Microbial communities and activities of forest floor fractions in degraded pine forests after thinning treatments

Alessandra Lagomarsino[1], Alessandro Elio Agnelli[1], Gianluigi Mazza[2], Ugo Chiavetta[2], Caterina Bartoli[3], Carlo Viti[3], Roberta Pastorelli[1]

[1]*Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria (CREA) Research Center Agriculture and Environment (AA) Via di Lanciola, 12/A 50125 Firenze, Italy*

[2] *Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria (CREA) Research Center for Forestry and Wood (FL). Viale Santa Margherita 80 52100 Arezzo, Italy*

alessandra.lagomarsino@crea.gov.it

[3] *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Università di Firenze. Piazzale delle Cascine, 18 50144 Firenze, Italy*

Abstract

Summary

Litter decomposition is a key process of biogeochemical cycling in forest ecosystems, which has an impact on biodiversity, nutrient availability, green-house gas emissions, soil organic matter and carbon sequestration. The objective ot the study was the evaluation of forest floor composition at three decomposition stage, considering: i) C and N amount; ii) the abundance and composition of microbial communities (bacteria and fungi); iii) the hydrolytic enzyme activities; iv) the CO_2 emissions. We hypothesized changes of structural and functional diversity during the decomposition processes, affecting in turn carbon and nitrogen cycling.

The study has been conducted in a degraded peri-urban pine forest of central Italy in the frame of the FoResMit LIFe project. Forest floor has been collected under three management regimes: i) traditional thinning, ii) selective thinning and iii) no thinning. Leaf (L), fragmented (F) and humified (H) litter fractions have been separated considering the different decomposition stage.

A significant decrease of carbon content and C/N ratio has been observed along decomposition process. This matched with changes in bacterial community composition, as well as with the increase of fungal richness and diversity, and enzyme activity. Forest floor contributed between 18 and 37% of total CO_2 efflux from soil, on average. Thinning increased forest floor pool without affecting microbial composition, diversity and activity.

Material and methods

The study area is the peri-urban forest of Monte Morello (43°51′20″N; 11°14′23″E), located in the Sesto Fiorentino municipality and close to the urban area of Florence in Tuscany Region. This forest is the result of the

reforestations activities realized from 1909 to 1980; specifically experimental plots are 50 to 60 years old. The main tree species used are *Pinus nigra* J.F.Arnold, *Pinus brutia* Ten. subsp. brutia, *Cupressus* sempervirens, *Fraxinus ornus* L., *Quercus cerris* L. and *Quercus pubescens* L.. After reforestation the stands have been abandoned with consequences on trees stability, mortality, absence of regeneration, marked susceptibility to adversities and increase of fire risk (Cenni et al., 1998). Between September and December 2016, three silvicultural treatments have been tested in replicated plots of 1.5 ha approx. with the objective of restoring the ecological stability and enhance the resistance and resilience of the forest: traditional (negative selection) and selective (positive selection) thinning, *plus* three control plots without intervention (Paletto et al., 2017).

Forest floor has been collected in January 2017, after thinning operations, by pressing a 600-700 cm^2 steel sheet sampling frame 10 cm deep (or similar) into the forest floor and collecting all litter material above the soil. After sampling, forest floor has been separated into three components: leaf – L (fresh or slightly discoloured, with no or weak breaking up, material), fragmented – F (medium to strongly fragmented material with many mycelia and thin roots), humified – H (completely decomposed amorphous material). Samples have been dried and homogenized at 0.5 mm with a cut-mill for carbon and nitrogen content determination with an elemental analyzer (Flash 2000, Thermo Fisher).

CO_2 emissions were measured in two replicates for each of the 9 plots with a portable IRGA (EGM4, PP system) from collars with a without forest floor from April to December 2017.

Enzyme activity was measured according to the methods of Marx et al. (2001) and Vepsäläinen et al. (2001), based on the use of fluorogenic methylumbelliferyl (MUF)-substrates. Soil was analysed for N-acetyl-β-glucosaminidase, β-glucosidase, butyrate esterase, acid phosphatase, arylsulphatase, β-xylosidase, cellulose and acetate esterase activity.

