a comprehensive view of the decomposition process

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Abstract

Litter decomposition is an ecosystem process that is regulated by a multitude of factors and by their complex interactions. Current decomposition paradigms do not always offer a coherent view of the process because it can be hardly understood without a ~~comprehensive~~ thorough analysis of interacting factors. Thus, there is a need to further understand the mechanics of litter decay with a comprehensive approach, especially in temperate forest ecosystems where decomposition plays a crucial role in regulating them as source or sink of CO_2. Therefore, the aim of this work was to identify the interactions between chemical, biological and soil fauna variables in order to discern driving variables and the changes in their interactions during long-time (1300 days) beech leaf litter decomposition. In order to investigate patterns of variation and co-variation within and between datasets, we used Two-block Partial Least Squares, helping us to interpret the decomposition process with a systemic approach. Our key findings showed that the decomposition process of beech litter ~~in Mediterranean area~~in two Mediterranean forests was driven by litter quality at the beginning and in the later stages of decomposition, while edaphic and climatic factors were implied in the central steps, with a dramatic change of scenario around 2.5 years. Simultaneous and interacting changes in chemical variables, extracellular enzyme activities, and soil fauna were shown, with a significant role of lignocellulosic components and enzymes involved in their degradation, Mn residual weight, and abundance of Collembola.

Keywords: Extracellular enzyme activities; Leaf litter decomposition; Lignin and cellulose; Litterbags; Systemic approach

1 Introduction

Litter decomposition is an ecosystem functioning process that is regulated by a multitude of factors and by their complex interactions. Several researches carried out on decomposition dynamics suggested main controls either on the effect given by climate and litter quality (Kasurinen et al., 2007), or the nature and abundance of decomposer organisms and/or pedofauna (Fujii and Takeda, 2017; García-Palacios et al., 2013).

Litter quality affects both the decomposition rate and the limit value of decomposition (i.e. the limit for the accumulated mass loss when the decomposition may ultimately approach the rate zero and thus leave a recalcitrant or stabilized residue), the amount of humus produced and its chemical features (Berg, 2014). Great importance has been given to N and/or Mn (Berg et al., 2015; Innangi et al., 2015b) and the ratio of easily decomposable vs. recalcitrant compounds (Berg, 2014; Cotrufo et al., 2015). Thus, the C:N ratio, as well as the cellulose:lignin and cellulose:lignin:N ratios, are useful indices that would predict the decomposition rate (García-Palacios et al., 2016; Trap et al., 2013). Nevertheless, during decomposition, new organic matter originates by structural and chemical changes of original dead organic matter as well as by-products of soil biota, and these secondary molecules may be resistant to decomposition (Danise et al., 2018).

Climate affects the decomposition rates and the limit value of decomposition as well (Berg et al., 2010; Kasurinen et al., 2007). The effects can be either direct, e.g. by leaching of soluble compounds (Dise et al., 2009; Innangi et al., 2017a), or indirect by controlling species composition and activity of microbial and fauna communities (Aubert et al., 2010).

Litter decomposition rates appear also related to the complexity of the soil microbial and fauna communities (Rouifed et al., 2010). Microbial communities are the main ~~and direct~~ agents of the process through the secretion of extracellular enzymes (Burns et al., 2013), with a dominant role of fungi (Schneider et al., 2012). The diversity of the communities and their succession during decomposition ensures the degradation of organic matter and mineralization processes (Voříšková and Baldrian, 2013). Thus, extracellular enzyme dynamics, which are involved in the degradation of the major structural constituents of plant material, may provide information on specific metabolic and functional aspects of microbial communities (Sinsabaugh et al., 1991) as well as assess changes of microbial soil communities in response to environmental or chemistry variations (Fioretto et al., 2018). Soil fauna participates in the fragmentation of plant detritus and stimulate the activity of bacterial and fungal colonies (Hättenschwiler et al., 2005). In forest ecosystems soil and litter arthropod communities play a major role in the decomposition of fresh organic matter and in the formation of the humus profile (Rouifed et al., 2010). In particular, Oribatid mites and Collembola are important members of the detrital system in temperate forests (Menta et al., 2014).

Thus, there is a need to further understand the mechanics of the decomposition process with a comprehensive approach (García-Palacios et al., 2016), especially in temperate forest ecosystems when decomposition plays a crucial role in regulating forests as source or sink of CO_2 (Meier and Leuschner, 2010; Pan et al., 2011). This is particularly true under climate change scenarios, ~~particularly~~ especially in Mediterranean ecosystems that are highly susceptible to shifts in temperature and precipitation (Innangi et al., 2015a). Accordingly, beech forests have been extensively studied, given their substantial C stock and vulnerability (Chiesi et al., 2010; Curcio et al., 2017; Innangi et al., 2015a).

~~Thus, l~~Litter decomposition in beech ecosystems has been studied extensively, but there are few studies that address the ecological phenomenon of decomposition with a systemic approach. Therefore, the aim of this work was to identify the interactions between chemical, biological and soil fauna variables in order to discern driving variables and the change in their interactions during long-time (1300 days) leaf litter decomposition. To achieve this goal, we have studied beech leaf litter decomposition in two Mediterranean forests, which were already previously studied under different aspects, from decomposition regimes to carbon stocks and microbial activity (De Marco et al., 2016; Fioretto et al., 2018; Innangi et al., 2015b). Leaf litter was collected and incubated in the same sites, but a transplant experiment was also carried out by incubating the litter coming from each of them on the opposite site. In order to address the need of a comprehensive view of the decomposition phenomenon, we did not focus extensively on single variables, which have been amply studied in soil ecology research, but we have concentrated on Two-block Partial Least Squares (2B-PLS) regression, a data analysis process ~~which~~ that allowed us to discriminate and interpret patterns of variation and co-variation between sets of variables throughout the decay process.

2 Material and methods

2.1 Site descriptions

The two beech forest sites have been chosen according to their strong diversity in terms of soil characteristics and climate, yet with the same age (70–80 years at the beginning of the experiment) and management. A thorough description of these forests is given in (De Marco et al., 2016; Fioretto et al., 2018; Innangi et al., 2015b), yet a brief summary of the main forest features is ~~given~~provided.

