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Research paper

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"Second generation of planted hardwood forests in the EU"

Sprouting suppression and mushroom production after inoculation of *Juglans x intermedia* stumps with edible fungi species.

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Abstract - Removal of stumps and suppression of sprouts after harvesting by conventional methods, such as using heavy machinery or herbicides, alters the physico-chemical characteristics of soil, may cause environmental damage and can be very costly. In this study, the performance of inoculation with edible fungi as a biological alternative for stump degradation, has been examined in walnut plantations of five Spanish provinces. Stumps were inoculated with two species of edible fungi: *Pleurotus ostreatus* (Jacq. Ex Fr.) P. Kumm and *Lentinula edodes* (Berk) Pené. Compared with untreated controls, the two biological treatments resulted in a significant and evident reduction of the sprouting probability, which was stronger than the result obtained with chemical treatments. Inoculated stumps also produced edible sporocarps, averaging 15.58 g per stump during the first year. This article constitutes the basis for the development of a sustainable, environmentally friendly and cost-effective product, which is a bioeconomy-based solution for stump degradation in intensive plantations.

Keywords - stump degradation, saproxylic species, logistic models, walnut, nature-based solutions.

Introduction

After harvesting a tree plantation, a large amount of woody debris remains in place, mostly stumps and medium/small-sized branches. This is regarded as a nuisance by forest managers, because removing wood debris and stumps is costly. If the branches and stumps are left untouched in place, naturally occurring decay processes by rot fungi may take a long time, typically more than 25 years (Onofre et al. 2001). It is even worse for sprouting broadleaf tree species, as these stumps can easily become trees again (Hamberg and Hantula 2018). Coppice forests are based on this sprouting ability either from roots or from stumps, *e.g.* holm oak forests managed for firewood production. However, if the main objective is producing timber, managers usually have to clear stumps and other debris to efficiently start a new production cycle. In addition, in conifer forest regeneration areas, sprouting of spontaneous broadleaf species (like birch, rowan or aspen) can compete with conifer seedlings (Hamber et al. 2011, Harding and Raizada 2015, Hershenhorn et al. 2016).

Detached branches from logged trees can be shredded and used to produce compost or biomass that will generate energy, thus yielding some economic revenue. Alternatively, branches can be shredded *in situ* and left for natural decay, which will enrich the soil in organic matter. While the latter would not generate a direct economic revenue, it may have an impact on the growth and health of trees planted in future cycles (Franklin et al. 1997).

Managing the stumps requires a different approach, because they are frequently removed to facilitate the establishment of the next rotation. Stump removal using heavy machinery is expensive (Coder 2003, Andrade et al. 2012). As an example, the cost of mechanical stump removal in a *Eucalyptus* plantation in its third rotation could be US $1,400.00 per hectare (Pavan et al. 2010), and annual cost for extraction of stumps from a hectare of broadleaf forest in Spain could vary from 772.11 to 1282.5 € ha^{-1} (TRAGSA 2019). In addition, this treatment can result in deleterious ecological effects such as alteration of soil structure and litter disturbance (Coder 2003). This may cause a reduction in soil productivity by depleting soil nitrogen, carbon and sulphur availability, although possibly increasing phosphorous availability (Hope 2007), with up to 10 years needed to restore the original soil condition.

The use of chemical and biological approaches

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has been proposed as an alternative to mechanical stump removal. Chemical herbicides have been commonly used to avoid stump sprouts and root suckers, but their use is heavily restricted. This situation encourages the development of environmentally friendly methods for preventing stumps from sprouting and for accelerating their decay (Green 2003). Biological stump degradation has been proposed as an alternative solution. This technique consists of inoculating stumps with mycelium or spores from wood decay fungi. Biochemical activity of fungal enzymes degrades wood and prevents or reduces sprouting (Dumas et al. 1997, Becker et al. 2005, Bellgard et al. 2014), resulting in the improvement of physic-chemical characteristics of the soil and nutrient enrichment, while increasing the biodiversity and richness of microorganisms (Tian et al. 2010).

