

Ectomycorrhiza succession patterns in *Pinus sylvestris* forests after stand-replacing fire in the Central Alps

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Abstract Fires shape fundamental properties of many forest ecosystems and climate change will increase their relevance in regions where fires occur infrequently today. In ecosystems that are not adapted to fire, post-fire tree recruitment is often sparse, a fact that might be attributed to a transient lack of mycorrhizae. Ectomycorrhizal (EcM) fungi play an important role for recruitment by enhancing nutrient and water uptake of their hosts. The questions arise whether and for how long the EcM community is transformed by fire. We investigated the resistance and resilience of EcM fungal communities on a chronosequence of 12 *Pinus sylvestris* stands in Valais (Switzerland) and Val d'Aosta (Italy) affected by fire between 1990 and 2006. Soil samples from burnt and non-burnt forests were analyzed with respect to EcM fungi by means of a bioassay. The number of EcM species was significantly lower in samples from recently (2–5 years) burnt sites than non-burnt forest, and increased with time since fire reaching levels of adjacent forests after 15–18 years. Community composition changed after fire but did not converge to that of non-burnt sites over the 18 year period. Only *Rhizopogon roseolus* and *Cenococcum geophilum* were abundant in

both burnt sites and adjacent forest. Our data indicate fire resistance of some EcM fungal species as well as rapid resilience in terms of species number, but not in species composition. As long as the function of different EcM species for seedling establishment is unknown, the consequences of long-term shifts in EcM community composition for tree recruitment remain unclear.

Keywords Fire disturbance · Recruitment · Resilience · Resistance · Scots pine

Introduction

Forest fires are disturbances of far reaching spatial and temporal extent (Pausas and Keeley 2009) and result in a wide variety of ecological effects at the scale of regions (Bowman et al. 2009), landscapes (Turner and Romme 1994) and individual forest stands. Fires alter physical and chemical soil properties (Neary et al. 1999; Certini 2005), affect soil microbial populations (Vázquez et al. 1993; Mataix-Solera et al. 2009), shape plant community composition (Bond and Keeley 2005) and create spatial heterogeneity in forest ecosystems (Turner et al. 2003; Bradstock 2008). Wildfire prone parts of the world span ecosystems ranging from tropical savannas to boreal forests, with fire occurrence being most abundant in biomes with relatively high biomass productivity and concomitant weather patterns that promote biomass burning (Krawchuk et al. 2009). In Central European forests, fire activity is currently rather low. As a results of human activity, fires nevertheless regularly occur in the dry, inner-alpine valleys such as the Val d'Aosta (Italy), the Valais (Switzerland) and the upper Inn valley (Austria; Bovio et al. 2005; Gossow et al. 2007; Zumbrunnen et al. 2009). Both the

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occurrence and the extent of forest fires are expected to increase in these regions due to climate and land-use change (Zumbrunnen et al. 2009), especially under the projected increase of extreme drought events (Schär et al. 2004; Beniston et al. 2007).

In the Central Alps, regeneration failure of *Pinus sylvestris* L. after fire has been reported repeatedly (Delarze and Werner 1985; Maumary et al. 1995; Moser et al. 2010). Moser et al. (2010) concluded that microclimatic conditions, especially water availability during summer period, hamper *P. sylvestris* recruitment at its Southern distributional limit. Ectomycorrhiza (EcM), the symbiosis between fungi and roots of woody plants, is suggested to play an important role in water uptake by forest trees and in the resistance of forests towards water shortage (Garbaye 2000). The formation of an extensive mycelium by EcM fungi allows symbiotic plants to explore a considerably larger volume of soil, to access water in soil pores that are smaller than its own fine roots and to transport it to the hosts' roots (Duddridge et al. 1980; Brownlee et al. 1983; Lamhamedi et al. 1992). Moreover, the dense fungal sheath covering the short roots of EcM plants may protect them from desiccation (Pigott 1982; di Pietro et al. 2007). Biotic interactions between EcM fungi and seedlings may be important for seedling growth and thus tree recruitment success, especially in an environment where water availability is highly variable, as is the case in the inner-alpine valleys. Species belonging to the family of Pinaceae, in common with several other major plant families, depend obligately on symbioses with EcM fungi for successful seedling establishment (Molina et al. 1992; Read 1998) and the presence of EcM fungi has been shown to increase survival and to enhance growth of conifer seedlings (Mikola 1970; Amaranthus and Perry 1987; Nuñez et al. 2009). Seedling establishment success may be affected by EcM fungal community composition since EcM species differ in their ability to facilitate different aspects of seedling growth (Jonsson et al. 2001). Consequently, successful tree recruitment in fire prone regions may depend on the resistance of EcM species to fire and the speed at which they are able to re-establish from surviving and post-fire dispersed inoculum.

The response of organisms to high-severity fire disturbance can be described in terms of resistance, i.e. the extent to which a community is displaced by disturbance, and resilience, i.e. the rate of recovery of a community following disturbance (Pimm 1984; Attiwill 1994). In the context of community composition, resistance is measured as similarity in species composition before and after disturbance, whereas resilience is defined as the time needed for the species composition on a site to return to its original state after a disturbance (Halpern 1988; Moretti et al. 2006; Bruelheide and Luginbühl 2009). The impact of fire disturbance on plants has been investigated both in terms of

resistance and resilience of species richness or abundance of certain functional groups (Keeley et al. 1981; Delarze et al. 1992; Esposito et al. 1999; Kazanis and Arianoutsou 2004). As a general pattern, succession of post-fire plant communities has been found to be a function of survival of propagules (e.g. seed bank) and dispersal of new propagules onto that site (Whelan 1995). For tree species, an additional aspect emerges: the availability of EcM fungi. Most of the studies investigating the effects of forest fires on EcM focused on resistance of EcM communities rather than resilience (reviewed in Cairney and Bastias 2007). Field studies found that species number decreases following fire (Dahlberg et al. 2001; Smith et al. 2004; Smith et al. 2005) and EcM community structure changes (Jonsson et al. 1999; Stendell et al. 1999; Grogan et al. 2000; Buscardo et al. 2010). Experiments with soil heating have further demonstrated that EcM species react differently to fire effects (Izzo et al. 2006; Peay et al. 2009; Kipfer et al. 2010). Regarding EcM community resilience (sensu Attiwill 1994), there are two chronosequence studies that have evaluated EcM communities over long periods (≥ 100 years; Visser 1995; Twieg et al. 2007), but the first decades after fire disturbance were only poorly resolved. In both studies, however, it is concluded that changes in EcM community composition are most dramatic in these first decades after a fire event.

