The impact of mountain hay meadow management on litter decomposition and root colonisation by arbuscular mycorrhizal fungi

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Summary

1. Application of liquid (slurry) instead of solid manure, and irrigation with aerial sprinklers instead of traditional gravitational, ground irrigation are two new management practices that are spreading among alpine hay meadows, aiming at increasing forage production. The effects of this agricultural intensification have been documented for plant and animal communities, but almost nothing is known about their impact on belowground processes. We thus examined the influence of these new farming practices on litter decomposition and root colonisation by arbuscular mycorrhizal fungi (AMF).

2. Using a randomised block design, six management treatments were experimentally applied in subalpine hay meadows (replicated at 11 study sites in SW Switzerland), namely: 1) management with neither irrigation nor fertilisation, used as control; 2) aerial irrigation only, by the means of sprinklers; 3) fertilisation only, with slurry (liquid manure); 4-6) aerial irrigation combined with fertilisation at, respectively, low, medium, and high levels of intensity. Litter decomposition was measured 1) by the relative residual mass (remaining after a given period of time) of buried green and rooibos tea bags and 2) with two indices: the decomposition rate (*k*) that describes the speed of decomposition, and the stabilisation factor (*S*) that characterises the environmental factors inhibiting litter decomposition. Root colonisation by AMF was analysed directly from collected soil samples as well as from «trap cultures» where AMF were amplified in pots by growing them on *Plantago lanceolata*, *Trifolium pratense* and *Lolium perenne*.

3. After four months of burying, the relative residual mass of tea bags was significantly lower in high-input than in control plots (41% and 44%, respectively,

for green tea; 69% and 74%, respectively, for rooibos), which yielded higher *k* and lower *S* values. Furthermore, *S* was lower under irrigation alone. These results suggest a faster rate of litter decomposition when meadow management is intensified.

4. Management intensification negatively affected root colonisation by AMF (direct analysis of soil samples), which was 22% lower, on average, in high-input compared to low-input plots. In the trap cultures, lower AMF concentrations were measured under medium and high management intensity for *Trifolium pratense* (minus 45% and 47 %, respectively, compared to control plots). When irrigation and fertilisation were applied alone they did not affect root colonisation by AMF.

5. *Synthesis and applications*. This study demonstrates that the intensification of hay meadow management practices accelerates soil litter decomposition and hinders root colonisation by AMF. Possible consequences from faster decomposition are altered nutrient cycles with a lower carbon sequestration potential, while decreased root colonisation by AMF may lead to restricted nutrient transfer, reducing plant growth and health. In line with former studies of the response norms of aboveground organisms to hay meadow management intensification, the present results suggest that moderate intensification may represent a good compromise in terms of biodiversity preservation, ecosystem functions and forage production.

Management intensification accelerates litter decomposition in mountain grasslands

Master thesis

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Summary

1. Litter decomposition is a key ecosystem process and plays a fundamental role in ecosystem functioning as it drives nutrient and carbon cycling and is therefore highly linked to aboveground processes. The effect of management intensification in mountain grasslands on this important soil process remains poorly known.

2. Using a randomised block design, six management treatments were experimentally applied in mountain hay meadows (n = 11) situated in Valais (SW Swiss Alps): 1) extensively managed control plot (no irrigation, no fertilisation); 2) plot only irrigated using sprinklers; 3) plot only fertilised with slurry (liquid organic manure); 4-6) as well as plots with low-, medium-, and high- input levels of water and fertiliser. Decomposition was measured by the weight loss of buried tea bags using the Tea Bag Index (TBI) approach. The relative mass remaining was analysed plus the two parameters of the TBI: the decomposition rate (*k*) describing the speed of decomposition and the stabilisation factor (*S*) characterising the inhibiting environmental effect on decomposition (see Keuskamp et al. 2013 Methods Ecol Evol).

3. After four months, the relative mass remaining was lower in intensively compared to control plots, respectively 41% and 44% for green tea, and 69% and 74% for rooibos tea, resulting in increased *k* and decreased *S* values. Furthermore, *S* was lower under irrigation alone. These results suggest faster decomposition with management intensification. Moreover, irrigation alone led to lower *S* values. Surprisingly, fertilisation applied separately did not significantly affect decomposition.

4. Soil pH positively correlated with *k* after four months of burial. A change in the decomposer community along with increased pH values due to intensification may explain faster decomposition.

5. *Synthesis and applications*. Our results demonstrate that soil litter decomposition is accelerated by intensified farming practices. Possible consequences from faster decomposition are leaky nutrient cycles with a low carbon sequestration rate. The latter is in accordance with decreased *S* values, which is a measure for stabilisation of organic carbon in plant litter, in our high-input plots. Ultimately, this study contributes to a better understanding of how different management strategies impact litter decomposition.

Keywords: agricultural intensification, alpine meadows, ecosystem processes, fertilisation, irrigation, litter, soil

Introduction

Numerous important ecosystem processes, in particular nutrient and carbon cycling along with plant nutrition and growth, are regulated by the belowground community and are not only crucial for ecosystem functioning (Bardgett & van der Putten 2014), but also for sustainable agriculture (Barrios 2007). A key role is attributed to decomposition because of its major impact on both carbon and nutrient cycling (Heimann & Reichstein 2008). In fact, a considerable proportion of primary production is not directly consumed by herbivores but decomposed as dead organic matter, called detritus (Cebrian & Lartigue 2004). The decomposer community, mainly consisting of bacteria, fungi and invertebrates, breaks down the detritus into molecules available for many organisms. This crucial recycling of materials through the decomposers activity provides two important functions. On one hand, essential elements are converted from an organic to an inorganic form, e.g. mineralisation of the nutrients such as nitrogen in the form of nitrate, which are then available for plants. On the other hand, decomposition builds up soil organic matter comprising a cellular fraction, i.e. plant, microbial and animal residues but also nutrient-rich humus (Swift, Heal & Anderson 1979). Consequently, decomposition is not only essential for soil formation, but also for the existence of various plant and animal communities as it facilitates the nutrient transfer to higher trophic levels (Swan & Kominoski 2012).

Intensified farming practices implemented over recent decades, especially high nitrogen inputs, are known to reduce the diversity and abundance of belowground species (van der Heijden 2010; de Vries *et al.* 2013; Geisseler & Scow 2014). In particular, a shift towards bacteria instead of fungi dominated food

webs (Wardle 2002) that support rapid and leaky nutrient cycles with a low net accumulation of soil carbon (Cebrian 1999; Wardle 2002) has been reported. This is in contrast to systems with lower nutrient concentrations in which nutrients are conserved by slow decomposition rates and nutrient cycles and therefore also result in higher soil carbon sequestration (Cebrian 1999; Wardle 2002; Wardle *et al.* 2004). Likewise, in grasslands, traditionally extensively managed subalpine and montane hay meadows have been highly intensified (Tasser & Tappeiner 2002; Bowman *et al.* 2006; Marini *et al.* 2008; Tonn & Briemle 2010; Riedener, Rusterholz & Baur 2013). The application of liquid (slurry) instead of solid manure (Sommer & Hutchings 2001; Velthof, Kuikman & Oenema 2003) and the progressive replacement of traditional ground irrigation with water channels by aerial irrigation with sprinklers (Crook & Jones 1999; Leibundgut 2004) are two examples of novel and common practices that were introduced to increase hay production. These management practices are known to reduce the diversity of soil biota, and may thus influence the decomposition process (Matson *et al.* 1997). Decomposition rates seem to increase under fertilisation and peak when fertilisation is combined to irrigation (Sanchez 2001). However, the effect of irrigation alone on decomposition rates are less clear; there is either no effect (Sanchez 2001) or greater litter mass loss with increasing irrigation inputs (Guo & Sims 2001; Zhang *et al.* 2008).