Species of wood-decay fungi occur naturally in temperate forests, but they can be locally scarce. Fungi spores or mycelium need to reach the stump and colonise it, which can take years to happen (Becker et al. 2005). For this reason, the process of stump decay can be unreliable and often take too long if left to develop naturally. Stump degradation can be accelerated by controlled inoculations, with either a single species or a mix of species with complementary wood-decay features. By placing actively growing mycelium over the surface of a stump, the colonization is largely guaranteed and the process is accelerated. Previous experiences have demonstrated the efficiency of this approach, mostly focused on sprouting suppression, being *Chondrostereum purpureum* the most extensively used species (Lygis et al. 2012, Hamberg et al. 2011, 2015, 2016 and 2018). Other species used include *Pycnoporus sanguineus* (Andrade et al. 2012), *Phellinus gilvus* (Da Silva et al. 2010), *Laetiporus sulphureus* or *Trametes versicolor* (Alonso et al. 2007). The list is surprisingly short considering the high number of wood decay species that can be found in nature (Buée et al. 2009). It is also surprising the lack of studies focused on edible species, while many wood decay fungi (e.g. *Pleurotus ostreatus*, *Lentinula edodes*, *Flammulina velutipes*) produce edible mushrooms which could be harvested and generate income as non-wood forest products.

In this study, we benefit from previously described methods for stump inoculation with wood decay fungi, exploring novel applications resulting in synergistic outcomes on wood decay, sprout suppression and production of edible fungi. Specifically, the effect of stump inoculation with edible wood decay fungi in walnut plantations (*Juglans x intermedia Carr.*) was assessed and compared to natural degradation and to an alternative chemical treatment. The aim of this study was to provide useful information for sustainable forest management, by using a nature-based approach for stump degradation while also generating a product, i.e. mushrooms, that could contribute to profitability.

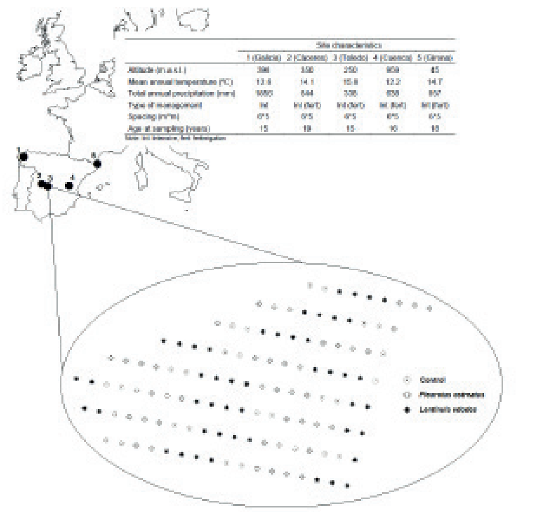

Figure 1 - Location of different walnut plantations.

Materials and methods

Experimental design and data collection

The study was carried out in five plantations, owned by Bosques Naturales company, across Spain (Fig. 1). In each plantation, walnut trees were harvested resulting a total of 910 stumps (120 in Cuenca, 120 to Cáceres, 130 in Girona, 120 in Toledo and 420 in Lugo).

Two stump degradation treatments were applied: a chemical treatment (Q) in 144 stumps, by using herbicides [Roundup], and a biological treatment (B) with two species, B1 with *Pleurotus ostreatus* (Jacq.) P. Kumm. in 363 stumps, and B2 with *Lentinula edodes* (Berk.) Pegler. in 168 stumps. In addition, an untreated control was also considered in 235 stumps.

The two fungal species used in this study were white-rot decay fungi of the *Basidiomycota phylum*, within the Agaricales order. *Pleurotus ostreatus* is one of the most cultivated species in the world (Sánchez 2010). It grows naturally on the surface of stumps and trunks of softwoods such as poplar, beech or willow. Also known as oyster mushroom, it has also been used industrially for mycoremediation purposes. *Lentinula edodes* is an emerging cultivated species, known and appreciated in Asia for centuries. Also known as shiitake, it grows in group, on the wood in decomposition of deciduous trees, as the shii, chestnut, oak, maple, beech, poplar, hornbeams and blackberries. Those species were chosen due to their properties among wood-decay

Table 1 - Transect types and treatments applied by site.

Site	Transect type	Number of transects	Treatment
Lugo	2, 3	42	*Pleurotus ostreatus* biological treatment
			Lentinula edodes biological treatment
			Chemical treatment
			Control
Girona	2	13	*Pleurotus ostreatus* biological treatment
			Chemical treatment
			Control
Cuenca	1	12	*Pleurotus ostreatus* biological treatment
			Control
Cáceres	1	12	*Pleurotus ostreatus* biological treatment
			Control
Toledo	3	12	*Pleurotus ostreatus* biological treatment
			Lentinula edodes biological treatment
			Control

fungal species, also known as lignicolous fungi, and the production of edible fruiting bodies.