In this study, we investigated the community composition of EcM fungi on a two-decade chronosequence of fire sites in *P. sylvestris* forest stands from two valleys in the Central Alps, the Val d'Aosta (Italy) and the Valais (Switzerland). Since fire severity may influence EcM survival, we only included sites affected by stand-replacing fire. As a result, *P. sylvestris* recruitment was sparse or missing at several sites, and we have chosen an indirect bioassay approach to assess EcM communities where soil samples from the fire sites and adjacent non-burnt forests were sown with *P. sylvestris*, and colonized roots were analyzed for EcM fungi. The following questions were addressed: (1) To what extent are EcM species number reduced and community composition changed after fire disturbance? (2) At what rates do species number increase after the fire? (3) Do EcM fungal communities converge to pre-fire composition? (4) Are burnt sites primarily colonized by species that survived the fire, by abundant species from the adjacent forest, or both?

Materials and methods

Study sites

The study sites are located in two inner-alpine valleys: Val d'Aosta (Italy) and Valais (Switzerland; Online

Resource 1). Both valleys are similar in terms of climate and vegetation composition (Braun-Blanquet 1961; Ozenda 1988). They are characterized by a continental-like climate. In Val d'Aosta, average summer precipitation is 291 ± 84 mm, and winter precipitation is 243 ± 105 mm. Average summer temperature is $17.7 \pm 0.7^\circ\text{C}$, and winter temperature $4.4 \pm 0.7^\circ\text{C}$ (meteorological station in St. Christophe, 545 m a.s.l.; Agenzia Regionale pel la Protezione dell' Ambiente). For the Central Valais, an average summer precipitation of 320 ± 77 mm and an average winter precipitation of 277 ± 93 mm have been measured in the same period. Average summer temperature is $16.6 \pm 0.7^\circ\text{C}$ and winter temperature is $4.5 \pm 1^\circ\text{C}$ (meteorological station in Sion, 482 m a.s.l.; MeteoSwiss). In Val d'Aosta, a total of 366 fires have been recorded between 1986 and 2002, destroying 1,666 ha of forest. Most of these fires occurred in the colline-montane zone (<1,400 m a.s.l.; Bovio et al. 2005). In the Valais, 906 fires (equivalent to 1 fire per km^2 forested area) are documented for the period between 1904 and 2006, with an increase in the number of large fires in the last decades. The fire regime in this area is dominated by anthropogenic influence with 85% of fires with known source of ignition caused by humans (Zumburrunen et al. 2009). Based on charcoal analyse in lake sediments, it is assumed that large-scale fires were infrequent in these ecosystems in the past, but a sharp increase in charcoal concentration has been found since the beginning of the 20th century (Bendel et al. 2006).

Based on the forest fire databases of the Nucleo Antincendi Boschivi, Aosta, and the WSL, Birmensdorf, all

recorded fire sites in the Val d'Aosta and the Valais were selected that satisfied the following criteria: former *P. sylvestris* forest affected by a stand-replacing wildfire (i.e. no surviving trees) with a burnt area ≥ 2 ha to avoid edge effects. This resulted in 12 fire sites, which burnt between 1990 and 2006 (Table 1). Stand age of the forests within which the fires occurred ranged from 60 (pastured until mid of the 20th century) to 160 years (Table 1). Since fires were stand-replacing, seedling banks were destroyed and post-fire recruitment had to start from seeds. This process is slow and can be hindered by rapid re-colonization of herbaceous vegetation (Moser et al. 2010). As a result, natural regeneration of *P. sylvestris* on the study sites was sparse or missing. Cover of EcM forming plant species (coniferous trees, deciduous pioneer trees and shrubs) was low, except in the three oldest sites Boesse, Del and Ronteron (Table 1). Areas where nursery-grown saplings had been planted were excluded from sampling. There are no records of previous fires in the selected sites. An exception is the site in Muin, which has been partly burnt in 1952 (G. Cesti, personal communication). Since this sample did not act as an outlier in our analyses, we decided to leave it in the dataset.

Soil sampling

To assess EcM communities of both burnt and non-burnt forests, we sampled soils along transects in paired burnt and non-burnt sites. Sampling took place between the 22 October 2008 and 15 November 2008. At each forest fire

Table 1 Location and forest stand characteristics of the 12 study sites in Valais (Switzerland; CH) and Val d'Aosta (Italy; I)

Site	Longitude	Latitude	Time since fire (yrs)	Forest stand age (yrs) ^a	Elevation (m a.s.l.)	Aspect	Slope (%)	Area (ha) ^a	<i>Pinus sylvestris</i> recruitment ^b	Cover of EcM plants (%) ^c
1 Menfrey (I)	7°30'48.11"	45°45'55.43"	2	60–70	1,029	SSE	40	25	0	2
2 Lens (CH)	7°26'34.66"	46°16'18.51"	3	140–160	1,035	S	45	6	2	4
3 Muin (I)	7°29'05.47"	45°45'55.74"	3	50–70	991	S	45	91	0	22
4 Leuk (CH)	7°38'07.56"	46°19'26.53"	5	140–160	1,035	WSW	53	310	0	2
5 Blavesse (I)	7°34'53.05"	45°45'48.07"	5	80–100	1,300	WSW	13	299	0	2
6 Grand Villa (I)	7°31'11.39"	45°46'51.64"	7	100	1,550	SSE	53	79	1	4
7 Avion (I)	7°26'34.85"	45°45'46.30"	11	60–70	1,300	S	46	2	0	12
8 Morgex (I)	7°00'12.92"	45°46'03.68"	11	120–150	1,046	SSW	48	12	2	3
9 Stalden (CH)	7°52'19.73"	46°15'10.86"	12	60–70	946	SSE	65	4	1	8
10 Del (I)	7°33'01.64"	45°46'05.61"	13	60–70	1,400	S	35	14	1	50
11 Ronteron (I)	7°28'22.25"	45°46'04.38"	18	60–70	1,007	W	60	23	2	63
12 Boesse (I)	7°36'53.58"	45°45'55.42"	18	60–70	1,400	NW	61	201	3	27