Pradaccio (44.24 °N, 10.01 °E, 1350 m a.s.l.), is located on the northern Italian Apennines, within the "Guadine-Pradaccio" National Reserve (Emilia-Romagna region). The site has a mean temperature of 6.0 °C with total average rainfall of 2900 mm per year. Parent material is sandstone, giving the soil an extremely acidic reaction (pH = 4.0). The other forest, Laceno (40.47 °N, 15.05 °E, 1150 m a.s.l.), lies in the southern Italian Apennines within the Regional Park of Monti Picentini (Campania region). The forest has an overall average rainfall of 2300 mm per year and a mean annual temperature of 8.7 °C. The parent material is carbonate, and the soil is strongly acidic (pH = 5.5).

2.2 Litter collection and litterbag preparation

Newly shed litter was collected between the second half of September and the end of November 2011 by equally-spaced 6 net traps (80 cm Ø) on a surface of about 1 ha in each site. Litter was collected several times until fall was complete. Given that leaves were the most abundant fraction of total litter, we included only leaf litter in the litterbags.

Before their inclusion in the litterbags, aliquots of litter was dried in an oven at 75 °C until constant weight to evaluate dry weight. For the decomposition experiment, 576 standard terylene litterbags of 20 cm × 10 cm were prepared, with a mesh size of 1 mm × 1.5 mm, allowing interaction with most of the soil fauna except the largest animals (Bokhorst and Wardle, 2013). Each litterbag was filled with approximately 4 g of dried newly shed litter.

For the microarthropods study, instead, 108 modified terylene/plastic litterbags of 25 cm × 16.5 cm and different mesh sizes on the upper and lower surface of the litterbag were prepared. Top side had a mesh size of 2 cm × 2 cm, to allow the entry of most soil fauna, while the bottom one had a mesh size identical to the standard litterbags. Each litterbag was filled with approximately 6 g of dried newly shed litter.

Of the 288 standard litterbags and 54 modified litterbags prepared with litter from the forest site of Laceno, half of them were placed on the surface or organic soil in Laceno forest (LL) and the other half in Pradaccio forest (LP) in 6 randomized microsites in December 2011. Similarly, the litterbags enclosing the litter coming from Pradaccio were located at Pradaccio (PP) and Laceno (PL).

2.3 Litterbag collection and processing

Standard litterbags were sampled in both sites at 200, 535, 680, 935 up to a maximum of 1300 decomposition days, while modified litterbags for soil fauna were collected at 535, 935, and 1300 decomposition days. Each time, 3 litterbags per each leaf material were harvested from the 6 microsites in both forests (overall 18 bags).

After collection, each standard litterbag was opened in laboratory and carefully cleaned with a soft brush in order to remove large fauna and particles of soil. Afterwards, a little amount of the litter (about 0.5 g) from each litterbag was weighted, dried at 75 °C for 48 h and again weighted to evaluate its dry weight and ~~then~~ to calculate the residual mass in each litterbag. Subsequently, the residual material of the three litterbags coming from each microsite was pooled to obtain a homogeneous sample for each incubation microsite. An aliquot was dried and ground until passing a 0.5 cm screen, while the rest was stored at −80 °C until enzyme activity measurements. By considering M as the dry mass in g of litter from each bag and t_x as the days of decomposition, residual weight was measured as $[M(t_x)/M(t_0)] \times 100$. Decomposition trends were evaluated according to the single exponential model (Olson, 1963), obtaining the decomposition constant k (days^{-1}) as $-\{\ln [M(t_x)/M(t_0)] \times [1/t_x]\}$.

2.4 Soil fauna extraction and classification

In the laboratory, soil particles and living plant parts were removed from the surface of the litterbags. Animals were extracted by each modified litterbag using a Berlese-Tüllgren funnel at 35 °C for 10 days. The specimens were collected in a preserving solution (3:1 ethanol/glycerol v/v) and identified to class level for Myriapoda and order level for Hexapoda, Chelicerata and Crustacea (Menta et al., 2014) using an optical microscope. The organisms belonging to each taxon were counted in order to estimate their ~~presence~~ abundance in the litterbag.

2.5 Chemical analyses

Organic matter was measured by loss-on-ignition method by incinerating samples in a muffle furnace at 550 °C for 4 h (Fioretto et al., 2018). Total organic carbon and total nitrogen were measured by a CNS elemental analyser (Fioretto et al., 2018). In order to measure the content of Mn, samples were cold-mineralized and analytical determinations were performed by ICP-AES without ultrasonic nebulization (Bussotti et al., 2005; Innangi et al., 2015b). Cellulose and lignin were evaluated spectrophotometrically (Danise et al., 2018; Innangi et al., 2017a). All chemical variables were measured as mg/g and then they were expressed as residual mass in the litterbag, i.e. the ratio of the concentration per residual weight at t_x divided the concentration per weight of the original litterbag.

2.6 Extracellular enzyme activities

Extracts for cellulase, xylanase and chitinase activities were prepared by suspending 1 g of fresh litter in 10 ml of 0.05 M sodium acetate buffer at pH 5.5. For peroxidase and laccase 0.5 g of fresh litter were suspended in 10 ml of sodium acetate buffer 0.05 M pH 5.0. The extracts for acid and alkaline ~~phosphatase~~phosphomonoesterases were prepared by suspending 0.5 g of fresh litter in 10 ml of modified universal buffer at pH 6.5 and 11, respectively. The samples were homogenized while kept on ice using a Polytron Heidolph Diax 600 for about 20–30 s. The homogenate was then centrifuged for 20 min at 17,000 *g* at 4 °C, the supernatant recovered and used as enzyme extract. For the measurement of the dehydrogenase activity, the colorimetric assay was performed directly on 0.3 g homogenized litter in TRIS buffer 1 M pH 7.0.