Commercial mycelium of both species was obtained from a spawn company. The ability of the strains to grow on walnut wood was assessed in a laboratory trial. It consisted of preparing wood shavings, which were soaked, drained, sieved and mixed with cereal oat bran, which is a common practice in commercial mushroom production to supplement wood based substrates with some source of nitrogen and other nutrients. The mix, together with lime powder was sterilised in an autoclave at 121ºC for two hours. Then, for each of the two assayed species (*Pleurotus* and *Lentinula*), 24 substrate packages (1-kilogram wet weight) were inoculated with 50 grams of mycelium under sterile conditions. The ratio between dry and drained wet wood shavings was 1:4. To test the ability of each fungal strain on walnut wood, a trial was set to grow the mycelium under close to natural conditions. Packages with inoculated walnut wood shavings were incubated for three months at 20ºC and then transferred to a fructification room, were temperature was moderate (16ºC) and humidity high (>70%). The plastic bags, in which the substrate was incubated, were removed and the contents were placed on racks over water containers. Mushroom fructification started readily after the mycelium was exposed to low CO_2 concentration.

After the laboratory trial showed the suitability of the commercial inoculum, we applied the treatment to the stumps in the field. A cross-sectional disc (5-10 cm thick) was cut in each stump using a chainsaw and the mycelium was inoculated between the stump and the cross-sectional discs. Finally, the stump was covered with a biodegradable plastic bag and soil to protect the inoculated stump from external conditions.

The stump degradation treatments were applied to rows of 10 aligned stumps forming a transect. Within each transect, treatments were applied to contiguous groups of stumps, hereafter referred to as blocks. There were three different types transects, depending on the sequence of treatments applied (Fig. 2). Transect type 1 consisted of four stumps inoculated with *Pleurotus* and six controls. Transect type 2 consisted of four stumps inoculated with *Pleurotus*, four with the chemical treatment and two controls. Transect type 3 consisted of four stumps inoculated with *Pleurotus*, four stumps inoculated with *Lentinula*, and two controls. Not all transect types were installed in all sites. Type 1 was installed in Cáceres and Cuenca, type 2 in Lugo and Girona and type 3 in Lugo and Toledo. The resulting design is shown in Tab. 1.

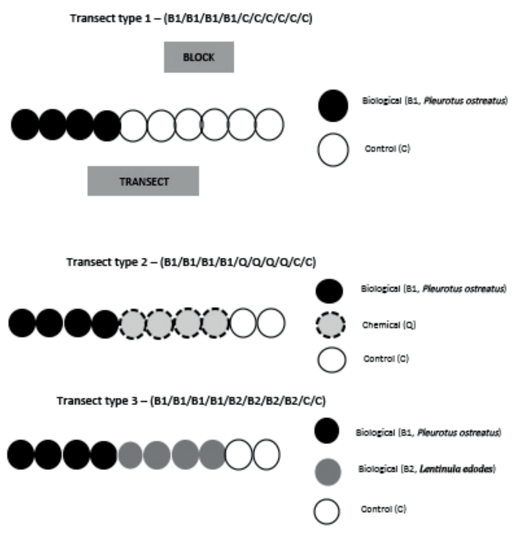

Figure 2 - Transect types considered in the study.

Table 2 - Logistic regression models used in the analysis.

Model	Equation
1	$log(\omega) = log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 I_{CU} + \beta_2 I_{LU} + \beta_3 I_{GE} + \beta_4 I_{TO} + \beta_5 I_{B1}$
2	$log(\omega) = log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 I_{GE} + \beta_2 I_{B1} + \beta_3 I_{Chemical}$
3	$log(\omega) = log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 I_{TO} + \beta_2 I_{B1} + \beta_3 I_{B2}$

Note: ω is the odd of sprouting, $\omega = \varrho/(1 - \varrho)$, and p is the probability of sprouting. Dummy variables represent treatments and sites, where: ICU is 1 if the stump belongs to Cuenca. ILU is 1 if the stump belongs to Lugo. IGE is 1 if the stump belongs to Girona. ITO is 1 if the stump belongs to Toledo. IB1 is 1 if the stump was treated with *Pleurotus ostreatus*. IChemical is 1 if the stump was treated with chemical. IB2 is 1 if the stump was treated with *Lentinula edodes*.