^a Estimates by the local forest services

^b Number of transects on the burnt sites where *P. sylvestris* seedlings were present within 1 m distance on each side of the transect (4 transects/site were sampled)

^c EcM plant species included *Quercus* spp., *Betula pendula*, *Populus tremula*, *Salix appendiculata*, *Prunus mahaleb*, *Sorbus aria*, *Viburnum lantana*, *Rosa* spp., *Berberis vulgaris*, *Juniperus communis*, *Pinus sylvestris*, *Larix decidua*, *Arctostaphylos uva-ursi*

site, four parallel transects, each 30 m long and separated by 50 m, were established along the slope contours in the burnt area and another four similarly established in the adjacent non-burnt forest. To avoid edge effects (Dickie and Reich 2005), the nearest distance of a transect to the forest edge was always around 20 m. Thus transects from each pair of burnt and non-burnt locations were separated from each other by around 40 m. On account of this relatively small distance we assume that plant and EcM species composition prior to the burn have been similar at burnt and control sites, although we cannot fully exclude that differences in soil moisture exist. Since *P. sylvestris* forests in the Central Alps are generally restricted to shallow soils they all host similar understory vegetation (Keller et al. 1998). The use of four transects allowed to sample areas of identical size, despite the fact that total area of the burnt sites differed (Table 1). Every 3 m, a soil sample of approximately 0.5 L was taken between 0 and 15 cm soil depth with a shovel. This sampling procedure was chosen because soils of most sites were too stony to be excavated by a soil corer. The eleven samples of one transect were pooled, packed in a sealed plastic bag and stored at 10°C until further processing. Between each transect, sampling tools were cleaned with ethanol to avoid cross-contamination. The same sampling scheme was also applied in the adjacent non-burnt forest, giving a total of eight bioassay samples per location.

Bioassay and morphotyping

Within 7 days after removal, 1 L of each soil sample was filled in three plastic pots and sown with ten surface-sterilized seeds of *P. sylvestris* per pot. The pots were placed in a growth chamber with day/night cycles of 16/8 h, temperature of 24/18°C, respectively, relative humidity of 60%, and mean irradiance of 640 lux during the day cycles. 12 additional pots were filled with autoclaved (120°C, 20 min) soil to assay for possible contaminants in the growth chamber. Seedlings were watered with deionized water every 3–4 days. When most of the seeds had germinated, seedlings were thinned to 4 per pot. After 6 months, seedlings were harvested and the roots were carefully rinsed under tap water. Root samples originating from the same transect were pooled for further analysis. The root tips were examined under a dissecting microscope and classified into morphotypes based on morphological characteristics such as color, mantle texture, type of ramification, presence of rhizomorphs, etc. as described in Agerer (1987–1997). From each sample, one representative of each morphotype was used for molecular identification. Mean colonization rate of bioassay seedlings was $83 \pm 9\%$.

DNA extraction, amplification and sequencing

DNA was extracted from the root tips using a DNeasy Plant Mini Kit according to the manufacturer's protocol (Qiagen 2006), and 5 μl of the extract was amplified following the protocol of Gardes and Bruns (1993) using the primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). PCR products were visualized with gel electrophoresis, and successful amplifications were cleaned on MultiScreen filter plates (Millipore, Billerica, USA). 5.5 μl PCR product was used together with 1 μl of the Terminator Ready Reaction Mix for cycle sequencing with the ITS1F-primer according to the protocol of Applied Biosystems (AppliedBiosystems 2002). Products were purified with a Sequencing Reaction Cleanup Kit (Millipore, Billerica, USA) and sequenced in one direction using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). Obtained sequences with a high degree of similarity (cutoff of 98%) were clustered into groups and were visually optimized using the programs Sequencing Analysis 5.1 (Applied Biosystems, Foster City, USA) and DNA Dynamo (Blue Tractor Software Ltd., North Wales, UK). The consensus sequence of groups or—where clustering failed—individual sequences were then entered for a query in the UNITE sequence database (Kõljalg et al. 2005) and the NCBI GenBank and analyzed using the BLASTn algorithm (Altschul et al. 1997). Consensus sequences or individual sequences showing less than 95% identity with a relevant database entry were identified at family or ordinal level, 95–97% at genus level, and greater than 97% at species level. Where two or more sequences or sequence clusters with less than 98% identity between each other matched the same database entry, a number was added to the name to mark the difference between the two, e.g. *Tomentella* sp. 1 and *Tomentella* sp. 2. Each sequence or cluster of sequences that differ from others by more than 98% is hereafter referred to as “operational taxonomic unit” (OTU), regardless of whether it has been identified to species, genus or family level. A list with the species names and the accession numbers of the corresponding database entry of the best match is given in Online Resource 2. Sixty-six percent of root tips could be identified successfully, either after one attempt of DNA extraction and sequencing or, where needed, after a second attempt on a backup root tip with the same morphotype from the same root sample. Two unidentified ascomycete species occurred also in the control pots with autoclaved soil, and were considered to be contaminants in the growth chamber and therefore were excluded from further analysis. Another four species, *Neonectria macrodidyma*, *N. radicola*, *Oidiodendron citrinum* and *Pseudodictyosporium wauense*, were excluded because they are not considered to be EcM fungi.

