Which criteria should be considered when appraising ectomycorrhizal communities for forest research?

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Forests host a large part of terrestrial biodiversity and provide a wide range of ecosystem services; they regulate local, regional and global climate, store carbon, purify air and freshwater. Plants and their networks with associated ectomycorrhizal fungi play a crucial role in biogeochemical cycles, biodiversity, climate stability and economic growth. Due to the complexity of the natural environment, the evaluation of any type of change is often very difficult, since it may not be clear which environmental component will be affected by the stressor, what type of change will occur and what the exposure will be.

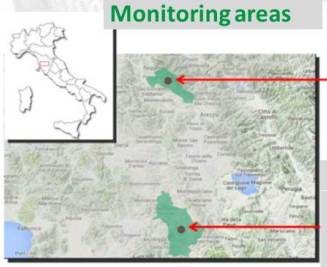
SelpiBioLife project was established to evaluate the effects of an innovative silvicultural treatment on different biological groups (flora, fungi, bacteria, carabids, nematods and microarthropods) in *Pinus nigra* plantations in order to analyze management effects.

For this project the Before-After-Control-Impact (BACI) design was applied. This was to overcome the problem of attributing changes to an impact rather than natural variability.

Aim of the work:

demonstrate whether the sampling criteria were appropriate to describe exhaustively the composition of ectomycorrhizal communities BEFORE treatment in the BACI design.

Monitoring areas



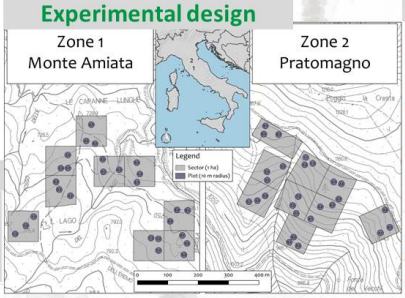
Zone 2

"Pratomagno-Valdarno"- «Pian della cucina» Municipality: Loro Ciuffenna (AR) PRATOMAGNO

Zone 1

«Madonna delle Querce» - «Il Lago» Municipality: Castiglione d'Orcia (SI) AMIATA

Experimental design



Materials and methods

Analysis of ecto-mycorrhizal fruiting body (EMFb) community: diversity and abundance of ectomycorrhizal fruiting bodies (EMFb) were determined using mycocoenological approach.

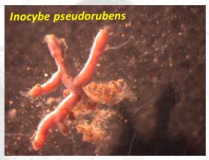
Analysis of ecto-mycorrhizal root tips (EMRt) community: soil sampling was set up to test ectomycorrhizal root tips (EMRt) community. Morphotypes were recognized and molecularly identified by means of a direct PCR approach.

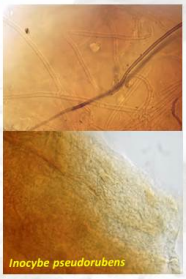
Statistical analysis: species richness and composition as well as the effect of spatial scale on EMRt and EMFb communities were assessed using rarefaction technique, permutation tests for multivariate analysis of variance (PERMANOVA), Non-metric multidimensional scaling (NMDS), Mantel's tests and similarity Percentage Analysis (SIMPER).

Results

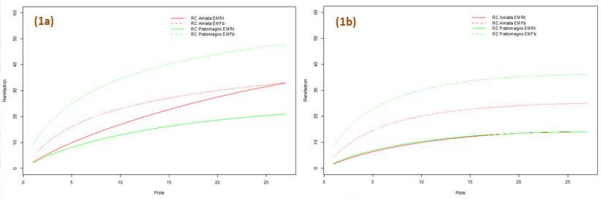
EMFb and EMRt diversity

	Total	Zone 1 - Amiata	Zone 2 - Pratomagno
Number of plot	54	27	27
EMFb richness	70	33	48
Number of EMFb	2527	1250	1277
EMRt richness	54	26	28
Number of EMRt	2946	1717	1229





Rarefaction curves **(1a)** supported the lowest species richness in EMRt respect to EMFb community. However, while EMRt in Pratomagno showed an asymptote, Amiata reached no saturation indicating that the total number of species likely exceeded the number sampled. Concerning patterns inside EMFb, Amiata increased less steeply respect to Pratomagno, which resulted in the highest estimates of richness at a given extent.