For each stump, quantitative and qualitative characteristics were recorded to assess the stump degradation efficiency after applying the treatments several months later. Data on whether the stump sprouted was collected. For those that sprouted, the viability of the sprouts (0 not viable, 1 viable) was recorded and the proportion of the area of the stump covered by sprouts was estimated (0 for 0% coverage, 1 for 25%, 2 for 50%, 3 for 75% and 4 for 100%) in order to know their origin and distribution, what could be related to the efficiency of the treatment. The number of all sprouts and their height (in meters) were also recorded. Treatment quality indicated the state of treatment and was measured by observing the success of inoculation, whether protection and initial conditions were maintained and observing the presence of damage. It was categorized by 0 for bad quality, 1 for ordinary and 2 for a good quality treatment.

In addition, in the stumps that received the biological treatment in Lugo, Toledo and Girona, the number of fruiting mushrooms and their dry weight in each stump was recorded during the fruiting session (weekly for 10 weeks from October to December in 2018).

Statistical analysis

First, descriptive statistics were computed to summarize the main characteristics of the sprout degradation capacity, such as the proportion of stumps sprouting and their viability, the mean height of sprouts and the mean number of sprouts, and the main characteristics of mushroom production, including mean number of sporocarps and mean weight.

Logistic regression models were fitted to estimate the probability of sprouting as a function of the treatments. Because the details of the design, i.e., the stumps were clustered into blocks and transects, different model structures and random effects configurations were tested: transect (E1), transect and block within transect (E2) and block within transect (E3). The final models were chosen based on the best statistical performance (based on the AIC criterion [Akaike information criterion]) with a reasonable biological interpretation. Since the experimental design was unbalanced and not all treatments were applied in all sites, three logistic models were fitted to: a) compare the effect of the B1 (biological treatment with *Pleurotus ostreatus*) treatment vs control in all 5 sites (Tab. 2, Model 1); b) compare the effect of the B1, Q (chemical treatment) and control treatments in Lugo and Girona (Tab. 2, Model 2) and c) compare the effect of the B1 (biological treatment with *Pleurotus ostreatus*), B2 (biological treatment with *Lentinula edodes*) and control treatments in Toledo and Lugo (Tab. 2, Model 3). All the models include two covariates, a factor for treatment and a factor for site effects, to account for differences in sprouting among sites. Site was considered as a fixed effect because there was a relatively small number of sites, which makes it impossible to estimate a variance component for exact location (Crawley 2002) and to avoid fitting a three-level hierarchical models (as transects and blocks are nested within site).

The probabilities of sprouting in stumps by treatments were calculated with the formula where was the odds estimated from the logistic models and *p* was the probability. Since all stumps with chemical treatment in Lugo resprouted, the effect of the treatment on the regrowth was estimated separately in Girona, due to the singularity of the logistic model. All the statistical analyses were performed in RStudio, with package “lme4”, (R version 3.5.3, RStudio version 1.1.463).

Table 3 - Number of sampled stumps with sprouts by treatment and site.

Site	Treatment	Re-sprouts		Total
		0	1	
Cáceres		24	96	120
	B1	11	37	48
	C	13	59	72
Cuenca		3	117	120
	B1	3	45	48
	C	0	72	72
Lugo		150	270	420
	B1	95	73	168
	B2	52	68	120
	C	3	82	85
	Q	0	47	47
Girona		23	107	130
	B1	8	43	51
	C	1	23	24
	Q	14	41	55
Toledo		15	105	120
	B1	12	36	48
	B2	3	45	48
	C	0	24	24

Note: B1 is the *Pleurotus ostreatus* treatment, B2 is the *Lentinula edodes* treatment, Q is the chemical treatment, C is control treatment.

Results

Descriptive statistics

Out of the 910 stumps analysed in the study 24% did not sprout. The proportion of stumps that did not sprout was 35% for the B1 treatment (biological treatment with *Pleurotus ostreatus*), 33% for the B2 (biological treatment with *Lentinula edodes*), 14% for chemical treatment, and 6% for Control (Tab. 3). Lugo was the site with highest percentage of non-sprouting stumps (35%). The quality of the treatment was good in 85% of the treatments. The distribution of the sprouts across the stump area was relatively homogeneous: 24% of the stumps had no area occupied by shoots, 18% were distributed by 25% of the stump area, 21% by half, 17% by 75%, and 20% were distributed by the entire stump area.

Despite the sprouting, 25% of the sprouts turned out to be not viable in Cuenca, and 13% in Lugo. The site with the lowest number of sprouts was Lugo, with a mean number of 1.9 sprouts per stump, followed by Cáceres. The shortest shoots appeared in Lugo, with an average of 0.6 meters (Tab. 4).

Effect of the treatments on the probability of sprouting

According to the AIC, a random effect of block nested within transect was best for models 1 and 3, and a random effect of transect was best for model 2 (Tab. 5).