Cellulase (EC 3.2.1.4) and xylanase (EC 3.2.1.32) activities were determined according to (Schinner and von Mersi, 1990) with minor modifications (Fioretto et al., 2000). As for β-1,4-poly-N-acetyl glucosamidinase (i.e. chitinase, EC 3.2.1.14) and dehydrogenase (EC 1.1.1.x), the activities were measured according to (Verchot and Borelli, 2005) and (Von Mersi and Schinner, 1991), respectively. Peroxidase (EC 1.10.3.x) and laccase (EC 1.10.3.2) activity was evaluated according to (Leatham and Stahmann, 1981) with minor modifications (Di Nardo et al., 2004), while acid phosphomonoesterase (EC 3.1.3.2) and alkaline phosphomonoesterase (EC 3.1.3.1) was performed according to (Eivazi and Tabatabai, 1977). All activities are expressed as µmol ~~/~~ g d.w. ~~/~~ h and were measured in triplicate on each sample from each incubation microsite.

2.7 Statistical analysis

All variables were inspected for outliers and for normality. All data are represented as mean ± standard error of the mean. Previous studies in the same two locations focused on significant differences between variables by means of three-way analysis of variance (Innangi et al., 2015b) or chemical and seasonal patterns of enzyme activities by means of linear mixed models (Fioretto et al., 2018). Here, we did not focus on significant differences between single variables, but patterns of covariance between datasets were tested using Two-block Partial Least Squares (2B-PLS), contrasting chemical variables vs. biological activities, chemical variables vs. pedofauna, and biological activities vs. pedofauna. The aforementioned analysis falls within the broader range of partial least square regression, which is well suited to analyzing a large array of related predictor variables, with a sample size not as large as the number of independent variables, or when approaching complex phenomena that must be defined as a combination of several variables obtained independently (Carrascal et al., 2009). Given two matrixes with different variables but same number of observations, 2B-PLS will construct pairs of variables as linear combinations of the variables within each of the two sets, which are constructed so that the new variables account for as much as possible of the covariation between the two original sets of variables (Rohlf and Corti, 2000). This technique was recently applied to forest ecosystems to investigate litter to topsoil chemical variations (Innangi et al., 2017a). The R packages ‘ggplot2’

and ‘plsdepot’ were used to perform the analyses.

3 Results

3.1 Chemical variables

Newly shed litter from the two forest sites showed several differences in chemical composition. As a matter of fact, leaves from Pradaccio had higher N and Mn concentrations ($12.28 \pm 0.77\,mg\,g^{-1}$ d.w. and $0.14 \pm 0.01\,mg\,g^{-1}$ d.w. against $10.17 \pm 0.22\,mg\,g^{-1}$ d.w. and $0.06 \pm 0.001\,mg\,g^{-1}$ d.w. of Laceno, respectively). Moreover, Laceno also showed a lower content of cellulose but a similar content of lignin ($17.9 \pm 0.5\%$ d.w. and $21.3 \pm 0.6\%$ d.w., respectively) than that from Pradaccio ($20.0 \pm 0.6\%$ d.w. and $19.5 \pm 0.6\%$ d.w., respectively). As for all other nutrients and their dynamics during decomposition until 680 days, the results are reported in Innangi et al. (2015b).

On the one hand, the overall mass loss followed a single exponential model (Fig. 1). Decomposition constant k was highest for Laceno’s litter decomposing in the autochthonous site ($0.00081\,days^{-1}$), while it was lower when the same plant material was decomposing in Pradaccio ($0.00069\,days^{-1}$). As for the litter from Pradaccio, once again a faster decomposition constant was recorded when decay happened in Laceno compared to Pradaccio (0.00071 vs. $0.00066\,days^{-1}$, respectively). On the other hand, the trends for residual masses of lignin, cellulose, N, and Mn showed differences throughout the years (Fig. 1).

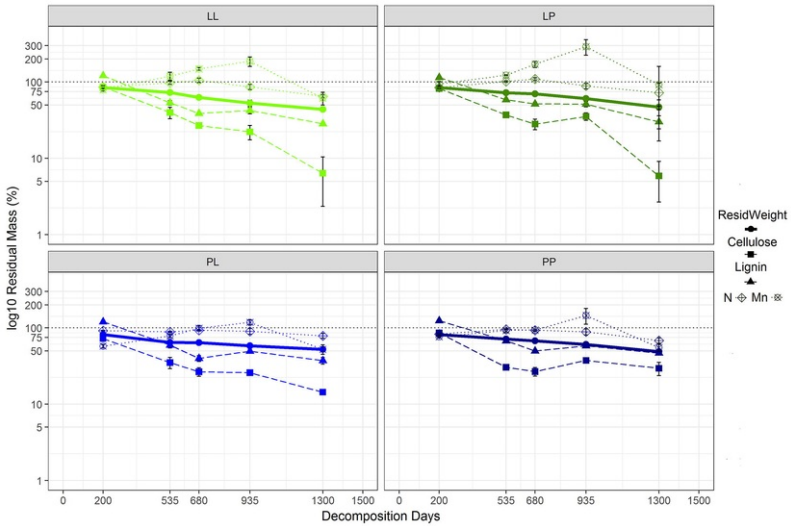

Fig. 1 Overall Residual weight and residual masses for all chemical variables. Data are expressed as percentage compared to the initial amount. Residual weight is represented as bold continuous line, cellulose and lignin as continuous lines, N and Mn as dotted lines. To optimize visualization, logarithmic transformation was applied to the data. Values are represented as mean ± standard error of the mean.

The degradation of cellulose had already started when we made our first measurements. However, between about 200 and 680 days of incubation maximum degradation rate was reached. At this time, 40–30% of initial mass remained. On the contrary, lignin showed an initial increase of the absolute amount followed by a rapid degradation until 680 days, when it reached a residual value about 40–50%. Subsequently, the degradation of lignin and cellulose slowed down and then reprised in the last year of incubation. Generally, by comparing the litters of Laceno and Pradaccio, cellulose degradation rates were similar and differed only in the last year. Accordingly, Pradaccio’s litter incubated in Laceno (PL) lost more cellulose than in the autochthons condition (PP). As for lignin degradation, the rate was greater for LL and LP, and the differences between litter from Pradaccio and Laceno increased with time. Generally, residual lignin for LL and LP was around 30%, while it was 50% for PP and 40% for PL.