The impact of fertilisation and irrigation on plant and invertebrate communities in mountain grasslands has already been investigated (Riedener, Rusterholz & Baur 2013; Andrey *et al.* 2014; Melliger *et al.* 2014; Riedener *et al.* 2015; Andrey, Humbert & Arlettaz 2016; Humbert *et al.* 2016) while belowground responses remain largely unknown (Wardle *et al.* 2004). Today, there is a lack of

knowledge about the impact of management, climate, as well as ecosystem and soil type on decomposition rates at the global scale (Zhang *et al.* 2008; Keuskamp *et al.* 2013). Keuskamp *et* al. (2013) highlighted the need for experimental studies on the impact of different management strategies aiming to create a global soil map of decay rates (2013). In terms of management it is known that intensification changes the belowground community but its consequences on agro-ecosystem functions remains poorly understood. Designing agriculture in a way to maintain a balance between production and soil functioning therefore remains a challenge but is necessary for sustainable agricultural production in the long-term (Matson *et al.* 1997).

To fill this knowledge gap, we tested whether mountain grassland intensification (irrigation with sprinklers and/or fertilisation with slurry) changes the decomposition speed. Six management treatments were therefore experimentally applied in extensively managed hay meadows (n= 11) located in the Swiss Alps. Decomposition was measured implementing a simple, standardised and cost-effective Tea Bag Index (TBI) approach developed by Keuskamp *et al.* (2013) by using tea bags as litterbags. Litterbags have been widely used to measure mass loss and compare decomposition rates between sites, litter types and treatments, e.g. climate change or different land use types, in respect of carbon and nutrient cycles (Prescott 2010; Bonan *et al.* 2013). Green and rooibos tea bags were buried in each plot and the weight loss was measured after two and four months. Accordingly, we calculated the relative mass remaining but also the two parameters of the TBI: the decomposition rate (k) describing the turnover time of the labile carbon pool in plant litter, i.e. the speed of decomposition and the stabilisation factor (S), a measure for the stabilisation of organic carbon in plant litter, i.e. the inhibiting

environmental effect on decomposition. Using those two types of tea allowed measuring *k* and *S*, which indicate two different stages of plant decay (see Appendix 2 in Supporting Information, for decay curves), after only one measurement in time. The initial decomposition rate constant *k* in the first phase was determined from the slowly decomposing rooibos tea (woody litter) while stabilisation (*S*) in the second phase was calculated from the easily decomposable green tea (leaf litter).

Our study meadows were extensively managed for a long time (≥ 10 years) before the experiment started and the management treatments were already applied for five years by the time we measured decomposition. An impact of the treatments on the speed of decomposition was therefore assumed. We expected increased decomposition rates in response to the intensity gradient (Wardle *et al.* 2004) peaking in high-input plots with a combination of both irrigation and fertilisation (Sanchez 2001). Furthermore, we predicted faster decomposition due to fertilisation alone (Sanchez 2001). The impact of irrigation alone seems to be less clear therefore we assumed either no or an increasing effect on plant decay (Guo & Sims 2001; Sanchez 2001).

To better understand the mechanisms how intensified management practices affect soil processes we measured several other soil parameters (pH, humus-, phosphorus- and nitrogen content) and tested whether they differed among experimental plots. As a consequence from the expected faster decomposition, we predicted higher levels of humus as it is formed through the process of decomposition and accumulates over time (Swift, Heal & Anderson 1979; Berg 2000). We predicted higher phosphorus and nitrogen levels in fertilised plots (F, I+F 1/3, I+F 2/3 and I+F 3/3), peaking in high-input plots (I+F 3/3) due to increased

nutrient inputs. Additionally, we expected an in increase in pH in according to higher fertiliser inputs (F, I+F 1/3, I+F 2/3 and I+F 3/3) because the organic fertiliser used in our study contains Calcium (CaO) and Magnesium (MgO). Fertilisation treatments may therefore act like "liming"; an agricultural practice applied to raise soil pH (Haynes & Naidu 1998). For pH, we further assessed whether there is a direct relationship with decomposition rates. There is a lack of studies investigating the role of soil pH on decomposition but it is known that the belowground community composition is influenced by pH (Rousk *et al.* 2010), which in turn affects the decomposition process (Wardle *et al.* 2004). Changes in pH due to intensification may therefore directly influence decomposition. We predict a positive correlation between soil pH and decomposition rates. On one hand because bacteria seem to dominate over fungal communities in systems with faster decomposition (Couteaux , Bottner & Berg 1995; Wardle *et al.* 2004) and on the other hand, because bacteria seem to be positively related with pH (Rousk *et al.* 2010).

Materials and methods

STUDY SITES

In 2010, eleven montane or subalpine hay meadows were selected in Valais, SW Switzerland (Appendix 1). Central Valais, the location of our study sites, is characterised by a continental climate with cold winters and dry as well as hot summers. The selected meadows were extensively managed for at least ten years before the study started with no or very low fertiliser and water applications and

only one mowing event per year. Additionally, the meadows were situated along an altitudinal gradient between 790-1740 m above sea level. For details on meadow selection see Andrey *et al*. (2016).

STUDY DESIGN

A randomized block design was used in which every meadow (the block, n = 11) harboured six experimental plots of 20 m diameter (314 m^2) with a buffer zone of at least 5 m between plots. Six different management treatments (= intensity levels of irrigation and/or fertilisation) were randomly assigned to the plots: 1) control plot (C): extensively managed without fertilisation and irrigation; 2) plot only irrigated (I); 3) plot only fertilised (F); as well as plots that were irrigated and fertilised at low (I+F 1/3), medium (I+F 2/3), and high (I+F 3/3) intensity levels. C-plots were mown once a year according to standards of traditional extensively managed hay meadows while all the other plots were mown twice a year. Irrigated plots were watered weekly with sprinklers from mid-May to the beginning of September, unless it was raining strongly. Slurry, consisting of dried organic manure NPK pellets and mineral potassium oxide (K_2O) dissolved in water, was applied in May and August. The maximum input of slurry (I+F 3/3) was calculated according to the theoretical hay production potential of each meadow to reach maximum productivity. The applied doses of slurry represented a site-adapted intensification gradient ranging from extensively (C) to intensively (I+F 3/3) managed and are listed in the study of Andrey *et* al. (2016). Plots only fertilised (F) received medium inputs of slurry, thus the same amount as I+F 2/3 plots. In contrast, irrigation treatments were not site-adapted but equal in all plots of a specific treatment.