Model 1

There was a clear effect of the biological treatment B1 (biological treatment with *Pleurotus ostreatus*) on the probability of stump sprouting after harvest compared to the control ($p<0.0001$, Tab. 6). The odds of sprouting in the untreated control stumps were 17.41 times larger than in stumps treated with *Pleurotus ostreatus* (95% CI for the odds ratio, 6.86 to 44.17). Interestingly, the probability of sprouting in control treatments was almost 100% in all locations (Tab. 7).

Table 4 - Mean number and mean height of the sprouts by stump by treatments.

Treatment	Mean n sprouts	Mean height (m)
Pleurotus ostreatus biological treatment	1.91	0.91
Lentinula edodes biological treatment	1.48	0.60
Control	3.93	1.66
Chemical treatment	2.97	1.63

Note: B1 is the *Pleurotus ostreatus* treatment, B2 is the *Lentinula edodes* treatment, Q is the chemical treatment, C is control treatment.

Table 5 - Considered random effects configurations for each model.

Model	Random effects	AIC
1.1	Transect	523.5
1.2	Transect + treatment in transect	505.7
1.3	Treatment in transect	503.7
2.1	Transect	160.7
2.2	Transect + treatment in transect	162.5
2.3	Treatment in transect	162.7
2.4	Transect (Girona dataset)	122.6
3.1	Transect	391.6
3.2	Transect + Treatment in Transect	391.2
3.3	Treatment in transect	389.2

Note: nc: not converge; AIC: Akaike Information Criterion.

Model 2

There was not a significant difference in the odds of sprouting between the control and B1 (biological treatment with *Pleurotus ostreatus*) treatments, nor between B1 and Q treatments in Lugo and Girona, but there was a marginal difference between the effects of the control and chemical treatments (p=0.0983, Tab. 8). However, there was a difference in the probability of sprouting between Girona and Lugo (p=0.00585).

The results of the model for Girona alone indicate that, compared with the control, the treatments resulted in a decrease in the probability of sprouting and that the effect was greater with Q than with B1. However, the effect was not significant for B1 treatment (p=0.19) and only marginally significant for Q treatment (p=0.0524) (Tab. 9).

Model 3

There was strong evidence that both biological treatments decreased the probability of sprouting compared with the control (p<0.0001 for both B1 [biological treatment with *Pleurotus ostreatus*] and B2 [biological treatment with *Lentinula edodes*]). The odds of sprouting in the control treatment were 99.4 (95% CI for the odds ratio, 20.9 to 472.3) and 22.7 (95% CI for the odds ratio, 5.00 to 103.2) times greater as large as those in stumps treated with *Pleurotus ostreatus* and *Lentinula edodes*, respectively (Tab. 10). The *Pleurotus ostreatus* treatment was more effective preventing sprouting than the *Lentinula edodes* treatment (p<0.0001). The odds of sprouting in stumps treated with *Lentinula edodes* were 4.37 times as large as in stumps treated with *Pleurotus ostreatus* (95% CI for the odds ratio, 2.33 to 8.21).

In summary, results showed evidence for the effect of biological treatment on reducing the probability of sprouting in walnut stumps. Both biological treatments showed better performance than chemical and control treatments, albeit *Pleurotus ostreatus* was more efficient than *Lentinula edodes*.

Mushrooms yields

There were 1157 records in Lugo, 109 in Girona and 864 in Toledo (Fig. 3). The number of sporocarps in a single stump ranged between 0 and 43. The mean number of sporocarps per stump was 0.47, but it was very different depending on the site (0.52 in Lugo, 1.79 in Girona and 0.23 in Toledo). The mean weight of mushroom per stump was 15.58 g, but ranged between 18.25 g in Lugo, 107.24 g in Girona and 0.43 g in Toledo. Week 46 (second week of

Table 6 - Solution for the biological and control treatment model comparing the effect of the *Pleurotus ostreatus* (B1) and control treatments across all sites (model 1.3, table 2).

Fixed effects	Estimate	Std.Error	z value	Pr(>lzl)	Significance codes
(Intercept)	3.2313	0.5050	6.399	<0.0001	
site Cuenca	3,1581	0.8833	3.576	0.0004	***
site Lugo	-0.3370	0.4751	-0.709	0.4781	
site Girona	1,8598	0.7353	2.529	0.0114	*
site Toledo	1,1452	0.6728	1.702	0.0887	.
treatment B1	-2.8573	0.4749	6.016	<0.0001	***

Note: Std. Error is the standard error of the estimate, z value is the value of the Z test, Pr(>lzl) is the P-value associated with the test. Reference level for place is Cáceres and for the treatment is the control. The estimate for site Cuenca is the difference in the log odds of sprouting in Cuenca, site Lugo is that for Lugo, site Girona is that for Girona, site Toledo is that for Toledo. The estimate for treatment B1 is the difference in the log odds between treatment B1 and the control. Significance codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 ''; 1.