The dynamics of N and Mn followed the same trend as that reported by Innangi et al. (2015b) until 680 decomposition days. As for N (Fig. 1), the immobilization process that persisted for approximately two years, was followed by a release in all conditions, although such release was more prominent in LL and LP (~65%) compared to PP and PL (~75%). Conversely, there was a conspicuous immobilization of Mn in all conditions at 935 decomposition days, followed by a large release at 1300 days (Fig. 1). Such trend was extreme in LL and LP, where Mn reached values up to 300% of the initial amount at 935 days, while in PL and PP such increase reached up to 150%. Subsequently, all conditions tended to 50–70% of initial Mn amount at 1300 days.

3.2 Biological activities

As a general remark for biological activities, it has to be noted that the third sampling, corresponding to slightly less than 2 years of decomposition (between 680 and 935 decomposition days), was a definitive time of change. Thus, two phases can be distinguished, the first from 0 to 2 years and the second from this moment until the end of the study period (1300 days). All enzymes showed different trends according to the decomposition location, with higher activities in Pradaccio for most enzymes with the exception of peroxidase that was higher in Laceno.

The activities of the cellulase and xylanase (Fig. 2a) were largely overlapping and showed two different trends according to the locations of decomposition. Accordingly, both litters decomposing in Laceno (i.e. LL and PL) showed a cellulase trend with two relative peaks at 535 and 935 days and a xylanase trend with a fast increase until 535 days, followed by a proximately stable trend afterwards. Generally, the activity of xylanase was higher than cellulase. On the contrary, litter decomposing in Pradaccio (LP and PP) showed a marked peak at 935 days for both activities.

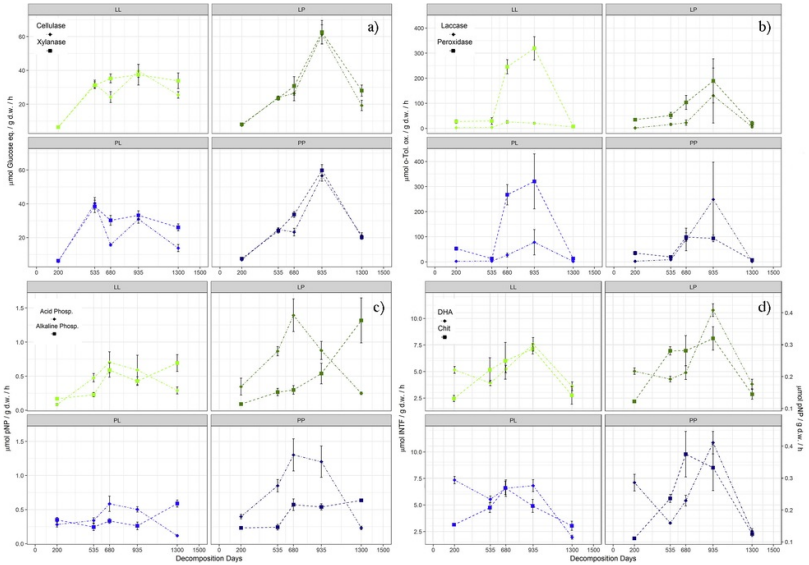

Fig. 2 Dynamics of cellulase and xylanase (a), laccase and peroxidase (b), acid and alkaline phosphomonoesterase (c), and dehydrogenase (DHA) and chitinase (d). Data are expressed a µmol Glucose equivalent × g d.w.$^{-1}$ × h^{-1} for cellulase and xylanase, µmol *ortho*-toluidine oxidized × g d.w.$^{-1}$ × h^{-1} for laccase and peroxidase, µmol *para*-nitrophenol × g d.w.$^{-1}$ × h^{-1} for acid and alkaline phosphomonoesterase, µmol iodonitrotetrazolium formazan × g d.w.$^{-1}$ × h^{-1} for DHA (left axis) and µmol *para*-nitrophenol × g d.w.$^{-1}$ × h^{-1} for chitinase (right axis). Values are represented as mean ± standard error of the mean.

Laccase and peroxidase showed large differences according to the decomposition site as well (Fig. 2b). Thus, trends were different for LL and PL compared to LP and PP. In Laceno, there was a sharp peak of activity for peroxidase between 680 and 935 days, with a considerably lower activity of laccase. In Pradaccio, instead, the activity of peroxidase and laccase were more comparable, with a relative peak only at 935 days. Noticeably, in PP, the activity of laccase was higher than that of peroxidase at 935 days, although with a considerable variability between samples.

Phosphomonoesterases, instead, showed a more complex pattern (Fig. 2c). Whereas LL and PL showed similar trends, LP and PP were different. In the case of litter decomposing in Laceno, the activity of acid phosphomonoesterase was higher than alkaline phosphomonoesterase between 535 and 935 days, yet such differences were not particularly strong. In the case of LP, the activity of acid phosphomonoesterase showed a clear peak at 680 days followed by a drop at 1300 days. Conversely, alkaline phosphomonoesterase increased almost linearly, with a peak at 1300 days. As for PP, the peak of acid phosphomonoesterase was constant between 680 and 935 days, while there was no peak for alkaline phosphomonoesterase but a roughly constant activity between 680 and 1300 decomposition days.

The trends for dehydrogenase and chitinase were similar in LL-PL and LP-PP (Fig. 2d). Accordingly, in Laceno, the activity of dehydrogenase decreased between 200 and 535 days, but increased at 935 days followed by another drop at 1300 days. Chitinase, instead, increased from 200 to either 680 (PL) or 935 (LL) decomposition days, dropping at 1300 days. A bell-shaped trend was evident also in LP and PP, especially for dehydrogenase, which peaked strongly at 935 days and fell at 1300 days. Chitinase peaked at 680 days for PP, while such peak was a 935 days for LP.

3.3 Soil fauna

Overall, we found 15 taxa of soil arthropods for a total of 17,175 individuals (Table 1). Acari and Collembola showed the highest abundance, together ~~contributed~~ contributing to over 95% of the soil fauna. Psocoptera, Diptera larvae, Coloeoptera (adults and larvae) were well represented, while others were rare (e.g. Thysanoptera or Hymenoptera) or very rare (e.g. Chilopoda or Diplura). Diplopoda and Opiliones were never found in all circumstances.

Table 1 Counting of the soil fauna taxa within the combination of litter material and location along the decomposition process. All values are number of individuals per litterbag. Values represent mean ± standard error of the mean (N = 6).