Similarly to the F treatment, I plots received medium inputs of water, thus the same amount as I+F 2/3 plots.

Decomposition was measured following a simple and standardised Tea Bag Index (TBI) approach developed by Keuskamp *et al.* (2013) where commercially available tea bags are used as litterbags (see TBI subsection below). We performed our experiments with tetrahedron-shaped synthetic bags with sides of 5 cm containing green tea (dried leaves of *Camellia sinensis*; Lipton, EAN 87 22700 05552 5) or rooibos tea (dried leaves of *Aspalathus* linearis; Lipton, EAN 87 22700 18843 8). The initial weight (precision 10^{-4} of a g) of each tea bag (6 pairs x 6 plots x 11 meadows = 396 pairs = 792 tea bags) was measured at the beginning of the experiment. Six pairs (consisting of one bag of green tea and one bag of rooibos tea) per plot were buried on a radius 2 m away from the plot centre at 8 cm depth according to the protocol of Keuskamp *et al.* (2013) between end of April and beginning of May 2016, before fertilisation application (Appendix 1). Each pair was attached to a nail (7 mm x 210 mm) through a rope (Polypropylene, 1 mm) for recovery. Three pairs were collected after two months (end of June / beginning of July) and the remaining three pairs after four months (end of August / beginning of September). Directly after collection tea bags were dried at 40 °C for at least 48 h to stop further biological activity and keep the bags until further processing. Later in the lab, before measuring the final weight of each bag (precision 10^{-4} of a g) tea bags were again oven dried (24 h at 70 °C) to make sure they were all completely dry.

RELATIVE MASS REMAINING

For every tea bag, the fraction (relative mass) remaining $W(\mathrm{t})$ after burial time t (days) was calculated using the following equation (Keuskamp *et al.* 2013):

$$W(\mathrm{t}) = \frac{final\ weight}{initial\ weight} \qquad \text{eqn 1}$$

where *final weight* is the dry weight of the tea inside the bag after decomposition and *initial weight,* the dry weight of the tea inside the bag before burial. The weight of the bag, cord and label was subtracted before calculating W.

TEA BAG INDEX (TBI)

During decomposition, easily degradable compounds in plant litter are more rapidly decomposed compared to more recalcitrant compounds. The decay curve is thereby characterised by two stages: a fast initial phase and a slower second phase (Appendix 2). The TBI comprises two parameters describing those two different phases. The speed of initial decomposition in phase one is described by the decomposition rate (k), a measure for the turnover time of the labile carbon pool in plant litter. The inhibiting effect of environmental conditions on decomposition in the second phase is described by the stabilisation factor (S), representing the stabilisation of organic carbon in plant litter. Consequently, the decomposition rate constant k can only be assessed from initial stages of decomposition whereas the labile (decomposable) proportion a, which is needed to know to assess the extent of stabilisation S, cannot be estimated before most of the labile fraction is lost. Using

just one type of plant material would thus assume time series to estimate both *k* and *S*. To overcome this problem, the approach of Keuskamp *et al.* (2013) uses two litter bag types differing in decomposability. In this way, *k* and *a* can be calculated in one step. The fast decomposing green tea (leaf litter) allows assessing how much of the labile fraction is decomposed and how much is stabilised in the second phase (*S*). In turn, the slowly decomposing rooibos tea (woody litter) is still in the first phase of decomposition at that time and its weight loss indicates the initial decomposition rate (*k*).

Decomposition rates were calculated using the following equations (Keuskamp *et al.* 2013):

$$W_r(t) = a_r e^{-kt} + (1 - a_r) \qquad \text{eqn 2}$$

where W_r(t) is the relative mass remaining of the rooibos tea after burial time *t* *(*days), *k* is the decomposition rate constant and a_r is the predicted labile (decomposable) fraction of the rooibos tea. Fractions of the labile proportion stabilise with time and become recalcitrant (persistent). The extent of this stabilisation process, which is the difference between observed and expected mass loss, depends on environmental conditions and leads to a discrepancy of the real decomposed fraction *a* from the hydrolysable (chemically labile) proportion *H* (Appendix 2). The stabilisation factor *S* therefore indicates the inhibiting effect of the environment on the breakdown of the labile fraction:

$$S = 1 - \frac{a_g}{H_g} \qquad \text{eqn 3}$$

where a_g is the decomposable and H_g the hydrolysable proportion of green tea. H_g is a constant estimated by Keuskamp *et al.* (2013) after chemical composition analysis and a_g is simply the decomposed fraction of green tea after burial time t (days) based on the mass loss in the field:

$$a_g = 1 - \frac{final\ weight\ green\ tea}{initial\ weight\ green\ tea} \qquad \text{eqn 4}$$

As rooibos tea decomposes slowly, its decomposable fraction (a_r) could not be assessed in the field after a short burial time. Thus, it was estimated from the hydrolysable fraction of rooibos tea (H_r) and the stabilisation factor (S) after the assumption that stabilisation factors are equal for both tea types. H_r, the chemically expected labile fraction of rooibos tea, is a constant estimated by Keuskamp *et al.* (2013) after chemical composition analysis.

$$a_r = H_r\,(1 - S) \qquad \text{eqn 5}$$

The decomposition rate k was finally calculated from mass loss of rooibos tea, using eqn 2 with the previously calculated parameters $W_r\,(t)$ and a_r and the burial time t (days).

SOIL PARAMETERS

Two months after fertilisation, and after mowing, five pooled samples were collected per plot at a distance of 7 m from the plot centre (Appendix 4) with a soil

core sampler (2 - 10 cm deep, Ø 8 cm). Samples were stored in a cooling room at 2.3 ° C. Analyses were performed at Sol Conseil (Nyon, Switzerland) on four soil parameters: (a) pH measured by suspending soil in water; (b) humus content (%); (c) phosphorus (mg / kg dried soil) measured using the H2O10 method (extraction in water); and (d) total nitrogen in the soil (N_{tot}) in percentage (lab protocols can be found at: http://www.sol-conseil.ch/fr/Laboratoire/Methodes/Resumes-de-methode.html).

STATISTICAL ANALYSES

Decomposition indices as well as soil parameters were analysed with linear mixed-effects models (LMMs) using the *lmer* function from the *lme4* R-package (Bates *et al.* 2015). For the decomposition indices, the two sampling sessions were analysed separately to test whether a burial time of two or four months is more appropriate to measure decomposition in alpine grasslands. In order to avoid pseudoreplication, the mean was calculated for the three replicates of a given plot, finally resulting in one value per plot and meadow (n =66). Response variables for decomposition were the relative mass remaining of green and rooibos tea, the decomposition rate *k* and the stabilisation factor *S*. For the soil parameters, response variables were pH, humus-, phosphorus-, and nitrogen content. For both data sets, management treatment effects were included in the model as fixed effects and meadows as a random effect to account for the variation between sampling sites. The *relevel* function in R, which allows changing the reference level of the fixed effects in the LMM, was used to examine pairwise differences among treatments.