Statistical analyses

Calculations were conducted using R version 2.11.0 (R Development Core Team 2010). Species lists of the four transects from the burnt sites and the non-burnt forest, respectively, were pooled. The mean number of OTUs between burnt sites and non-burnt forest was compared using analysis of variance with location as random effect. The number of OTUs on burnt sites in relation to the time since fire was analyzed by linear regression, with number of OTUs on burnt sites standardized by the number of OTUs on adjacent non-burnt forest sites. For this analysis, both the successfully identified as well as the unidentified species were included. To present the community composition, a non-metric multidimensional scaling (NMDS) on Bray–Curtis distances was performed using *vegdist* and *isoMDS* of the R *vegan* library (Oksanen et al. 2011). To test for differences in species composition between burnt sites and non-burnt forest, an analysis of similarity was performed using the *anosim* function of the R *vegan* library with 1,000 permutations. The overlap in species composition of burnt sites and non-burnt forest was expressed by the Jaccard index (Koleff et al. 2003), plotted against time and analyzed by a linear regression. The analyses on species composition were performed on a subset of data containing successfully sequenced species only (108 OTUs; see Online Resource 2).

Results

The number of OTUs were lower on burnt sites than in adjacent forest stands (ANOVA of 24 samples, $F_{1,11} = 19.03$, $P = 0.001$). The mean number of OTUs in the forests was 21.2 ± 3.5 (mean \pm SD), whereas 11.6 ± 1.5 OTUs were found in the most recently burnt sites (2–5 years since burn), and 19.5 ± 2.1 OTUs on the most ancient sites (>13 years). For the samples from burnt sites, there was a slight but significant increase in number of OTUs with increasing time since fire (linear regression, $P = 0.004$, $r_{\text{adj}}^2 = 0.53$). This trend was even more pronounced when the number of OTUs of each fire site was standardized by the number of OTUs in the adjacent forest ($P = 0.001$, $r_{\text{adj}}^2 = 0.61$; Fig. 1). 18 years after fire, the number of OTUs in the burnt sites has reached the number of OTUs in the non-burnt forests.

Samples from burnt sites differed in species composition from those from non-burnt forest sites as demonstrated by NMDS (Fig. 2) and confirmed by analysis of similarity ($P = 0.001$). The proportion of OTUs shared between pairs of burnt sites and the adjacent non-burnt forest was low: The Jaccard index varied between 0.05 (Morgex) and 0.38 (Avion), but no temporal trend could be detected (Fig. 3). Although the similarity between burnt and non-burnt sites

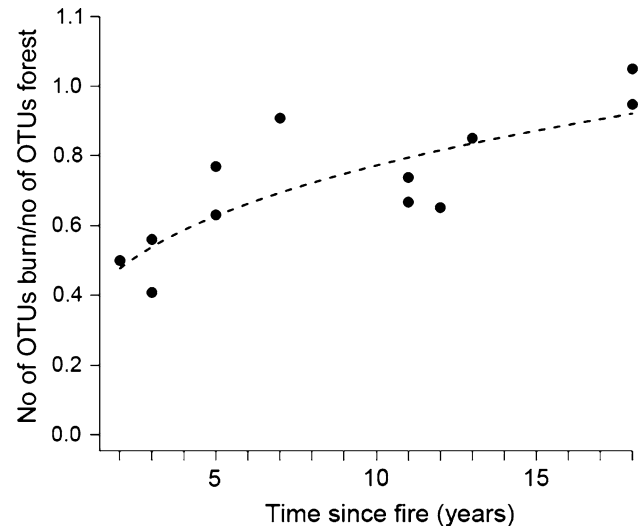


Fig. 1 Changes in ectomycorrhizal diversity in relation to time since fire. Data are the ratio of the number of OTUs detected on burnt and non-burnt forest sites ($N = 4$ pooled soil samples per burnt and non-burnt site, respectively). Line plotted is the ratio of OTUs = $0.39 \times$ time since fire $^{-0.95}$ and was fitted with log–log regression

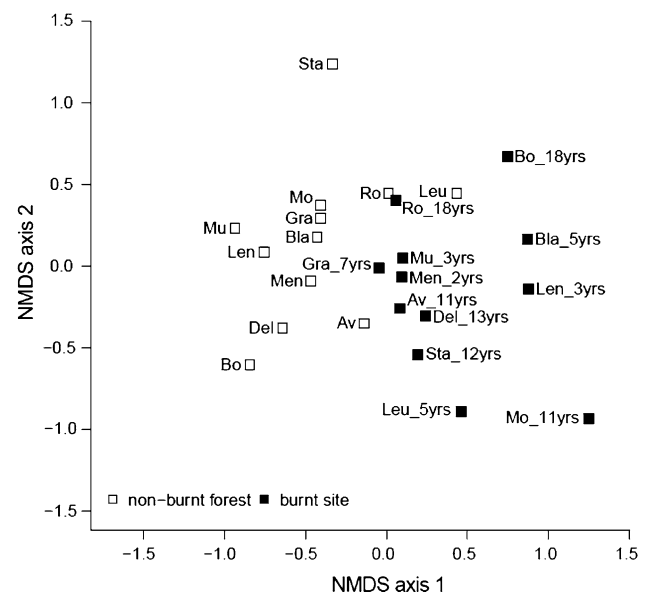


Fig. 2 Ectomycorrhizal community composition in burnt and non-burnt forest sites as determined by nonmetric multidimensional scaling (NMDS) using Bray–Curtis distances as a measure of dissimilarity. Dimensions: 294 operational taxonomic units (unidentified species included) \times 24 sites. Stress = 20.5 and non-metric fit $r^2 = 0.96$. Site_Yrs: sites are coded Av Avion, Bla Blavesse, Bo Boesse, Del Del, Gra Grandvilla, Len Lens, Leu Leuk, Men Menfrey, Mo Morgex, Mu Muin, Ro Ronteron, Sta Stalden and Yrs as years since fire for the burnt sites

is not clearly related to the age of the forest stands (linear regression, $P = 0.07$, $r_{\text{adj}}^2 = 0.22$), it is apparent that the forest stands of the 5 sites with the highest Jaccard index values are all less than 70 years old.