Table 7 - Estimated probabilities of sprouting in stumps treated with B1 by sites.

Site	*Pleurotus ostreatus* treatment	Control
Cáceres	0.5924	0.9620
Cuenca	0.9716	0.9983
Lugo	0.5092	0.9476
Girona	0.9032	0.9939
Toledo	0.8204	0.9876

November) was the most productive, with a mean number of mushrooms of 1.73 and a weight of 67.28 g (Fig. 3).

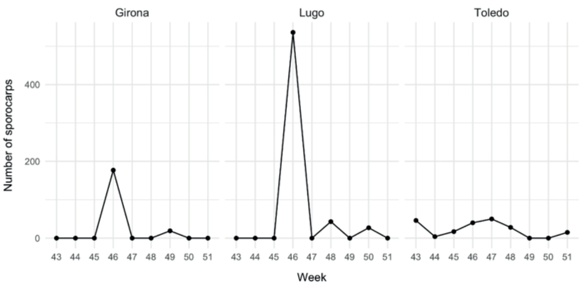

Figure 3 - Number of sporocarps picked by site and week.

Discussion

In this study, the effect of stump inoculation with edible wood decay fungi in walnut plantations has been assessed. Our results prove the great potential of these fungi for stump degradation and how they provide two beneficial effects: *i)* sprouting reduction and *ii)* production of edible mushroom. This article underpins wood decay fungi as a more efficient technique for stump degradation than natural degradation or chemical treatment.

Our findings show that biological stump degradation can be considered as a viable alternative for the elimination of stumps and the reduction of sprouts after logging a stand. In our studied plantations, all the trees that were not subjected to either biological or chemical treatment, did not die and continued growing as coppice. In forestry, most of the experiences of stump degradation have been carried out through chemical or heavy mechanization (Alonso et al. 2007). Mechanical extraction of stumps is estimated to cost over 700 € ha^{-1} for hardwood species in Spain (TRAGSA S.A. 2019). Regarding chemical treatments, herbicides can kill the stump but would never contribute to its degradation. Environmental concerns have led to a global reduction of the chemical herbicides used in forests (Dumas et al. 1997, Becker et al. 2005). Although in this paper, biological stump degradation has been only evaluated from an ecological point of view, future studies including a wide array of forest stand characteristics (species, density, age, etc.), may help to define appropriate forest management prescriptions in which biological stump degradation could be a viable economic and ecological alternative.

Our results showed that fungal inoculation reduced probability of a stump sprouting, the number of sprouts per stump and their height compared to an untreated control. Specifically, an untreated stump was 17.41 times more likely to resprout than inoculated peers. However, this general pattern was not uniform across tested sites, with Lugo and Cáceres displaying the lowest sprouting frequency found among the inoculated stumps, 0.51 and 0.59 respectively. This contrasted with much higher sprouting rates in Toledo (0.82), Girona (0.90) and Cuenca (0.97), where almost all stumps sprouted, and inoculation meant no advantage over control. These results can be explained by site-specific conditions regarding the plots themselves, and by intrinsic limitations of the experiments carried out

Table 8 - Solution for the model comparing the effects of the *Pleurotus ostreatus* (B1), chemical and control treatments in Girona and Lugo (model 2.1, table 2).

Fixed effects	Estimate	Std. Error	z value	Pr(>lzl)	Significance codes
(Intercept)	4.7951	1.0171	4.715	<0.0001	***
site Girona	-1,9984	0.7251	-2.756	0.0059	**
treatment B1	-1,0938	0.8185	1.336	0.1814	
treatment Q	-1.3373	0.8090	-1.653	0.0983	

Note: Std. Error is the standard error of the estimate, z value is the value of the Z test, Pr(>lzl) is the P-value associated with the test. Reference level for treatment is the control and Lugo for the site. treatment B1 is difference in the log odds of sprouting between the *Pleurotus ostreatus* (B1) treatment and the control, treatment Q is the difference in the log odds of between the chemical treatment and the control. Significance codes: 0 ‘***’; 0.001 ‘**’; 0.01 ‘*’; 0.05 ‘.’; 0.1 ‘’; 1.