To test whether there is a relationship between the decomposition rate constant *k* and soil pH, we fitted the log-transformed decomposition rate against pH. Again, meadows were included as a random effect to account for the variation between sampling sites. All statistical analyses were performed using R version 3.2.2 (R Development Core Team 2015).

Results

RELATIVE MASS REMAINING

After two months of burial, analyses performed on green tea bags revealed that there was less relative mass remaining when irrigated (I) or intensively managed (I+F 3/3) compared to the low-input treatment (I+F 1/3). In addition, fertilisation (F) applied separately did not influence weight losses. No differences were detected for rooibos tea after two months (see Appendix 3 in Supporting Information, for detailed model outputs).

After four months, relative mass remaining of green tea was lower in I and I+F 3/3 plots compared to C plots. Moreover, there was a difference between I+F 1/3 and I+F 3/3 treatments with less relative mass remaining in the latter. Likewise, less relative mass of rooibos tea was remaining in medium-input (I+F 2/3) and I+F 3/3- plots compared to control C-plots. Furthermore, the treatment I+F 3/3 led to lower levels of mass remaining compared to the F treatment (Fig. 2).

DECOMPOSITION RATE (*K*) AND LITTER STABILISATION FACTOR (*S*)

After two months of burial, we found no significant effect of management on *k*. However, *S* was lower in I+F 3/3 and I-plots compared to I+F 1/3-plots (see Appendix 3 in Supporting Information, for detailed model outputs).

After four months of burial, *k* was higher under intensive management (I+F 3/3) compared to C- plots (Fig. 1a). *S* was decreased in plots under the I+F 3/3 treatment compared to I+F 1/3-plots. Additionally, *S* values were lower in irrigated (I) and intensively managed plots (I+F 3/3) compared to control plots (C).

SOIL PARAMETERS

Soil pH was significantly higher in high-input plots (I+F 3/3) compared to control plots (C) and even showed a positive correlation with decomposition rates (estimate = 0.325, SE = ± 0.068, $t = 4.772$, P = < 0.001). The phosphorus content in the soil, peaked in F-plots but levels did not significantly differ between I+F 3/3 and C-plots or I+F 1/3-plots. For total nitrogen in the soil as well as humus content we did not detect any differences in response to the treatments (see Appendix 4 in Supporting Information, for detailed model outputs).

Discussion

This study experimentally tested whether the speed of litter decomposition is altered by grassland management intensification in the Swiss Alps. Despite the fact that decomposition plays a key role in nutrient and carbon cycling, global

decomposition data about the impact of different management is lacking (Keuskamp *et al.* 2013). Our results contribute to fill this knowledge gap and demonstrate that a combination of high inputs of slurry and irrigation with sprinklers (I+F 3/3) increased the decomposition rate after five years of application. Irrigation (I) and fertilisation (F) alone with medium inputs, did not change decomposition rates. Still, irrigation alone resulted in lower stabilisation of organic carbon in plant litter, indicating a lower inhibiting effect of environmental conditions on the decomposition process as well as a lower stabilisation of organic carbon in plant litter.

The present study is among the first applying the innovative TBI approach and therefore contributes to the collection of uniform decomposition data a global scale (Keuskamp *et al.* 2013). We measured the speed of decomposition in response to management intensification by weight losses of buried green and rooibos tea bags after two and four months. We consequently analysed the relative mass remaining as well as the resulting decomposition rates and stabilisation factors. In addition, we compared two different burial times and included soil pH in our analyses. Furthermore, the experimental design of this study is originality and allowed disentangling single treatment besides investigating the intensification gradient. Two novel and common management types (slurry and aerial irrigation) were therefore tested separately as well as combined at low, medium and high intensity levels in experimental plots in the field.

RESPONSE TO MANAGEMENT INTENSIFICATION GRADIENT

After five years of treatment application, more green and rooibos tea was decomposed in I+F 3/3-plots than in extensively managed control plots (C) confirming the conclusions of previous studies (Guo & Sims 2001; Zhang *et al.* 2008). Our results demonstrate this effect by less relative mass remaining after four months of burial (Fig. 1). The I+F 3/3 treatment consequently increased the decomposition rate *k* compared to C-plots. Environmental conditions under intensive management therefore act less constraining on the decay of organic material compared to traditional management showed by a lower stabilisation factor *S* (Fig. 2).

The decomposition speed increased along our experimental intensification gradient from no (C) to low (I+F 1/3), medium (I+F 2/3) and high inputs (I+F 3/3) illustrated by the weight losses of green and rooibos tea bags (Fig 1). However, this pattern was only statistically different between the most extreme treatments (I+ F 3/3 vs. C and I+F 1/3 for green tea; respectively I+ F 3/3 vs. C for rooibos tea). The decreasing stabilisation factor along the gradient supports this outcome and mirrors the negative effect of the environment on the decomposition process (Fig. 2).

SEPARATE EFFECTS OF IRRIGATION AND FERTILISATION

Against expectations, decomposition rates were not higher under either fertilisation or irrigation treatments alone (Guo & Sims 2001; Sanchez 2001; Zhang *et al.* 2008). Although *k* values were slightly higher compared to control conditions (C), the pattern was not significant after statistical analysis (Fig. 2a). Nevertheless, we

found a decreasing effect of irrigation on stabilisation factors (Fig. 2b). *S* is calculated from the rapid decomposing green tea which lost more weight in irrigated than in control plots. In contrary to green tea, rooibos tea decomposed much more slowly and the lower values of the relative mass remaining under irrigation were statistically not significant.

Eventually, an effect could maybe be detected under high- instead of only medium inputs of I and F treatments.

RELATIONSHIP BETWEEN THE PARAMETERS

The relative weight loss of each tea bag was first calculated to estimate the relative mass remaining for both green and rooibos tea. Assuming a two-phase decomposition model (Appendix 2), green tea reaches the second phase where decomposition is faster and stagnates after two to three months. In a second step, we therefore calculated how much of the labile (decomposable) fraction of the tea is decomposed and how much is stabilised (*S*) using the weight losses of the rapidly decomposing green tea. At the same point of time, the slowly decomposing rooibos tea is still in the first phase with constant and fast decomposition. Decomposition rates (*k*) were estimated in the third step using the mass fraction remaining as well as the decomposable fractions of rooibos tea after two respectively four months of burial. To overcome the problem that rooibos tea decomposes too slowly to estimate decomposable fractions in the field in such a short time, decomposable fractions of rooibos tea were calculated under the assumption that *S* is equal for both tea types. Decomposable fractions of rooibos tea were therefore calculated using *S* that was beforehand estimated from green tea.

In conclusion, the relative mass remaining was estimated for both tea types separately while *S* was calculated from decay of green tea only *k* included weight loss of both green and rooibos tea. This approach explains why differences in relative mass remaining for green tea as well as *S* are more pronounced as weight losses of green tea is greater than rooibos tea.