Decomp. Days	LL			LP			PL			PP		
	535	935	1300	535	935	1300	535	935	1300	535	935	1300
Acari	223 ± 24	531 ± 129	1083 ± 199	227 ± 24	1155 ± 164	301 ± 55	*	1049 ± 197	2162 ± 880	49 ± 16	1844 ± 145	685 ± 119
Collembola	83 ± 19	460 ± 152	428 ± 112	*	1714 ± 233	11 ± 9	98 ± 15	1424 ± 313	455 ± 244	*	2198 ± 240	1 ± 1
Chilopoda	1 ± 0	*	*	*	*	*	*	*	*	*	*	*
Pseudoscorpiones	1 ± 0	*	14 ± 7	*	1 ± 1	*	1 ± 1	7 ± 4	7 ± 7	*	5 ± 2	3 ± 2
Araneidae	*	2 ± 2	18 ± 10	*	8 ± 3	28 ± 27	*	7 ± 4	20 ± 11	*	5 ± 2	2 ± 1
Symphyla	*	*	4 ± 4	*	2 ± 2	*	*	*	*	*	4 ± 2	*
Diplura	*	2 ± 2	*	*	*	*	*	2 ± 2	*	*	*	*
Coleoptera Larvae	2 ± 1	12 ± 4	4 ± 4	*	64 ± 46	7 ± 5	2 ± 0	32 ± 14	34 ± 16	*	24 ± 8	3 ± 2
Coleoptera Adults	1 ± 0	2 ± 2	7 ± 4	*	41 ± 17	9 ± 4	1 ± 0	2 ± 2	*	*	16 ± 5	7 ± 4
Psocoptera	1 ± 0	*	*	1 ± 0	12 ± 6	*	*	6 ± 2	4 ± 4	*	13 ± 5	7 ± 4
Hemiptera	*	*	*	*	14 ± 8	*	*	*	4 ± 2	*	1 ± 1	*
Diptera Larvae	4 ± 1	7 ± 4	64 ± 32	2 ± 1	147 ± 103	13 ± 4	3 ± 0	32 ± 18	50 ± 26	2 ± 1	62 ± 20	16 ± 6
Hymenoptera	1 ± 0	4 ± 4	18 ± 6	*	1 ± 1	*	3 ± 2	*	11 ± 5	*	*	*
Lepidoptera Larvae	*	*	*	*	1 ± 1	*	*	2 ± 2	*	*	*	*
Dermaptera	*	2 ± 2	*	*	1 ± 1	*	*	*	*	*	*	*
Thysanoptera	*	*	11 ± 7	*	1 ± 1	7 ± 4	*	2 ± 2	28 ± 15	*	11 ± 4	5 ± 1
Total Individuals	313 ± 28	1021 ± 260	1649 ± 336	230 ± 24	3163 ± 298	376 ± 83	107 ± 14	2564 ± 459	2772 ± 1111	52 ± 16	4180 ± 284	730 ± 125
N Taxa	5 ± 0	4 ± 1	5 ± 1	3 ± 0	8 ± 0	4 ± 1	5 ± 0	6 ± 0	5 ± 0	2 ± 0	8 ± 1	4 ± 1

Generally, for location Laceno and both litters (LL and PL), the number of taxa was roughly stable around 5, while in Pradaccio (LP and PP) there was a peak of 8 taxa at 935 days. Total number of specimens showed differences in all comparisons, but the trend was not univocal. After 535 days, the number of specimens was higher in LL than in PL, and lower in PP than in LP. After 935 days, the trend was reversed, and after 1300 days, PP and PL were higher than LP and LL respectively. Comparing the two studied areas at the end of the experiment (1300 days), Laceno showed a noticeable higher number of specimens than Pradaccio for both litter types. This result was especially due to the abundance of Acari, but Collembola, Diptera larvae, Hymenoptera, Thysanoptera followed a similar trend. Acari represented the vast majority compared to Collembola at 535 and 1300 days, while Collembola became relatively more abundant at 935 days in Pradaccio (LP and PP). Laceno showed a different trend, but with a sensible effect of litter type as well. In LL, Collembola represented ~25% of the total at both 535 and 1300 days, becoming roughly even with Acari ad 935 days. Acari were absent at 535 days, and increased with time up to ~75% of the total at 1300 days in PL. Diptera larvae showed a different trend in the two sites. The other groups did not show a clear trend except Coleoptera (adults and larvae) that reached the highest abundances after 935 days in LL, LP and PP while they showed comparable value between 935 and 1300 days in PL.

4 Two-block partial least squares analyses

4.1 Chemical variables vs. biological activities

Explained variance in Axis 1 for Block 1 (Chemical Variables) was 46%, while Block 2 (Biological Activities) explained 19% of total variance (Fig. 3). On Block 1 (Chemical Variables), the most important variables were on the one hand, residual masses of lignin and cellulose and, on the other hand, residual Mn. The residual content of N explained no variance at all. As for Block 2, all biological activities were positively correlated, and, as a consequence, they were positively correlated with residual Mn as well. The most important variables were xylanase and cellulase, with decreasing importance to laccase and dehydrogenase, the latter explaining no variance. The scatterplot for Axis 1 put forward that the most distinct group was formed by all observations at 200 decomposition days, which had low residual Mn, high residual lignin and cellulose, and, at the same time, low biological activities, especially xylanase and cellulase. As decomposition proceed, a linear trend, with little difference between leaf material and decomposition location, was found. Such trend reached a peak at 935 days, when the observations from Pradaccio (PP and LP) were clearly separated from the others. Such gap at 935 days was shown mostly on Block 2 (Biological activities), thus it corresponded to a peak in enzymes such as xylanase, cellulase and alkaline phosphomonoesterase. After 1300 days, all data converged to a point similar to 680 days, although differences were now given mostly by plant material, as the points for PL-PP were distant from LL-LP, especially on Block 2 (Biological activities).

