A method to evaluate ectomycorrhizal fungus richness in black truffled trees

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INTRODUCTION

Tree´s ectomycorrhizal community is one of the factors that determines the success of truffles production. Specific sampling effort that must be done to study it remains uncertain in truffle plantations.

Figure 1.– An example of a soil core (20 x 3,2cm) extracted.

MATERIAL AND METHODS

A sampling in depth of nine evergreen oaks (*Quercus ilex*) from different plantations sited in Teruel province was carried out, with the aim of developing a sampling method using soil cores (**Fig. 1 and Fig. 2**) that assess reliably the species richness of each truffled tree. Three different categories of age (less than 6-years-old, 6-9-years-old and more than 9-years-old) and two of black truffle production (productive and non productive) were chosen (**Table 1**). In every tree 12-24 soil cores were taken, in order to cover the whole root influence area. Ectomycorrhizal morphotypes found in each soil core were differentiated by its anato-morphological characters and sequenced, sampling sequence was randomized (EstimateS program) and species richness was estimated by Clench model (Species accumulation program). Observation of the 70% of predicted species richness was used as an indicator of validity of the estimation done (Jiménez-Valverde & Hortal, 2003).

Figure 2.– Tree 7 after sampling.

Table 1.– Experimental design and results of the species richness study done on each tree.

Tree	Truffle producer	Age group	Samples taken each tree	ECM fungus richness		% observed	Samples to reach 70% of estimated richness	Sampling effort needed to add one new species
				Observed	Estimated			
1	Yes	<6 years	12	1	1,0	100,0%	1	∞
2	No			3	4,5	66,0%	Almost reached	∞
3	No			2	2,9	69,0%	Almost reached	11
4	Yes	6-9 years	24	7	9,1	77,3%	20	7
5	No			15	32,0	46,9%	Not reached	2
6	No			1	1,0	100,0%	1	∞
7	Yes	>9 years	24	3	4,7	64,4%	Almost reached	12
8	No			12	14,8	**81,1%**	14	7
9	No			12	12,1	**98,8%**	4	10

RESULTS AND DISCUSSION

Observed number of species per tree was from one to 15, with a strong dependency on the productive character and fewer on the age of the host tree (**Table 1**). Species accumulation curves (**Fig. 3,** see also **Fig. 4**) were stabilized quickly in asymptotes in three trees (number 1, 2 and 6), or approached them in five ones (numbers 3, 4, 7, 8 and 9) even when the observed number of species was high. Samples taken were not enough only for just one of them (tree number 5), but the increase of sampling effort that is needed is so much in trees containing a big number of rare species like this one.

CONCLUSIONS

A **correct estimation** of species richness in truffled trees can be done using this method. **The same number** of soil cores for each tree per age (12 or 24) must be taken.
The study of the cores one to one with a **gradual and progressive statistical analysis** of data is recommended in order to reduce ectomycorrhizae analysis effort

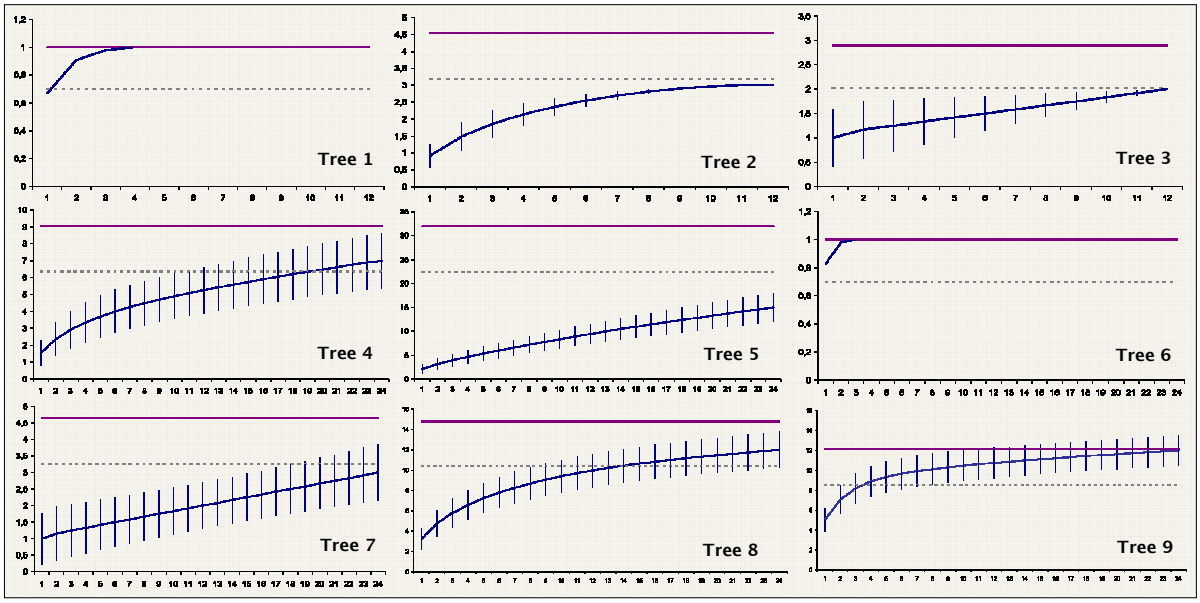

Figure 3.– Species accumulation curves obtained for each tree: ——— Estimated number of species (Clench model): ———
70% of estimated number of species (validated sampling): ····· X-axis: Sampling effort; Y-axis: Species detection

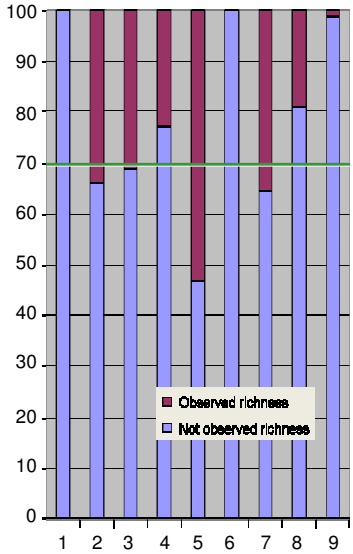

Figure 4.– Comparative observed *vs* estimated richness on each tree. 70% is the value chosen to validate estimated richness.

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