INCUBATION (BURIAL) TIME

A novelty of our approach compared to the base study of Keuskamp *et al.* (2013) is that we collected tea bags in two sessions, respectively after two and four months of burial instead of three months because we did not know decay rates in our sites. We decided to collect tea bags after two respectively four months instead of after only three months.

The three response variables (relative mass remaining, *k* and *S*) showed similar responses after two months (see Appendix 4 in Supporting Information, for detailed model outputs) compared to after four months (Fig. 1-2) but the effect was less pronounced after two months. We explain this finding by the smaller proportion of the plant material decomposed after only two months of burial. Therefore, in alpine grasslands an incubation time of two months may be too short to reach the limit value (flatter decomposition curve when stabilisation is reached) of green tea resulting in the overestimation of S (Keuskamp *et al.* 2013). In addition, standard errors of *k* were much larger after only two (Appendix 3) compared to after four months (Fig. 2). This outcome indicates that decomposition rates varied a lot between our study sites after only two months. However, what is striking is the fact that the standard error for I+F 3/3 plots remains after four months of burial (Fig. 2).

This outcome can be relevant for designing future tea bag experiments. In some of our I+F 3/3 plots at lower elevation sites that were exposed to higher ambient temperatures, *k* could not be calculated anymore due to outstanding high weight losses leading to missing values of *k*. Keuskamp *et al*. (2013) addressed this problematic and recommend a reduced incubation time for such "extreme" sites in order to prevent that the weight loss of rooibos tea approaches the limit value finally leading to an underestimation of *k*.

Eventually, we recommend a burial time of three months as two months seem to be too short and four months too long for future decomposition experiments in alpine grasslands, unless experiments would only be assessed at higher elevation (> 1000 m) to avoid losing too much material due to increased temperature.

SOIL PARAMETERS

A possible explanation for faster decomposition in our high-intensity plots is the higher pH potentially accelerating the decomposition process. As suggested by previous studies, bacteria dominated soil communities can cause faster decomposition. Bacteria seem to profit from increased pH levels and lead to systems with faster decomposition compared to fungi dominated systems (Couteaux , Bottner & Berg 1995; Wardle *et al.* 2004; Rousk *et al.* 2010). We found no significant differences between treatments in soil nitrogen or phosphorus content (Appendix 4). Despite this outcome, additional inputs of nitrogen and phosphorus could still contribute to the observed findings. Nutrients might be taken up very rapidly by plants instead of being accumulated in the soil. Surprisingly and against

expectations (Swift, Heal & Anderson 1979; Berg 2000), humus content was equal for all treatments (Appendix 4, Supporting Information). Still, the pattern illustrates an accumulation of humus in response to the intensification gradient (Appendix 4, Supporting Information) but the effect was not statistically significant. This either represents the absence of a true effect, or the effect could not be shown due to the relatively large variances in the data set. The present study showed that the speed of decomposition is accelerated by intensified farming practises but further research should focus on the causes and mechanisms behind this phenomenon as well as its consequences.

CONCLUSIONS

After five years of experimental application, intensive management, which combines high doses of slurry and irrigation with sprinklers, accelerated the speed of decomposition and decreased stabilisation of the organic carbon pool. A change in the decomposer community along with increased pH values due to intensification may explain faster decomposition in our study (Couteaux , Bottner & Berg 1995; Wardle *et al.* 2004; Rousk *et al.* 2010). Possible negative consequences of intensified management combining high inputs of slurry and water are leaky nutrient cycles (Kowalchuk & Stephen 2001; Bardgett *et al.* 2005) and lower carbon sequestration in the soil (Heimann & Reichstein 2008). The latter is in accordance with decreased *S* values, which is a measure for stabilisation of organic carbon in plant litter (Keuskamp *et al.* 2013) in our high-input plots. Besides alterations in the soil system, previous studies have shown that decomposition is highly linked to aboveground systems i.e. nutrient dynamics, plant community

composition (Wardle *et al.* 2004) and plant production (Bardgett *et al.* 2005). Such impacts are especially concerning in the face of the still ongoing intensification of mountain European grasslands (Tasser & Tappeiner 2002).

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Figure legends

Fig. 1

Relative mass remaining in green tea (a) and rooibos tea (b) after four months of burial. Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. Different letters indicate significant differences among treatments at an alpha rejection value set to 0.05. Mean values ± standard errors (SE) are presented.

Fig. 2

Decomposition rates *k* (a) and stabilisation factors *S* (b) after four months of burial in response to the different management treatments. For the treatment abbreviations see Fig. 1. Different letters indicate significant differences among treatments at an alpha rejection value set to 0.05. Mean values ± standard errors (SE) are presented.