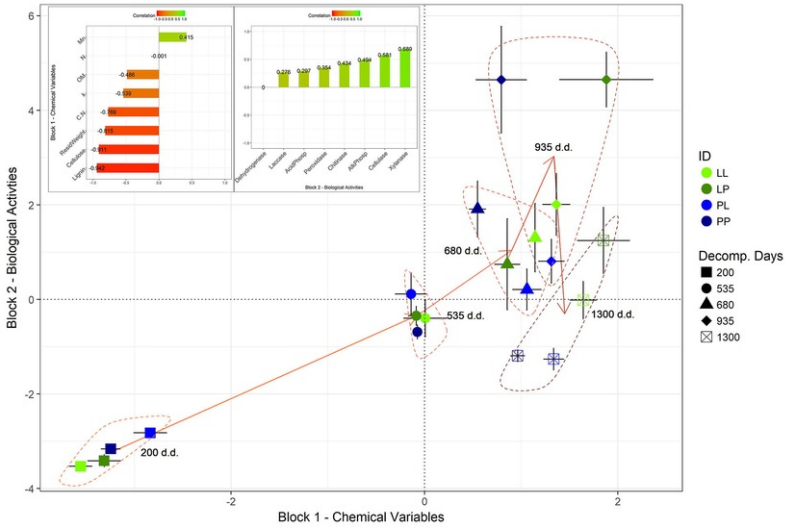

Fig. 3 Scatterplot of the mean scores (±standard error of the mean) for Axis 1 of the 2B-PLS. Block 1 (*x*-axis) represents chemical variables, while Block (*y*-axis) represents biological activities. Data are encircled according to decomposition days, while arrows are implemented to follow decomposition. Boxes within the graph represent correlations with Axis 1 for Block 1 (horizontal bars) and for Block 2 (vertical bars). Variables with same patterns of colors can be seen as positively correlated both within and between blocks, while opposite colors imply invers correlation both within and between blocks.

4.2 Chemical variables vs. pedofauna

Explained variance in Axis 1 for Block 1 (Chemical Variables) was 23%, while Block 2 (Pedofauna) explained 33% of total variance (Fig. 4). With the exception of the decomposition constant *k*, all variables in Block 1 (Chemical variables) were positively correlated, although residual Mn was clearly the driving variable. In Block 2 (Pedofauna), all variables were positively correlated, with particular importance of Collembola, that, as a consequence, were also positively correlated to residual Mn. In the scatterplot, a certain affinity between 535 and 1300 days emerged, compared to 935 days. Both at 535 and 1300 days, data from PP-LP were separated from LL-PL, corresponding to a low abundance of pedofauna and lower residual Mn but higher decomposition constant *k*. Conversely, a strong distinction was given for the data at 935 days. In this case, observations from Pradaccio (PP-LP) showed a sharp increase in both pedofauna, especially Collembola, along with residual Mn and lower decomposition constant *k*. As for observations from Laceno, PL was clearly separated from LL, the latter showing lower abundance of pedofauna compared to the former.

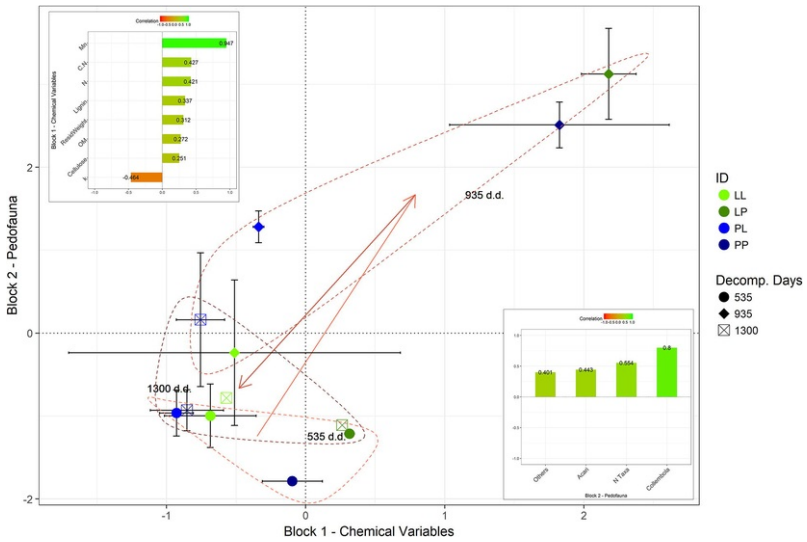

Fig. 4 Scatterplot of the mean scores (±standard error of the mean) for Axis 1 of the 2B-PLS. Block 1 (*x*-axis) represents chemical variables, while Block (*y*-axis) represents pedofauna. Data are encircled according to decomposition days, while arrows are implemented to follow decomposition. Boxes within the graph represent correlations with Axis 1 for Block 1 (horizontal bars) and for Block 2 (vertical bars). Variables with same patterns of colors can be seen as positively correlated both within and between blocks, while opposite colors imply inverse correlation both within and between blocks.

4.3 Biological activities vs. pedofauna

Block 1 (Biological activities) explained 46% of total variance in Axis 1, while Block 2 (Pedofauna) explained 40% of total variance (Fig. 5). In contrast with the first 2B-PLS, dehydrogenase was the most relevant variable in Block 1, followed by xylanase, cellulase and chitinase. Alkaline phosphomonoesterase had a weak inverse correlation with all other biological activities. Conversely, in similarity with second 2B-PLS, the variables for Block 2 were identical, with Collembola, followed by number of taxa, Acari and other taxa, explaining the variance. At 535 days, data were clearly separated according to location, with LL-PL separated from PP-LP. In line with previous results, 935 days showed the most important differences. Again, samples from Pradaccio (PP and LP) were remarkably separated from the others, showing high activity of dehydrogenase, xylanase, cellulase, and chitinase, corresponding to more abundance of Collembola and other pedofauna variables. Noticeably, PL data were separated from LL on Bock 2, showing lower abundance of pedofauna for the latter. Finally, at 1300 days, data reverted to values similar to 535 days but differences given by plant material were evident, as data from PL-PP were separated on Block 1 from LL-LP data.