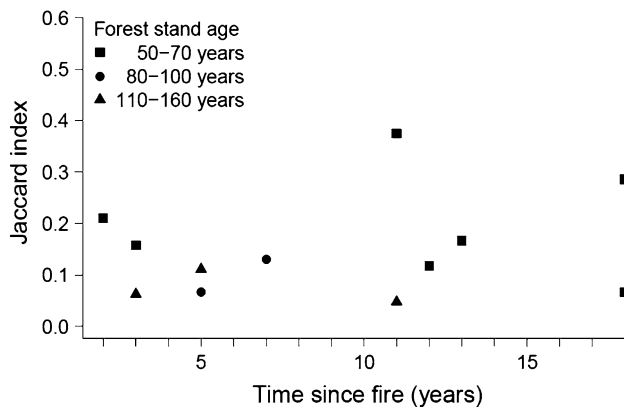


Fig. 3 Changes in similarity in species composition based on Jaccard's index of community similarity between burnt sites and non-burnt forest in relation to time since fire. Unidentified species were excluded

The most frequent species on the burnt sites were *Rhizopogon roseolus* (38 occurrences), *Cenococcum geophilum* (9), an unidentified ascomycete species (8) and an unidentified Pyronemataceae species (8, Online Resource 3). The first three species were also the most abundant species in the non-burnt forest, thus we assume that they either survived the fire or are among the very early post-fire colonizers. The unidentified Pyronemataceae, as well as *Hebeloma mesophaeum*, were also among the early post-fire colonizers, but neither of them was found in more than one forest site. Later colonizers not present in the forest sites include *Hebeloma crustuliniforme* (present 5–10 years after the burn) and an unidentified Inocybaceae species (present >10 years after the burn). Besides *R. roseolus* and *C. geophilum*, the EcM communities of the non-burnt forest were dominated by members of the Atheliaceae, Sebacinaceae and several *Tomentella* species, which were not present in the burnt sites. Overall, the variability in species composition between sites as well as between transects within sites was high: more than 50% of the successfully identified species from both burnt sites and non-burnt forests occurred in only a single transect (Online Resources 3 and 4).

Discussion

On forest fire sites in the Central Alps, the window of opportunity for conifer seedling establishment is narrow because competing herbaceous species colonize the fire sites in the first few years after fire (Moser et al. 2010). Moreover, conifer seed production is highly variable between years, with the majority of trees producing seeds synchronously in masting years. During non-masting years, the probability of encounter and re-association between plant and fungus is low and thus, the efficiency of EcM

facilitating the recruitment process is limited (Yamauchi et al. 2009). Given that the presence of EcM fungi is necessary for the establishment of obligately ectomycorrhizal *P. sylvestris* (Molina et al. 1992), successful post-fire pine recruitment requires either a fire-resistant EcM community or one that is resilient through rapid re-colonization, or a combination of both. We found that the number of species in terms of OTUs was reduced by 45% on recently burnt sites (2–5 years after the fire) compared to the corresponding adjacent non-burnt forest sites. Nevertheless, ten or more OTUs were recorded in these sites, indicating that a considerable number of EcM species survived the fire or re-colonized the soil rapidly after fire. Since sampling in the most recent fire site at Menfrey took place 2 years after the fire, we cannot distinguish between survival and very early re-colonization. Several of the species present at Menfrey are, however, known to survive artificial soil heating (*Cenococcum geophilum*, *Rhizopogon roseolus* and *Wilcoxina rehmsii*; Kipfer et al. 2010), suggesting that there is considerable resistance to fire among the EcM community. On the one hand, soils do not burn homogeneously, even not during a stand-replacing fire, and temperatures decrease rapidly across the soil profile, reaching detrimental temperatures only in the uppermost layer (DeBano et al. 1998). Thus, especially in deeper soil layers, EcM fungi may have to resist only moderate temperatures, but mycelium may also die because hosts are destroyed during stand-replacing fires. On the other hand, it is known that species in these genera have high re-colonization abilities after disturbance because of their abundant stock of resistant propagules (spores, sclerotia) in the soil (Baar et al. 1999; Taylor and Bruns 1999; Rincón and Pueyo 2010).

The number of OTUs increased with increasing time since fire, roughly doubling from around 11–19 OTUs in a time-frame of two decades after fire. Increasing number of OTUs is a result of newly arriving spores which are dispersed by wind (Allen 1987; Trappe and Luoma 1992), water run-off (Trappe and Luoma 1992), insects, soil-dwelling arthropods (resupinate fungi with fruitbodies consisting of a crust lying flat on the substrate; Lilleskov and Bruns 2005) and small mammals (hypogeous fungi with fruitbodies that are buried in the soil; Johnson 1996; Pyare and Longland 2001). In our study, dispersal by small mammals is probably of little relevance in promoting EcM community recovery because, with the exception of *R. roseolus* and *Tuber rufum*, species known to produce hypogeous fruitbodies were absent in the soil samples from burnt sites. Ectomycorrhizae of epigeous fungi (above-ground fruiters) were the predominant species, accounting for 88% of all identified species. Epigeous fungi are mainly wind-dispersed and spore rain largely depends on the abundance of fruitbodies in the surrounding forests in the

years after the fire (Ingold 1971). Consequently, EcM re-colonization may be determined by EcM community composition in the adjacent forest, which, in turn, may be affected by forest type and stand history. Not all abundant EcM species of the forest sites were found in the burnt sites, and many species present in the burnt sites were not found in the adjacent forest (Online Resource 3). These patterns may be largely due to the high spatial and temporal variation of species occurrences, as shown by the large number of OTUs encountered on single transects (Online Resource 4).