Fig. 1

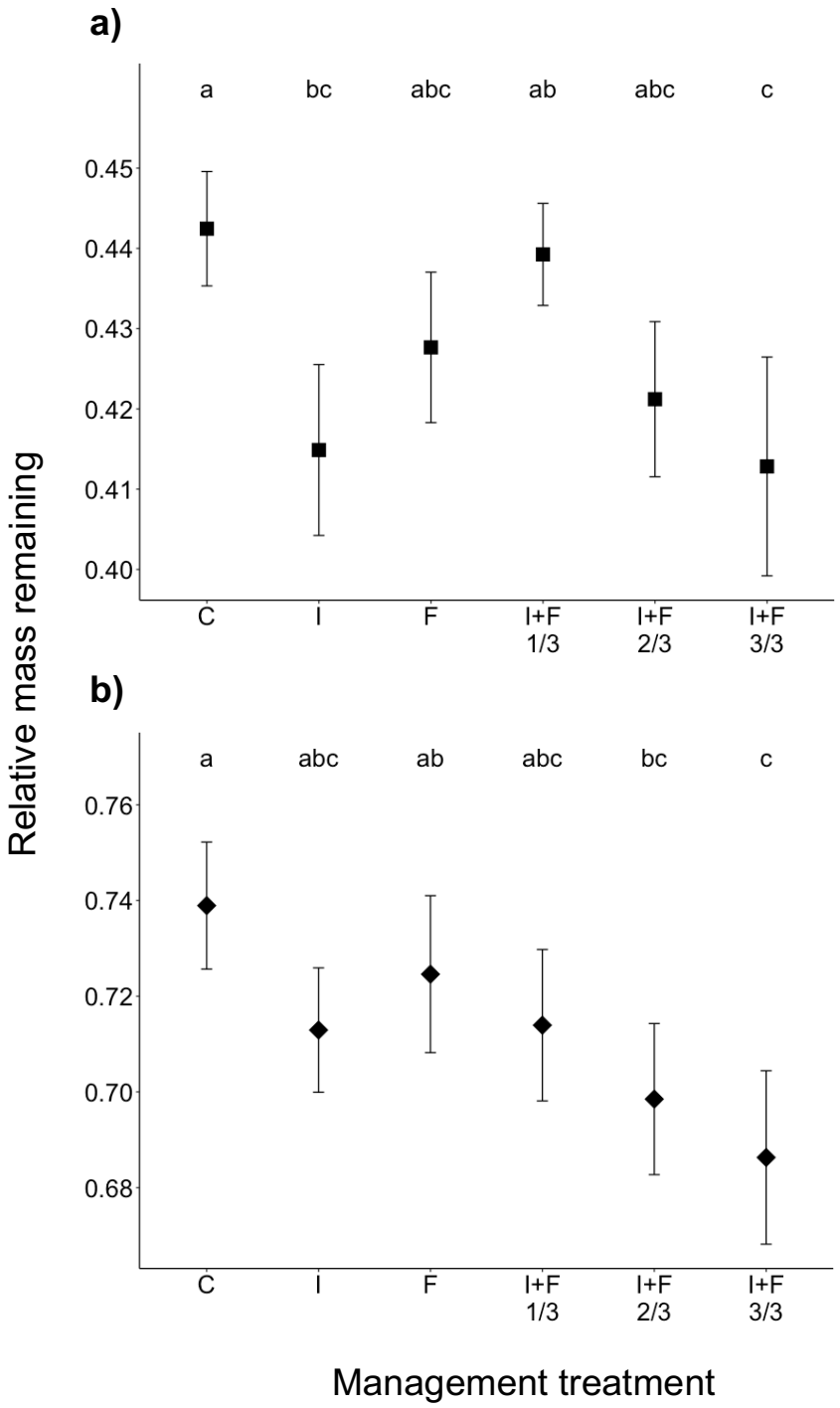
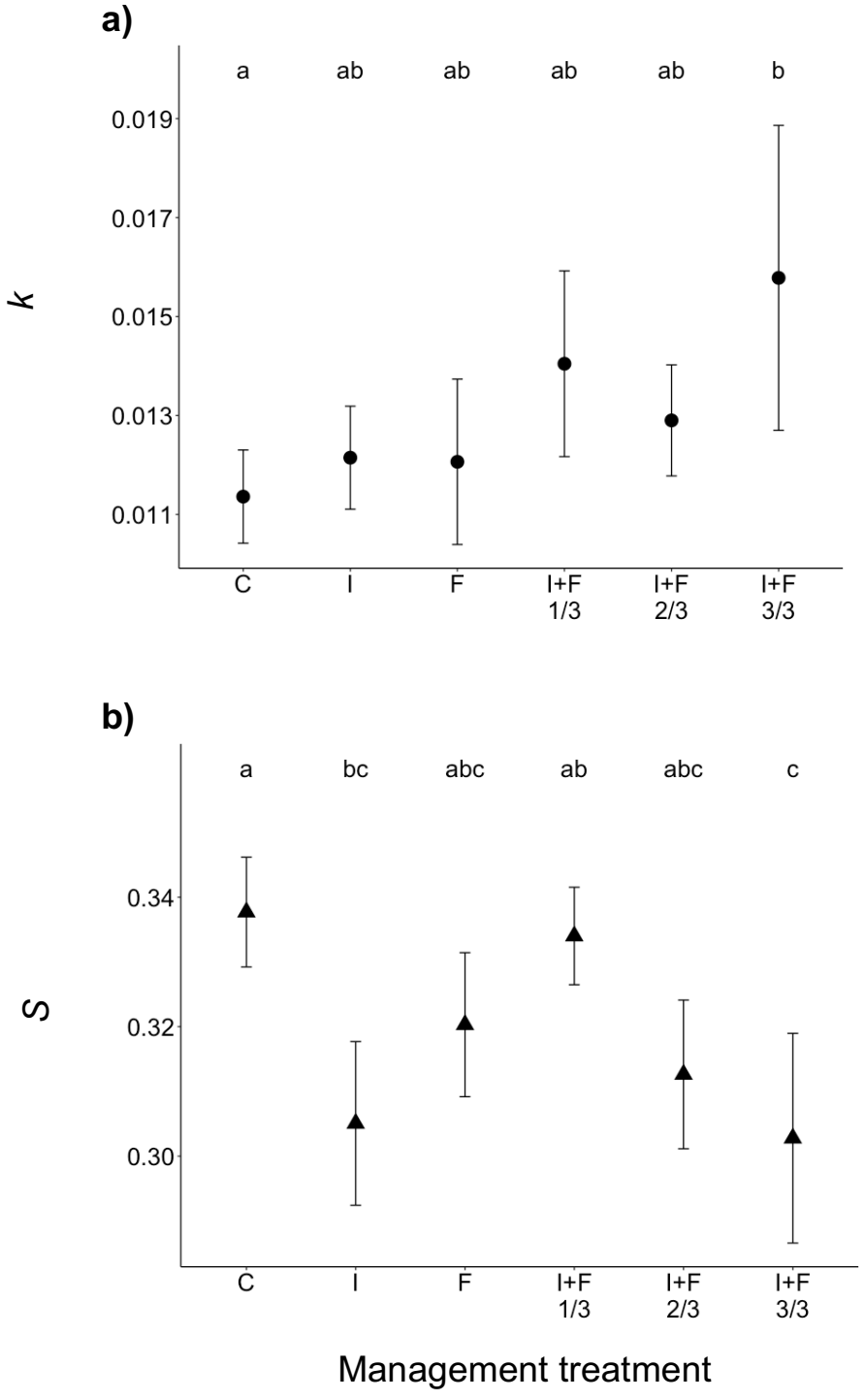

a)
a
bc
abc
ab
abc
c
0.45
0.44
0.43
0.42
0.41
0.40
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
b)
a
abc
ab
abc
bc
c
0.76
0.74
0.72
0.70
0.68
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
Relative mass remaining
Management treatment

Fig. 2


a)
a
ab
ab
ab
ab
b
0.019
0.017
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0.011
k
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
b)
a
bc
abc
ab
abc
c
0.34
0.32
0.30
S
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
Management treatment

Supporting Information

Appendix 1. Location of the different study sites (n = 11 replicates) and study design.

Fig. S1.1. Map of Switzerland with the eleven study sites (white dots) in the canton of Valais (black region).