The composition and structure of the bacterial and fungal communities were estimated by PCR-DGGE exploiting the 16S rDNA of bacteria and 18S rDNA of fungi. Bacterial and fungal community diversity was characterized through two indicators: richness (R, number of bands) and Shannon-Weiner index (H).

nMDS was used to represent the similarity distance between each DGGE profile in a two-dimensional space.

Results and discussion

The three components of forest floor showed well distinguished characteristics, with a decrease of C content along the litter decompositions level (Table 1). A correspondent increase of N % determined a decrease of C/N ratio. C is lost first during decomposition process and the concentration of N increases consequently. This pattern occurred independent of treatments and remained stable along time. Differently, both thinning treatments increased the amount of forest floor (significantly for L and F fractions).

	L				F				H			
	biomass kg m^{-2}	N %	C %	C/N ratio	biomass kg m^{-2}	N %	C %	C/N ratio	biomass kg m^{-2}	N %	C %	C/N ratio
control	0.32[a]	0.77	46	64	1.42 [a]	0.97	36	48	0.96	1.14 [a]	26	23
traditional	0.68[b]	0.82	45	60	2.19 [b]	1.00	36	56	1.34	1.24 [a]	27	22
selective	0.43[b]	0.75	46	64	1.93 [b]	1.12	40	57	1.17	1.39 [a]	29	21

Table 1. Biomass, C and N percentage and C/N ratio of forest floor

Forest floor contribution to CO_2 emissions was highly variable (between 2 and 72 %) and did not follow temperature trend. A lower contribution, on average, was observed in thinned plots.

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
control	4	48	37	24	26	39	21	25	63	37
traditional	12	21	23	30	17	19	4	6	20	18
selective	7	19	18	33	25	23	5	12	42	21

Table 2. Percentage contribution of forest floor to CO_2 emissions from soil

Enzyme activities and functional diversity increased along decomposition stage, whereas non significant differences due to thinning treatments were observed. Among enzymes, arylsulphatase was the most represented in H fraction (80 %).

	L		F		H	
	Enzyme activity	Simpson-Yule index	Enzyme activity	Simpson-Yule index	Enzyme activity	Simpson-Yule index
control	50.81	6.63	54.29	7.10	59.81	7.42
traditional	46.11	6.62	57.68	7.06	51.16	7.47
selective	42.72	6.49	50.54	6.95	56.08	7.39

Table 3. Enzyme activity (mean of 8 enzymes) and functional diversity in the forest floor

Bacterial communities did not change significantly in abundance and diversity, whereas the different decomposition stage alters significantly their composition. Differently, fungal communities richness and diversity increased significantly

along decomposition process, confirming results of arylsulphatase. Thinning treatments did not affect microbial communities composition and diversity.

	Bacteria						Fungi					
	R			H			R			H		
	L	F	H	L	F	H	L	F	H	L	F	H
control	23.7	20.7	20.3	3.13	2.91	2.90	8.3^{c}	11.3^{bc}	14.7^{abc}	2.08^{b}	2.38^{ab}	2.64^{a}
traditional	24.3	17.3	27.0	3.14	2.83	3.25	8.3^{bc}	12.3^{abc}	18.7^{a}	2.10^{b}	2.48^{ab}	2.87^{a}
selective	17.0	22.7	19.7	2.75	3.09	2.87	11.0^{bc}	12.7^{abc}	15.7^{ab}	2.37^{ab}	2.47^{ab}	2.69^{a}

Table 4. Richness (R) and Shannon index (H) calculated on bacterial and fungal DGGE profiles.

Overall, decomposition process was characterized by changes in chemical and microbiological composition, driving higher enzyme activities in more decomposed fractions.

Thinning reduced microbial respiration, possibly as a consequence of the enrichment of fresh, low decomposed, organic matter (L and F litter fractions), without affecting microbial diversity and composition.

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Keywords: pine forest management, forest floor decomposition, CO_2 emissions, enzymes, microbial diversity

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