Sample-based rarefaction curves of fungal communities for Zone 1 and Zone 2 (1a) and excluding of sporadic species (1b)

Excluding sporadic species **(1b)**, the slope of all rarefaction curves became nearly asymptotic, supporting the efficiency of sampling design. Furthermore, a complete overlay of EMRt rarefaction curves belonging to different Sites was found, showing a comparable pattern in cumulative species richness between study areas.

PERMANOVA revealed that Zone, Plot and Type factors significantly affected community composition as well as the interactions Zone x Type and Plot x Type. The Pair-wise Test showed significant differences between EMRt and EMFb for each Zone. The exclusion of sporadic species showed comparable results. **NMDS** confirmed this trend, showing a clear separation between fungal communities in terms of species composition. Furthermore, **Mantel's test** resulted in no correlation in distance matrices of community structure. The **SIMPER** indicated that the average dissimilarity between EMRt and EMFb communities was relevant in both Zones, showing a higher value in Zone 1 (Amiata average dissimilarity 96.25%; Pratomagno average dissimilarity 88.22%). In Amiata, *L. sanguifluus, P. niger, C. geophilum. T. psammopus* and *S. luteus* contributed the most to within-group similarity and between-group dissimilarities, while in Pratomagno, *I. geophylla, C. rutilus, R. xerampelina, S. granulatus* and *T. portentosum* made a higher contribution to average dissimilarity between EMRt and EMFb communities.

Average density of several prominent taxa in EMRt and EMFb communities of Zone 1 and Zone 2, including SIMPER results for contributions from the most important taxa towards the Jaccard dissimilarity distinguishing these two groups without sporadic species

	Taxon	Av. density EMRt	Av. density EMFb	Av. diss	Diss/SD	Contrib%
Zone 1 Amiata	*Lactarius sanguifluus*	0.33	0.26	9.32	0.57	9.65
	Phellodon niger	0.00	0.41	6.13	0.72	6.35
	Cenococcum geophilum	0.30	0.00	5.85	0.54	6.06
	Tricholoma psammopus	0.00	0.33	5.34	0.47	5.53
	Suillus luteus	0.07	0.30	5.59	0.60	5.80
Zone 2 Pratomagno	*Inocybe geophylla*	0.00	0.70	6.90	1.22	7.82
	Chroogomphus rutilus	0.00	0.70	6.83	1.37	7.74
	Russula xerampelina	0.48	0.93	6.21	0.80	7.04
	Suillus granulatus	0.00	0.56	6.12	0.95	6.94
	Tricholoma portentosum	0.07	0.48	4.49	0.86	5.09

Av average, Av diss average dissimilarity contribution of the most important taxa, Diss/SD dissimilarity divided by the standard deviation of the contribution of each taxa across all pairs of samples, Contrib% percentage of contribution of each taxa to the total of 96.25%

PERMANOVA results on the whole presence/absence dataset

Source of variation	*df*	MS	F
Zone	1	30,797	8.5637*
Type	1	29,519	8.3906*
Plot	16	3596.2	1.4652*
Zone x Type	1	15,583	4.4296*
Plot x Type	16	3,518.1	1.4334*
Residual	72	2,454.4	
Total	107		

*P<0.001

Results of PERMANOVA pairwise test for Amiata and Pratomagno Zones for each pair of levels of factor 'Type'

Type	Amiata	Pratomagno
	t	*t*
EMRt, EMFb	2.1459**	2.8237*

*P<0.001; **P<0.05

PERMANOVA results on the whole presence/absence dataset excluding of sporadic species

Source of variation	*df*	MS	F
Zone	1	33,409	9.7458*
Type	1	33,970	10.305 *
Plot	16	3,428	1.5868 *
Zone x Type	1	15,301	4.6416*
Plot x Type	16	3,296.5	1.5259*
Residual	72	2,160.4	
Total	107		

*P<0.001

Results of PERMANOVA pairwise test for Amiata and Pratomagno zones for each pair of levels of factor 'Type' excluding of sporadic species

Type	Amiata	Pratomagno
	t	*t*
EMRt, EMFb	2.4087**	2.9735*

*P<0.001; **P<0.05

In conclusion, these results suggest that the adopted sampling criteria are appropriate to exhaustively and quickly appraise the composition of ectomycorrhizal communities for forest research