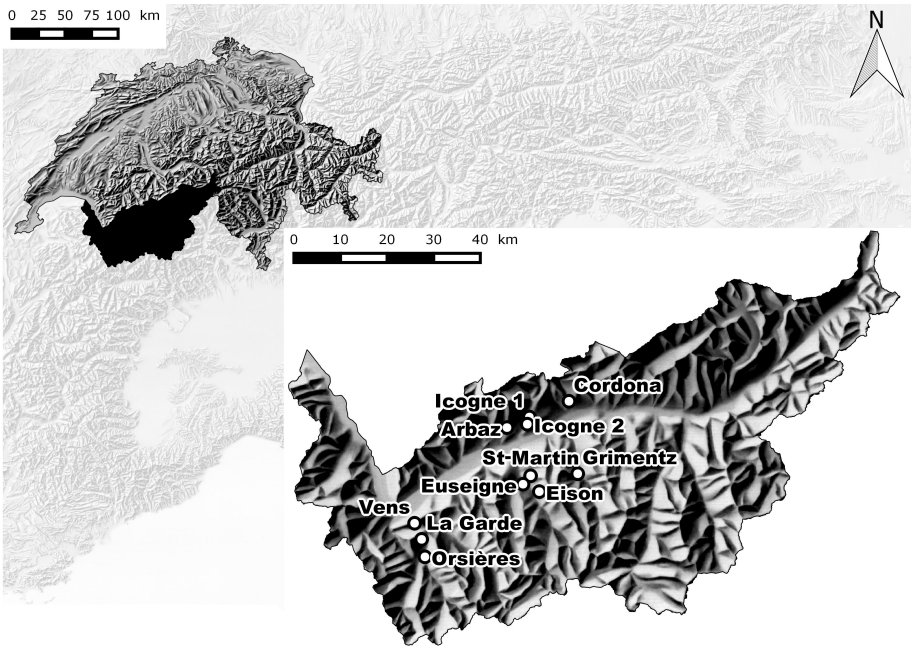
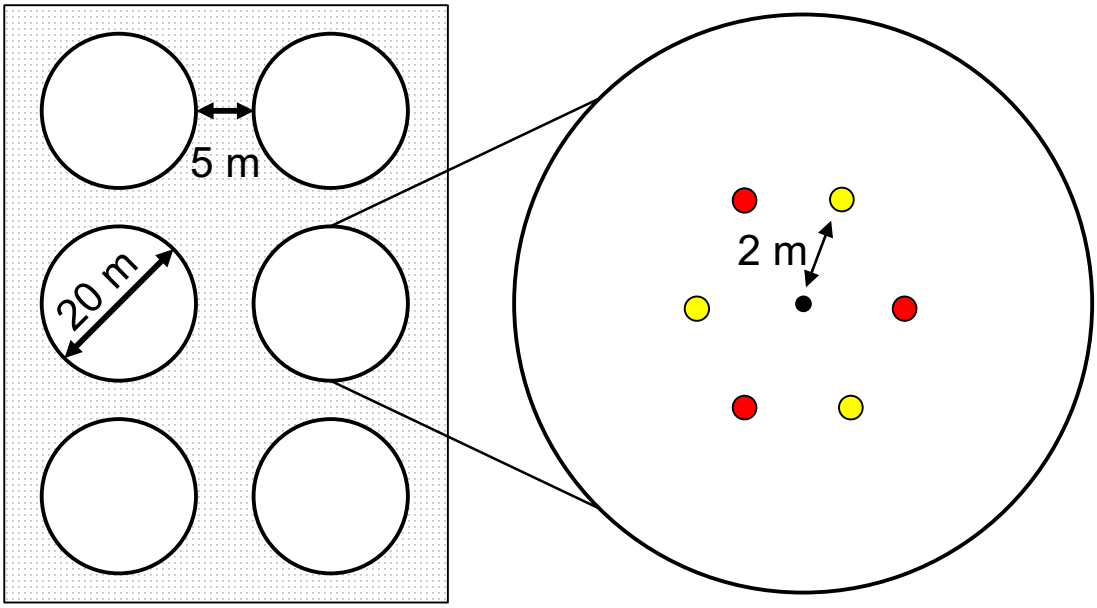


Table S1.1. The eleven study sites with altitude and geographical coordinates.

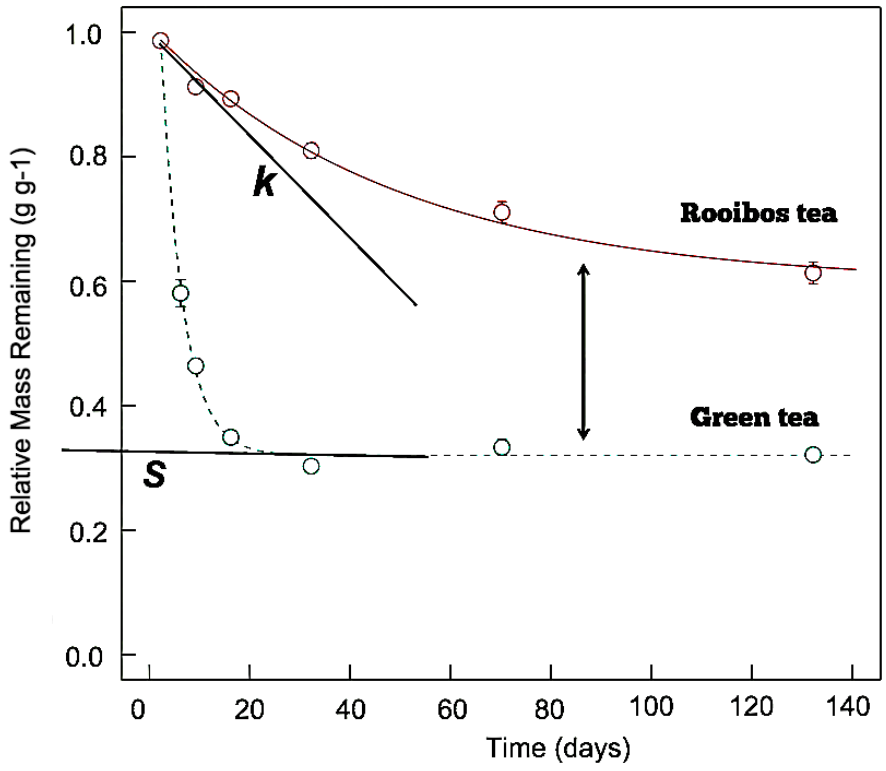
			Coordinates	
Sites	**Name**	**Altitude [m]**	**Latitude**	**Longitude**
1	Orsières	1022	46°1′44″N	7°9′8″E
2	Euseigne	1028	46°10′9″N	7°25′27″E
3	Icogne 2	880	46°16′42″N	7°26′10″E
4	La Garde	980	46°3′45″N	7°8′35″E
5	Vens	1373	46°5′7″N	7°7′24″E
6	Arbaz	1270	46°16′42″N	7°22′47″E
7	Icogne 1	1200	46°17′56″N	7°26′31″E
8	Cordona	1153	46°19′45″N	7°33′8″E
9	Eison	1768	46°9′18″N	7°28′10″E
10	Saint-Martin	1589	46°11′8″N	7°26′43″E
11	Grimentz	1738	46°11′22″N	7°34′35″E

Fig. S1.2 Experimental design. Each meadow harbours six different experimental plots of 20 m in diameter with a minimum of 5 m buffer zone between them. The six different management treatments were randomly allocated to the plots. Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. Six pairs of teabags (green and rooibos tea) were buried two meters from the plot centre (black dot). Teabags were collected in two sampling sessions: three pairs after two months (yellow dots) and the remaining three pairs (red dots) after four months.