While the number of OTUs on the burnt sites increased with increasing time since fire, there was no apparent tendency for community composition from burnt sites to converge to that of non-burnt forest over the same time frame (Figs. 2, 3). This corroborates the study by Visser (1995) and Twieg et al. (2007), who suggested that changes in EcM community composition are most dramatic in the first decades after a fire event. Differences in community composition between burnt and non-burnt sites are in accordance with the finding that the EcM community in North American oak savanna, an ecosystem dependant on low-intensity fires, is unique compared to that in adjacent mature oak forest (Dickie et al. 2009). Similarly, EcM community composition in a *Pinus muricata* stand changed markedly following a severe wildfire (Grogan et al. 2000). In contrast, Visser (1995) found that all morphotypes present at a 41 year-old wildfire site also occurred at a 122 year-old site with mature *Pinus banksiana* forest. The fact that, in our study, the similarity between burnt and non-burnt EcM communities varied considerably between sites (Fig. 2) and showed no temporal trend (Fig. 3) may have several reasons: first, the forest stand age may affect the EcM community composition in non-burnt sites, with community composition of younger stands more similar to disturbed sites than older stands due to species succession over time. This is supported by our finding that the sites with the highest similarity between forest and burn are all less than 70 years old (Fig. 3). Second, re-colonization is, to some extent, a stochastic process with early arriving species benefitting from low competition. Consequently, succession pathways may vary according to the availability of fungal propagules in the first months or years after fire. Third, the bioassay method used to assess EcM community composition can only detect species that readily form ectomycorrhizae with seedlings under growth chamber conditions. Species that exclusively colonize roots of mature trees such as members of the Russulaceae and Amanitaceae (see Taylor and Bruns 1999) are missed, but these species are less relevant to this study as our interest is with seedling establishment.

Our study on burnt *P. sylvestris* stands in the Central Alps shows that EcM species richness is both moderately

resistant and resilient to fire events. Pioneer tree species such as *Betula pendula*, *Populus tremula* and *Salix* spp. were found to re-colonize burnt sites (Wohlgemuth et al. 2010), and these early colonizers, together with the procumbent woody shrub *Arctostaphylos uva-ursi*, may facilitate the establishment of a EcM network belowground (Horton et al. 1999; Nara and Hogetsu 2004). Our data also show that fire events change EcM community composition in comparison to non-burnt forests. It may take far more than two decades for EcM communities to reach species composition similar to that before fire. To our knowledge, this is the first time that a relatively short but high-resolution chronosequence was investigated, and that species composition was compared to EcM communities from the adjacent, non-burnt forest. The question of whether EcM fungi account for the observed post-fire regeneration failure of *P. sylvestris* in the Valais cannot be answered conclusively. Regeneration failure was not caused by the absence of EcM fungi per se, since EcM fungi were present in all fire sites irrespective of time since burn. However, it remains unclear whether seedling growth varies with EcM community composition. Further studies should focus on the question of whether taxonomically different EcM communities also differ in their function, that is, whether changes in the composition of fungal communities during post-fire succession affect the facilitative effects of EcM fungi on seedling performance. In case different functionalities should emerge, long-term changes in EcM species composition after fire might be decisive for post-fire recruitment of *P. sylvestris*.

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References

- Agerer R (1987–1997) Colour atlas of ectomycorrhizae, 1st–11th (edn) Einhorn Verlag, Schwäbisch Gmünd
- Allen MF (1987) Reestablishment of mycorrhizas on Mount St Helens: migration vectors. *Trans Br Mycol Soc* 88:413–417
- Altschul SF, Madden TL, Schäffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Amaranthus MP, Perry DA (1987) Effect of soil transfer on ectomycorrhiza formation and the survival and growth of conifer