Table 9 - Solution for the model comparing the effects of the *Pleurotus ostreatus* (B1), chemical and control treatments in Girona (model 2.4).

Fixed effects	Estimate	Std. Error	z value	Pr(>lzl)	Significance codes
(Intercept)	3.210	1.039	3.090	0.0020	**
treatment B1	-1.448	1.093	-1.325	0.1853	
treatment Q	-2.076	1.070	-1.940	0.0524	.

Note: Std. Error is the standard error of the estimate, z value is the value of the Z test, Pr(>lzl) is the P-value associated with the test. Reference level for treatment is Control (C). Treatment B1 is the difference in the log odds of sprouting between the *Pleurotus ostreatus* (B1) treatment and the control, treatment Q is the difference in the log odds of between the chemical treatment and the control. Significance codes: 0 '***'; 0.001 '**'; 0.01 '*'; 0.05 '.'; 0.1 ''; 1.

at each location. In line with this hypothesis, the site with highest rainfall (and hence soil moisture), provided better conditions for stump colonization by fungi. Site-specific conditions influenced the behaviour of the inoculated fungi, which ultimately related to site humidity. While in Lugo, all the stumps treated with chemical treatment sprouted, in Girona (with the driest conditions) chemical treatments was marginally more effective than the two biological treatments. The reason in Girona may have been a result of the biological treatment not having enough time to develop the mycelium and begin to degrade the stump. In this sense, since there is an increasing pressure from society against using herbicides and for using environmentally beneficial alternatives (Bellgard et al. 2014), results of this study demonstrate the potential of *Pleurotus ostreatus* and *Lentinula edodes* as a nature-friendly and sustainable, as well as efficient alternative.

Wood rot fungi species possess a powerful set of enzymes for wood decomposition. They evolved adapting to access an abundant source of food which is nonetheless very stable chemically. From the vast number of naturally occurring species, only a few have been tested in stump inoculation experiments, most of them non edible. In this paper, *Pleurotus ostreatus* and *Lentinula edodes* saprophytic edible species, have shown their ability to reduce the resprouting of walnut species. These species, together *Pycnoporus sanguineus* and *P. chrysosporium* (Kerem et al. 1992, Sik and Unyayar 1998, Pu et al. 1998, Kodrík 2001, Poiting et al. 2003, Bari et al. 2015) were analysed previously to assess the potential of fungi in stump degradation of other tree species like *Quercus rubra* L. (Oriaran et al. 1989, Labosky et al. 1991), *Betula papyrifera* Marshall (Tai et al. 1990), *Paulownia elongata* S.Y. Hu (Tan et al. 1998), *Fagus sylvatica* L., *Pinus sylvestris* L. (Poiting et al. 2003) and *Picea glauca* (Moench) Voss (Roy et al. 2010). In *Eucalyptus* species, Silva et al. (2010) evaluated in vitro Eucalyptus sawdust degradation with several white-rot fungi and found that the most efficient decay was achieved with *Pycnoporus sanguineus* and *Phellinus gilvus*. Negrão et al. (2014) found a great ability of *Pycnoporus sanguineus* and *Lentinus bertieri* to degrade *Eucalyptus* hardwood. In *Eucalyptus* plantations in Brazil (*E. saligna*, *E. grandis*, *E. urograndis*, *E. urophylla*), *Pycnoporus sanguineus*, *Ganoderma* sp. and *Peniophora* sp. (Ferraz et al. 1998, Alonso et al. 2007), *G. applanatum*, *Lentinus bertieri*, *Xylaria* sp., *Lentinula edodes*, *Pycnoporus sanguineus* (Negrão et al. 2014) or *Gloeophyllum trabeum* (Aguiar et al. 2013) have been used as biological control agents.

Other researchers have evaluated the effect of fungi species in stump resprouting capacity. Bellgard et al. (2014) in *Salix fragilis* L. and *Salix cinerea* L. and Hantula et al. (2012) studied the efficiency of *Chondrostereum purpureum* to control sprouting in *Betula pendula* Roth, *Betula pubescens* Ehrn., *Populus tremula* L. and *Sorbus aucuparia* (L.) Crantz. The latter, found that the proportion of dead stumps with the best isolates exceeded 80% in

Table 10 - Solution for the model comparing the effects of the *Pleurotus ostreatus* (B1), *Lentinula edodes* (B2) and control treatments in Lugo and Toledo (model 3.3)