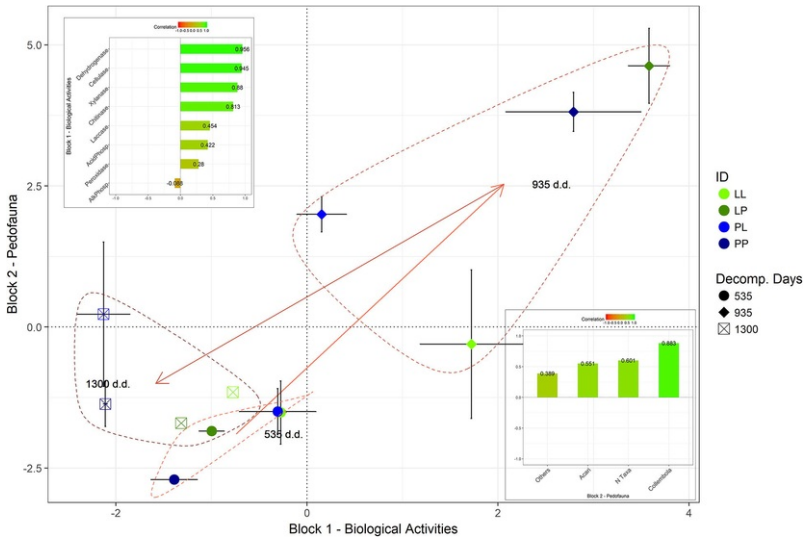

Fig. 5 Scatterplot of the mean scores (±standard error of the mean) for Axis 1 of the 2B-PLS. Block 1 (*x*-axis) represents biological activities, while Block (*y*-axis) represents pedofauna. Data are encircled according to decomposition days, while arrows are implemented to follow decomposition. Boxes within the graph represent correlations with Axis 1 for Block 1 (horizontal bars) and for Block 2 (vertical bars). Variables with same patterns of colors can be seen as positively correlated both within and between blocks, while opposite colors imply inverse correlation both within and between blocks.

5 Discussion

In line with previous research on the same forest locations, which have the same age, but are different in terms of parent material, pH, climate and several other factors (De Marco et al., 2016; Fioretto et al., 2018; Innangi et al., 2015b), we have found several differences both in terms of newly shed litter chemical composition and decomposition dynamics. Admittedly, beech leaves are known to be highly variable in terms of chemical composition and that a large number of factors concur to the chemistry of litter (Berg and McClaugherty, 2014).

Both beech litters showed a similar concentration of lignin and cellulose in the newly shed litter that was comparatively lower than some other studies on the same plant material, i.e. ~350 to 300 vs. 200 mg/g, respectively (Trum et al., 2015). Decomposition trends for cellulose and lignin were comparable, given their linkage in plant tissues and that access to cellulose, bound up in lignins and other plant constituents, happens only when these compounds are decomposed themselves (Kaspari et al., 2008). Admittedly, the decomposition of lignin in the initial stage seems contradictory to well-established decomposition models, yet it was recently shown that lignin degradation occurs particularly in the initial phase of litter decomposition and is hampered at later stages, when easily decomposable carbon sources decline (Klotzbücher et al., 2011).

The initial release of N up to 200 decomposition days can be explained by initial high microbial N demand along with leaching (Dise et al., 2009). Subsequently, an increase was observed, due to fungi's translocation of this element from the soil and/or from the more decomposed layers of litter, especially in Laceno's litter that was poorer in initial N (Cortez et al., 1996). As concerns Mn, its importance in litter decomposition has been widely recognized in the last years (Berg et al., 2013; Trum et al., 2015), given its relevance in enzymatic lignin decomposition operated almost exclusively by fungi (Schneider et al., 2012). Thus, we can assume that around 935 decomposition days there was a peak in fungi presence in all litterbags, which is consistent with PLFA concentration after 800 decomposition days recorded by Gavazov et al. (2014). In addition, the greater amount of Mn in LL-LP compared to PL-PP can be explained by a combination of factors. On the one hand, the higher Mn concentration in Pradaccio's newly shed litter can be explained by higher accumulation of Mn on acidic pH (Langenbruch et al., 2011). Yet, on the other hand, we detected greater accumulation of Mn in LL-LP because in Laceno's litter there is a deficiency of Mn that is mobilized from the environment (De Marco et al., 2016; Innangi et al., 2015b). Admittedly, Mn underwent higher immobilization in Pradaccio, under acidic pH, regardless of the original plant material.

As for the potential activities of extracellular enzymes, our results showed a large array of activities involved in the most important biogeochemical cycles, i.e. C (cellulase, xylanase, laccase, and peroxidase), N (chitinase), and P (acid and alkaline phosphomonoesterases), while dehydrogenase can be seen as an indicator of overall microbial biomass. In general terms, we have detected that most of the enzymes showed greater differences given by location rather than plant material. Thus, different trends could be detected for LL-PL compared to LP-PP, enforcing the idea that litter decomposition was regulated by local external factors rather than litter chemistry entering the forest floor (Andersson et al., 2004).

We have showed that in LL-PL the activities of both cellulase and xylanase showed two peaks at 535 and 935 days, a trend that was more evident in PL. Admittedly, the climate in Laceno has more affinities with a typical Mediterranean climate when compared to Pradaccio (Fioretto et al., 2018). Accordingly, in Mediterranean ecosystems, cellulase and xylanase have been showed to exhibit sharp seasonal patterns in their activities, peaking during autumn–winter (Fioretto et al., 2000). However, the peaks that we recorded did not match a precise pattern, being distributed between spring and summer. Peaks in cellulase and xylanase were recorded also for beech litter in a mountain ecosystem not far away from Laceno, although activities had the largest values in autumn and the smallest in spring (Papa et al., 2014a). Other studies showed cellulase peaks in late autumn (Kaiser et al., 2010) or no seasonal pattern at all (Papa et al., 2014b; Schneider et al., 2012). Conversely, in a colder site as Pradaccio, the activity of cellulase and xylanase peaked around two and a half years of decomposition, as shown in other studies on beech litter (Andersson et al., 2004).

The increase of laccase and peroxidase between 680 and 935 decomposition days was found in all litterbags. Noticeably, the peak was more prominent for peroxidase and was consistent with the increase in Mn amount. Thus, we can assume that the high peroxidase activity along with the Mn residual mass can be related to Mn-peroxidase activity, which is a key oxidative enzymes of litter degradation (Purahong et al., 2014).