- seedlings on old, non-reforested clear-cuts. *Can J For Res* 17:944–950
- Applied Biosystems (2002) BigDye® terminator v1.1 cycle sequencing kit protocol. Applied Biosystems, Foster City
- Attiwill PM (1994) The disturbance of forest ecosystems—the ecological basis for conservative management. *For Ecol Manage* 63:247–300
- Baar J, Horton TR, Kretzer AM, Bruns TD (1999) Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytol* 143:409–418
- Bendel M, Tinner W, Ammann B (2006) Forest dynamics in the Pfyn forest in recent centuries (Valais, Switzerland, Central Alps): interaction of pine (*Pinus sylvestris*) and oak (*Quercus* sp.) under changing land use and fire frequency. *Holocene* 16:81–89. doi:10.1191/0959683606hl906rpd
- Beniston M, Stephenson DB, Christensen OB, Ferro CAT, Frei C, Goyette S, Halsnaes K, Holt T, Jylhä K, Koffi B, Palutikof J, Schöll R, Semmler T, Woth K (2007) Future extreme events in European climate: an exploration of regional climate model projections. *Clim Change* 81:71–95. doi:10.1007/s10584-006-9226-z
- Bond WJ, Keeley JE (2005) Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends Ecol Evol* 20:387–394. doi:10.1016/j.tree.2005.04.025
- Bovio G, Camia A, Guglielmet E, Cesti G (2005) Piano regionale per la programmazione delle attività di previsione, prevenzione e lotta attiva contro gli incendi boschivi. Regione Autonoma Valle d'Aostam, Assessorato Agricoltura e Risorse Naturali e Corpo Forestale, Aosta
- Bowman DMJS, Balch JK, Artaxo P, Bond WJ, Carlson JM, Cochrane MA, D'Antonio CM, DeFries RS, Doyle JC, Harrison SP, Johnston FH, Keeley JE, Krawchuk MA, Kull CA, Marston JB, Moritz MA, Prentice IC, Roos CI, Scott AC, Swetnam TW, van der Werf GR, Pyne SJ (2009) Fire in the earth system. *Science* 324:481–484. doi:10.1126/science.1163886
- Bradstock RA (2008) Effects of large fires on biodiversity in south-eastern Australia: disaster or template for diversity? *Int J Wildland Fire* 17:809–822. doi:10.1071/WF07153
- Braun-Blanquet J (1961) Die inneralpine Trockenvegetation. Gustav Fischer Verlag, Stuttgart
- Brownlee C, Duddridge JA, Malibari A, Read DJ (1983) The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant Soil* 71:433–443
- Bruelheide H, Luginbühl U (2009) Peeking at ecosystem stability: making use of a natural disturbance experiment to analyze resistance and resilience. *Ecology* 90:1314–1325. doi:10.1890/07-2148.1
- Buscardo E, Rodríguez-Echeverría S, Martín MP, De Angelis P, Pereira JS, Freitas H (2010) Impact of wildfire return interval on the ectomycorrhizal resistant propagules communities of a Mediterranean open forest. *Fungal Biol* 114:628–636. doi:10.1016/j.funbio.2010.05.004
- Cairney JWG, Bastias BA (2007) Influences of fire on forest soil fungal communities. *Can J For Res* 37:207–215. doi:10.1139/X06-190
- Certini G (2005) Effects of fire on properties of forest soils: a review. *Oecologia* 143:1–10. doi:10.1007/s00442-004-1788-8
- Dahlberg A, Schimmel J, Taylor AFS, Johannesson H (2001) Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. *Biol Conserv* 100:151–161
- DeBano LF, Neary DG, Ffolliott PF (1998) Fire's effects on ecosystems. Wiley & Sons, New York
- Delarze R, Werner P (1985) Evolution après incendie d'une pelouse steppique et d'une pinède dans une vallée intra-alpine (Valais central). *Phytocoenologia* 13:305–321
- Delarze R, Caldelari D, Hainard P (1992) Effects of fire on forest dynamics in Southern Switzerland. *J Veg Sci* 3:55–60
- di Pietro M, Churin JL, Garbaye J (2007) Differential ability of ectomycorrhizas to survive drying. *Mycorrhiza* 17:547–550. doi:10.1007/s00572-007-0113-x
- Dickie IA, Reich PB (2005) Ectomycorrhizal fungal communities at forest edges. *J Ecol* 93:244–255. doi:10.1111/j.1365-2745.2005.00977.x
- Dickie IA, Dentinger BTM, Avis PG, McLaughlin DJ, Reich PB (2009) Ectomycorrhizal fungal communities of oak savanna are distinct from forest communities. *Mycologia* 101:473–483. doi:10.3852/08-178
- Duddridge JA, Malibari A, Read DJ (1980) Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. *Nature* 287:834–836
- Esposito A, Mazzoleni S, Strumia S (1999) Post-fire bryophyte dynamics in Mediterranean vegetation. *J Veg Sci* 10:261–268
- Garbaye J (2000) The role of ectomycorrhizal symbiosis in the resistance of forests to water stress. *Outlook Agr* 29:63–69
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118
- Gossow H, Hafellner R, Arndt N (2007) More forest fires in the Austrian Alps—a real coming danger? In: Borsdorf A, Sötter J, Veuillet E (eds) Managing alpine future, vol 2. Verlag der Österreichischen Wissenschaften, Innsbruck, pp 356–362
- Grogan P, Baar J, Bruns TD (2000) Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. *J Ecol* 88:1051–1062
- Halpern CB (1988) Early successional pathways and the resistance and resilience of forest communities. *Ecology* 69:1703–1715
- Horton TR, Bruns TD, Parker VT (1999) Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. *Can J Bot* 77:93–102
- Ingold CT (1971) Fungal spores: their liberation and dispersal. Clarendon Press, Oxford
- Izzo A, Canright M, Bruns TD (2006) The effects of heat treatments on ectomycorrhizal resistant propagules and their ability to colonize bioassay seedlings. *Mycol Res* 110:196–202. doi:10.1016/j.mycres.2005.08.010
- Johnson CN (1996) Interactions between mammals and ectomycorrhizal fungi. *Trends Ecol Evol* 11:503–507
- Jonsson L, Dahlberg A, Nilsson MC, Zackrisson O, Kårén O (1999) Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. *Mol Ecol* 8:205–215
- Jonsson LM, Nilsson MC, Wardle DA, Zackrisson O (2001) Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *Oikos* 93:353–364
- Kazanis D, Arianoutsou M (2004) Long-term post-fire vegetation dynamics in *Pinus halepensis* forests of Central Greece: a functional group approach. *Plant Ecol* 171:101–121
- Keeley SC, Keeley JE, Hutchinson SM, Johnson AW (1981) Postfire succession of the herbaceous flora in Southern California chaparral. *Ecology* 62:1608–1621
- Keller W, Wohlgemuth T, Kuhn N, Schütz M, Wildi O (1998) Waldgesellschaften der Schweiz auf floristischer Grundlage. Statistisch überarbeitete Fassung der "Waldgesellschaften und Waldstandorte der Schweiz" von Heinz Ellenberg und Frank Klötzli (1972). Mitt Eidgenöss Forsch Wald Schnee Landsch 73:91–357