Effects	Estimate	Std. Error	z value	Pr(>lzl)	Significance codes
(Intercept)	3.4289	0.7445	4.605	<0.0001	***
site Toledo	2.4205	0.3985	6.074	<0.0001	***
treatment B1	-4.5990	0.7953	-5.783	<0.0001	***
treatment B2	-3.1235	0.7721	-4.045	<0.0001	***

Note: Std. Error is the standard error of the estimate, z value is the value of the Z test, Pr(>lzl) is the P-value associated with the test. The control treatment was the reference level for the treatment test and Lugo was the reference level for site. Site Toledo is the difference in the log odds of sprouting betwee Toledo and Lugo, treatment B1 and treatment B2 difference in the log odds of sprouting between the *Pleurotus ostreatus* and *Lentinua edodes*, and control treatments, respectively. Significance codes: 0 '***'.

Betula pendula Roth and *pubescens* Ehrn.

Becker et al. (2005) assessed the effect of *Chondrostereum purpureum* to prevent the resprouting of *Alnus rubra* Bong. in Canada, finding that all the stumps died and there were not any sprouts 2 years after inoculation. Other studies (Dumas et al. 1997) evaluated the efficiency of the same fungi species in *Populus tremuloides* Michx and *Populus grandidentata* Michaux, and found a decrease of 63% in the resprout and a 39% in the stump mortality one year after inoculation.

On the other hand, other researchers have evaluated the success of biological stump degradation in terms of losses of degraded mass. Abreu et al. (2007) reported stumps mass losses of 22.6%, 16.2% and 11.3% thanks to the action of *Pycnoporus cinnabarinus* (Jacq.) Fr., *Pleurotus ostreatus* and *Schizophyllum commune* Fries, respectively, after 8 weeks of incubation in *Eucalyptus*. Calonego et al. (2010) showed mass losses of 34.3% after 12 weeks caused by *Pycnoporus sanguineus* in *Eucalyptus grandis* W.Hill. Finally, Boyle (1998) showed losses of 31.8% in *Acer sp.* and *Betula sp.* by the action of *Lentinula edodes* and *Pleurotus sajor caju* (Fr.). Our results did not record enough information to estimate mass losses because stump degradation was evaluated only several months after treatment. Further research focused on time degradation effects must be carried out in the future to improve the accuracy and efficiency of the biological technique.

Alternatively, limitations in the experimental design could be affecting the results of the study. The statistical analysis carried out allowed us to discuss the differences among treatments and to compare the treatments and the control in each site. The comparison between treatments in all sites was not possible due to the unbalancedness of the design, which may limit the ability to generalize the results. However, results showed an important site effect that was important to try to elucidate which factors may be causing those differences in effectiveness. Further studies must take into account this consideration in each site in order to conclude in this sense.

Moreover, longer-term studies could improve the results, allowing monitoring of the biological degradation of stumps and providing additional data to fully evaluate it as an alternative from ecological and economic points of view. Additional factors to be considered in future could be the date of the inoculation process as well as the humidity and temperature conditions after treatment. These factors could enable the establishment of different scenarios to predict the uncertainty (Herrero et al. 2019). Variables linked to degradation process are very important in the current context of climate change, since alterations in the climatic conditions are expected in the Mediterranean area. Increasingly irregular precipitation accompanied by extreme rainfall events (IPCC 2013), would likely affect the outcome of stump treatment with wood decay fungi.

The economic and social relevance of edible mushroom resources has increased in the last decades. The performance of alternative biological controls, specifically inoculation with edible fungi, has also provided income to forest owners, who manage their plantation with a new output with an important social and economic impact.

Conclusions

Our article shows the first results on stump degradation in walnut plantations and shows evidence of ability of biological treatment to reduce the probability of sprouting in walnut stumps. Biological treatments with *Pleurotus ostreatus* and *Lentinula edodes* showed better performance than control treatments. Moreover, *Pleurotus ostreatus* was more efficient than *Lentinula edodes*. These results provide useful information for sustainable forest management, by using a close to nature approach for stump degradation while also generating a product, *i.e.* mushrooms. This could contribute to profitability to the walnut chain, one of the most important and profitable hardwood species widely cultivated for timber and nut production.

Our findings support the stump inoculation of rot fungi as an alternative nature-based solution for stump removal after logging, with clear enhancement of stump degradation and sprouting reduction. This paper raises also awareness on the sustainability of resources with a big social and environmental prospective impact. Biological stump degradation, which constitute a toxic-free technique, demonstrates the potential of these two fungi species as biocontrol agents with valuable applications in forest management.

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