The complex pattern of phosphatases that we have observed can be explained by a combination of factors. As a matter of fact, it must be taken into account that the soil from Pradaccio is richer in available phosphorus compared to Laceno (12.3 vs. 8.0 mg/kg, respectively) whereas newly shed litter follows an opposite trend (0.40 vs. 0.62 mg/g, respectively) (Innangi et al., 2015b). Thus, the different dynamics can be explained in terms of leaves poorer in P decomposing in soil richer in P and vice versa. In addition, the current techniques for enzyme assays are unable to discriminate between the contribution of phosphatases associated with active microbial cells and those linked to soil colloids (Nannipieri et al., 2011), which can explain the complexity in the pattern of these enzymes in our samples.

As for the activities of dehydrogenase and chitinase, these two enzymes can be interpreted as important indicators of biomasses. Accordingly, dehydrogenase is an excellent proxy of overall microbial activity (Innangi et al., 2017b), while chitinase is often interpreted as representative of active fungal biomass (Andersson et al., 2004; Papa et al., 2014a). Thus, both enzymes point out to higher microbial activity between 680 and 935 days and a convergence to very low values at 1300 days. The activity of chitinase was higher in Pradaccio regardless of leaf material, and this can be explained by the larger abundance of fungi on lower pH. Noticeably, at the beginning of the incubation (200 days) dehydrogenase was sensibly higher than chitinase, suggesting a colonization of the litterbags by more generalist microorganisms (Sanderman and Amundson, 2003).

We have recorded a relatively low diversity of soil fauna in our litterbags that can be explained by the general trend of beech forests to show a lack of species attributed to their vegetation history and secondary plant chemistry (Petrakis et al., 2011). Moreover, the high concentrations of polyphenols in beech litter reduces the palatability for soil fauna (Scheu, 1993). The dominance of Acari and Collembola in forest litter is a known phenomenon (Hättenschwiler et al., 2005; Menta et al., 2014; Sanderman and Amundson, 2003). Between the two forests, we have found a general lower abundance of Collembola in Pradaccio compared to Laceno, given their occurrence at lower densities in acid forest soils (Meier and Leuschner, 2010). Salamon, Scheu, & Schaefer (2008) reported that the diversity of Collembola increased with forest age, which likely reflects increased amount and diversity of food resources (e.g. microflora). Dominant species/functional groups of Collembola, such as hemiedaphic species, appear to depend predominantly on abiotic factors, in particular soil pH and water content (Salamon et al., 2008), highlighting the importance of site characteristics on litter microarthropod community. Moreover, changes in the amount and quality of food resources for Collembola and in abiotic factors linked to forest age probably transmit to higher trophic levels of the fungal food chain, sustaining higher predator populations, such as centipedes (Salamon et al., 2008). Potapov et al. (2013) found that trophic fractionation of ^{15}N of two collembolan species differed significantly in relation to which fungus they were fed on.

In our study, we have recorded a relative peak of Collembola at 935 decomposition days, when they became as abundant as Acari. It has been shown that Collembola do not directly change decomposition rate, but their activity resulted in greater availability of litter-derived C to the soil microbial community, a modification of soil organic matter at the molecular level and a direct transport of C in the litter-soil environment (Chamberlain et al., 2006). The strong link between fungal activity and Collembola was shown by finding labelled C in fungal fatty acid biomarkers and in Collembola, but not in Acari and Enchytraeidae (Högberg et al., 2010). In contrast, Asplund et al. (2018) reported that the exclusion of microarthropods from beech litterbags had no significant effect on either mass loss, N release in beech litter decomposition and fungal community compositions and biomass.

State of the art decomposition paradigms do not always offer a coherent view of the process because it can be hardly understood without a comprehensive analysis of interacting factors (García-Palacios et al., 2016). Some studies indicated that the relative control over litter C and N loss by biotic and abiotic factors can change dramatically during the process of decomposition (García-Palacios et al., 2016), while other research highlighted that mass loss could be explained by variations of selected molecular classes for a wide range of plant molecular composition and climates (Incerti et al., 2011). Conversely, climatic controls on litter decomposition were found to be quantitatively more important than species or site of origin on a large geographical and climatic gradient (Portillo-Estrada et al., 2016).

On such a complex phenomenon, our multivariate approach by means of Two-block Partial Least Squares allowed us to discriminate patterns of covariance between and within three different datasets, namely chemical variables, biological activities, and pedofauna. The observation of these patterns can help us interpret the decomposition process with a systemic approach. On the one hand, some key-variables maintain a coherent trend within and between blocks. Accordingly, there is a clear importance of cellulase/xylanase, Mn, and Collembola with a peak of all variables at 935 decomposition days. On the other hand, some variables reveal little to no-importance when co-varying with one block while exhibited relevance when confronted with another block. For instance, residual N appeared to have little importance when confronted with biological activities, but acquired greater significance when

compared with pedofauna. Similarly, dehydrogenase was irrelevant with chemical variables, but had a strong co-variation with soil fauna. At the same time, we have recorded an importance of plant material at the beginning (200 decomposition days) and in the later stage of decomposition (1300 days), while location was highly discriminating in the central stages, especially 935 days. Noticeably, such effect of location was particularly strong for Pradaccio, which had a lower pH and colder-wetter climate compared to Laceno (Fioretto et al., 2018).

We conclude that, according to our results, the decomposition process of beech litter in Mediterranean area was driven by litter quality at the beginning and in the later stages of decomposition, while edaphic and climatic factors were implied in the central steps, with a dramatic change of scenario around 2.5 years. Decomposition was driven by simultaneous and interacting changes in chemical variables, extracellular enzyme activities, and soil fauna, with a significant role of lignocellulosic components and enzymes involved in their degradation, Mn residual weight, and abundance of Collembola.

6 Author contributions

AF originally formulated the idea, MI, CM, SP, FD, and AF conducted fieldwork, MI, TD, and FD performed chemical and biological analyses, CM and SP performed soil fauna analyses, MI performed statistical analyses, and MI, CM, and AF wrote the manuscript.

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Highlights

- Litter decomposition is a complex ecosystem process regulated by many factors.
- We studied chemical, biological and soil fauna variables during beech decomposition.
- Two-block Partial Least Squares was used to apply a systemic approach.
- Decomposition was driven by litter quality at the beginning and in the later stages.
- Interacting changes in chemical variables, enzymes, and soil fauna were shown.

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