- Kipfer T, Egli S, Ghazoul J, Moser B, Wohlgemuth T (2010) Susceptibility of ectomycorrhizal fungi to soil heating. *Fungal Biol* 114:467–472. doi:10.1016/j.fumbio.2010.03.008
- Koleff P, Gaston KJ, Lennon JJ (2003) Measuring beta diversity for presence-absence data. *J Anim Ecol* 72:367–382
- Köljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjølner R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol* 166:1063–1068. doi:10.1111/j.1469-8137.2005.01376.x
- Krawchuk MA, Moritz MA, Parisien MA, Van Dorn J, Hayhoe K (2009) Global pyrogeography: the current and future distribution of wildfire. *PLoS One* 4(4):e5102. doi:10.1371/journal.pone.0005102
- Lamhamedi MS, Bernier PY, Fortin JA (1992) Hydraulic conductance and soil-water potential at the soil root interface of *Pinus pinaster* seedlings inoculated with different dikaryons of *Pisolithus* sp. *Tree Physiol* 10:231–244
- Lilleskov EA, Bruns TD (2005) Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella subulilacina*, via soil food webs. *Mycologia* 97:762–769
- Mataix-Solera J, Guerrero C, García-Orenes F, Bárcenas GM, Torres P (2009) Forest fire effects on soil microbiology. In: Cerdà A, Robichaud PR (eds) *Fire effects on soils and restoration strategies*, vol 5. Science Publishers, Enfield, pp 133–175
- Maumary L, Vallotton L, Delarze R (1995) Evolution après incendie d'une pinède et d'une steppe dans une vallée intra-alpine (Valais central). *Phytocoenologia* 25:305–316
- Mikola P (1970) Mycorrhizal inoculation in afforestation. In: Romberger J, Mikola P (eds) *International review of forestry research*, vol 3. Academic Press, New York, pp 123–196
- Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF (ed) *Mycorrhizal functioning, an integrative plant-fungal process*. Chapman & Hall, New York, pp 357–418
- Moretti M, Duelli P, Obrist MK (2006) Biodiversity and resilience of arthropod communities after fire disturbance in temperate forests. *Oecologia* 149:312–327. doi:10.1007/s00442-006-0450-z
- Moser B, Temperli C, Schneiter G, Wohlgemuth T (2010) Potential shift in tree species composition after interaction of fire and drought in the Central Alps. *Eur J For Res* 129:625–633. doi:10.1007/s10342-010-0363-6
- Nara K, Hogetsu T (2004) Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. *Ecology* 85:1700–1707
- Nearly DG, Klopatek CC, DeBano LF, Ffolliott PF (1999) Fire effects on belowground sustainability: a review and synthesis. *For Ecol Manage* 122:51–71
- Núñez MA, Horton TR, Simberloff D (2009) Lack of belowground mutualisms hinders Pinaceae invasions. *Ecology* 90:2352–2359. doi:10.1890/08-2139.1
- Oksanen J, Guillaume Blanchet F, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2011) *vegan: community ecology package v. 1.17–9*. <http://CRAN.R-project.org/package=vegan>
- Ozenda P (1988) *Die Vegetation der Alpen im europäischen Gebirgsraum*. Gustav Fischer Verlag, Stuttgart
- Pausas JG, Keeley JE (2009) A burning story: the role of fire in the history of life. *Bioscience* 59:593–601. doi:10.1525/bio.2009.59.7.10
- Peay KG, Garbelotto M, Bruns TD (2009) Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing *Pinus muricata* seedlings. *J Ecol* 97:537–547. doi:10.1111/j.1365-2745.2009.01489.x
- Pigott CD (1982) Survival of mycorrhiza formed by *Cenococcum geophilum* Fr. in dry soils. *New Phytol* 92:513–517
- Pimm SL (1984) The complexity and stability of ecosystems. *Nature* 307:321–326
- Pyare S, Longland WS (2001) Patterns of ectomycorrhizal-fungi consumption by small mammals in remnant old-growth forests of the Sierra Nevada. *J Mammal* 82:681–689
- Qiagen (2006) *DNeasy 96 Plant Kit Handbook*. Qiagen, Crawley
- R Development Core Team (2010) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna
- Read DJ (1998) The mycorrhizal status of *Pinus*. In: Richardson DM (ed) *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge, pp 324–340
- Rincón A, Pueyo JJ (2010) Effect of fire severity and site slope on diversity and structure of the ectomycorrhizal fungal community associated with post-fire regenerated *Pinus pinaster* Ait. seedlings. *For Ecol Manage* 260:361–369. doi:10.1016/j.foreco.2010.04.028
- Schär C, Vidale PL, Lüthi D, Frei C, Häberli C, Liniger MA, Appenzeller C (2004) The role of increasing temperature variability in European summer heatwaves. *Nature* 427:332–336
- Smith JE, McKay D, Niwa CG, Thies WG, Brenner G, Spatafora JW (2004) Short-term effects of seasonal prescribed burning on the ectomycorrhizal fungal community and fine root biomass in Ponderosa pine stands in the Blue Mountains of Oregon. *Can J For Res* 34:2477–2491
- Smith JE, McKay D, Brenner G, McIver J, Spatafora JW (2005) Early impacts of forest restoration treatments on the ectomycorrhizal fungal community and fine root biomass in a mixed conifer forest. *J Appl Ecol* 42:526–535. doi:10.1111/j.1365-2664.2005.01047.x
- Stendell ER, Horton TR, Bruns TD (1999) Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada Ponderosa pine forest. *Mycol Res* 103:1353–1359
- Taylor DL, Bruns TD (1999) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. *Mol Ecol* 8:1837–1850
- Trappe JM, Luoma DL (1992) The ties that bind: fungi in ecosystems. In: Carroll GC, Wicklow DT (eds) *The fungal community*, 2nd edn. Marcel Dekker, New York, pp 17–27
- Turner MG, Romme WH (1994) Landscape dynamics in crown fire ecosystems. *Landsc Ecol* 9:59–77
- Turner MG, Romme WH, Tinker DB (2003) Surprises and lessons from the 1988 Yellowstone fires. *Front Ecol Environ* 1:351–358
- Twieg BD, Durall DM, Simard SW (2007) Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytol* 176:437–447. doi:10.1111/j.1469-8137.2007.02173.x
- Vázquez FJ, Acea MJ, Carballas T (1993) Soil microbial populations after wildfire. *FEMS Microbiol Ecol* 13:93–103
- Visser S (1995) Ectomycorrhizal fungal succession in Jack pine stands following wildfire. *New Phytol* 129:389–401
- Whelan RJ (1995) *The Ecology of Fire*, 1st edn. Cambridge University Press, Cambridge
- White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (eds) *PCR protocols—a guide to methods and applications*. Academic Press, New York, pp 315–322
- Wohlgemuth T, Hester C, Joss AR, Wasem U, Moser B (2010) Recruitment dynamics following the stand replacing fire near Leuk (VS). *Schweiz Z Forstwes* 161:450–459

- Yamauchi A, Nishida T, Ohgushi T (2009) Stochastic tunneling in the colonization of mutualistic organisms: primary succession by mycorrhizal plants. *J Theor Biol* 261:74–82. doi:[10.1016/j.jtbi.2009.07.021](https://doi.org/10.1016/j.jtbi.2009.07.021)
- Zumbrunnen T, Bugmann H, Conedera M, Bürgi M (2009) Linking forest fire regimes and climate—a historical analysis in a dry inner alpine valley. *Ecosystems* 12:73–86. doi:[10.1007/s10021-008-9207-3](https://doi.org/10.1007/s10021-008-9207-3)