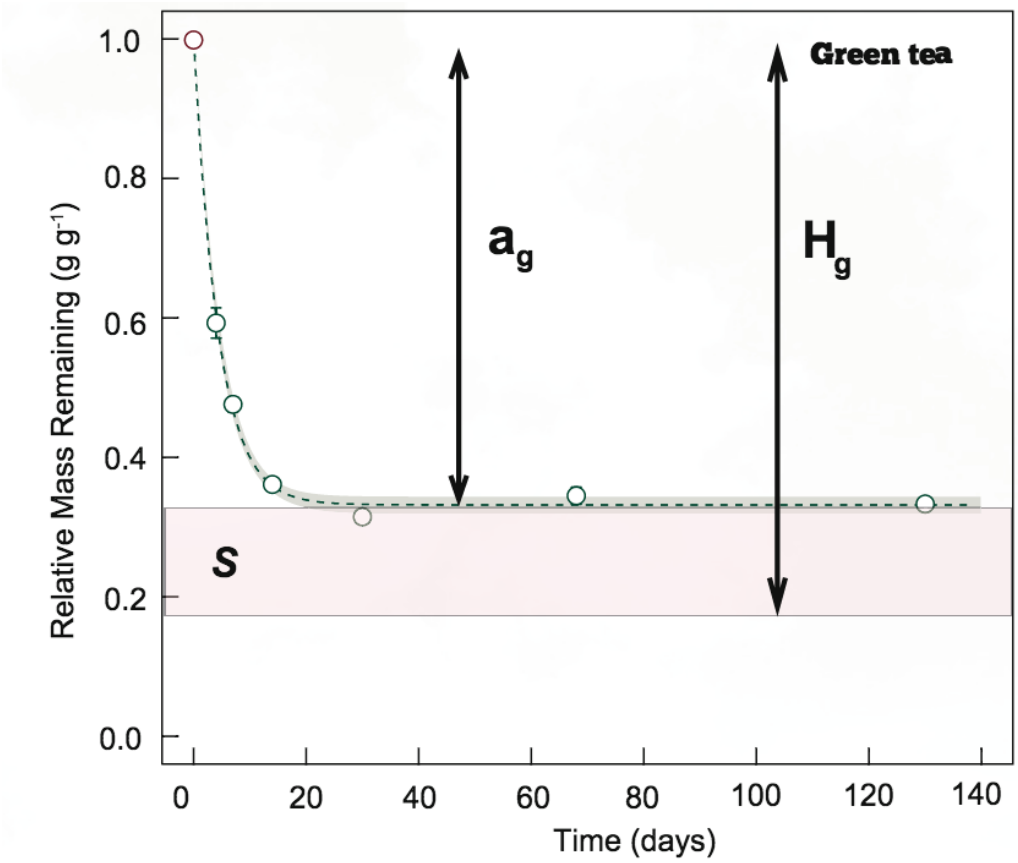
Appendix 2. Two-phase decomposition model

Fig. S2.1. Decomposition follows a two-phase model with a rapid first phase with a constant decomposition rate *k* and a more slowly second phase in which weigh loss decreases and approaches the limit decomposition value *a*. In this second phase, fractions of the labile (decomposable) proportion stabilise with time and become recalcitrant (persistent). The extent of this stabilisation process is environment-dependent and characterised by the stabilisation factor *S*. The TBI method uses two types of tea that differ in decomposability, to measure *k* and *S* in only one measurement in time. Weight loss of the rapidly decomposing green tea allows estimating the fraction that gets stabilised *S* while the initial decomposition rate *k* of the labile fraction is assessed from the decay of rooibos tea, which is still in the first phase by that point of time (Keuskamp *et al.* 2013).



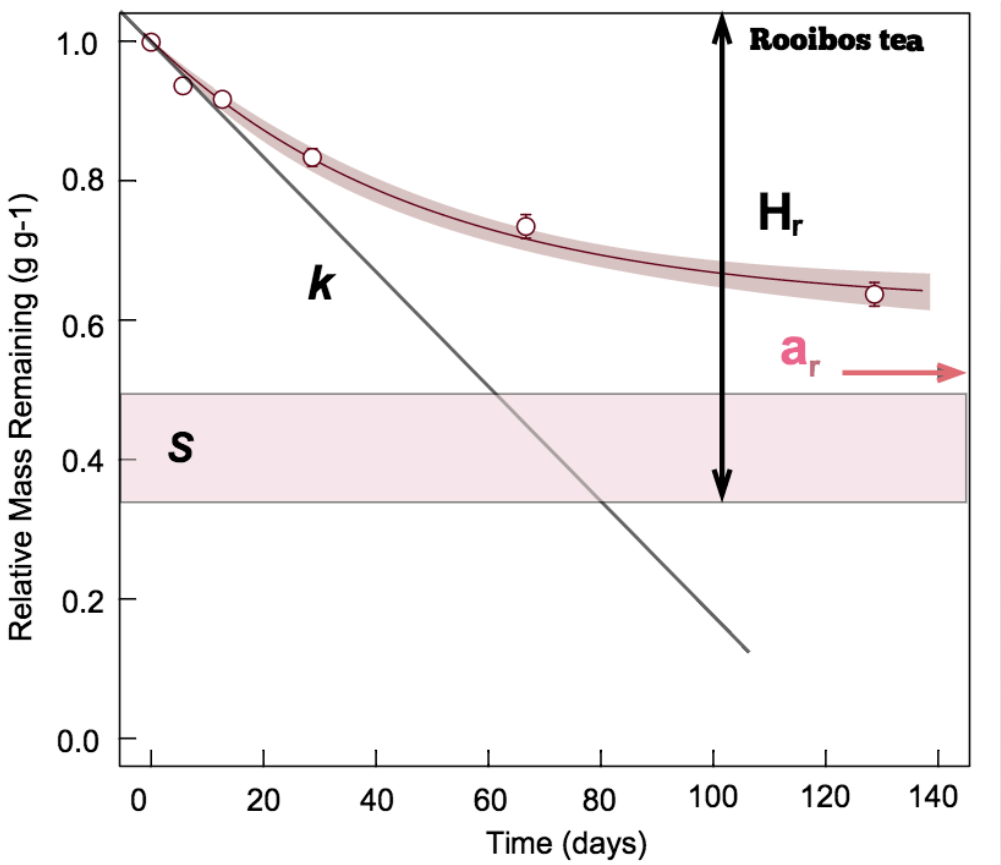
(Keuskamp, J.A., unpublished lecture material (2016))

Fig. S2.2. The stabilisation factor *S* was calculated from the difference between expected and observed weight loss of buried green tea using the observed mass loss in the field a_g and the expected hydrolysable fraction H_g estimated by Keuskamp *et al* (2013).



(Keuskamp, J.A., unpublished lecture material (2016))

Fig. S2.3. Using *S* calculated from weight loss of green tea and by assuming that S is equal for both tea types, the limit value for rooibos tea a_r was assessed, which finally allowed to estimate the initial decay rate *k* from weight loss of rooibos tea (Keuskamp *et al.* 2013).



(Keuskamp, J.A., unpublished lecture material (2016))

Appendix 3. Response of decomposition to different management treatments

RELATIVE MASS REMAINING

Fig. S3.1. Relative mass remaining in green tea (a) and rooibos tea (b) after two months of burial in response to the different management treatments. Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. Different letters indicate significant differences among treatments at an alpha rejection value set to 0.05. Mean values ± standard errors (SE) are presented.

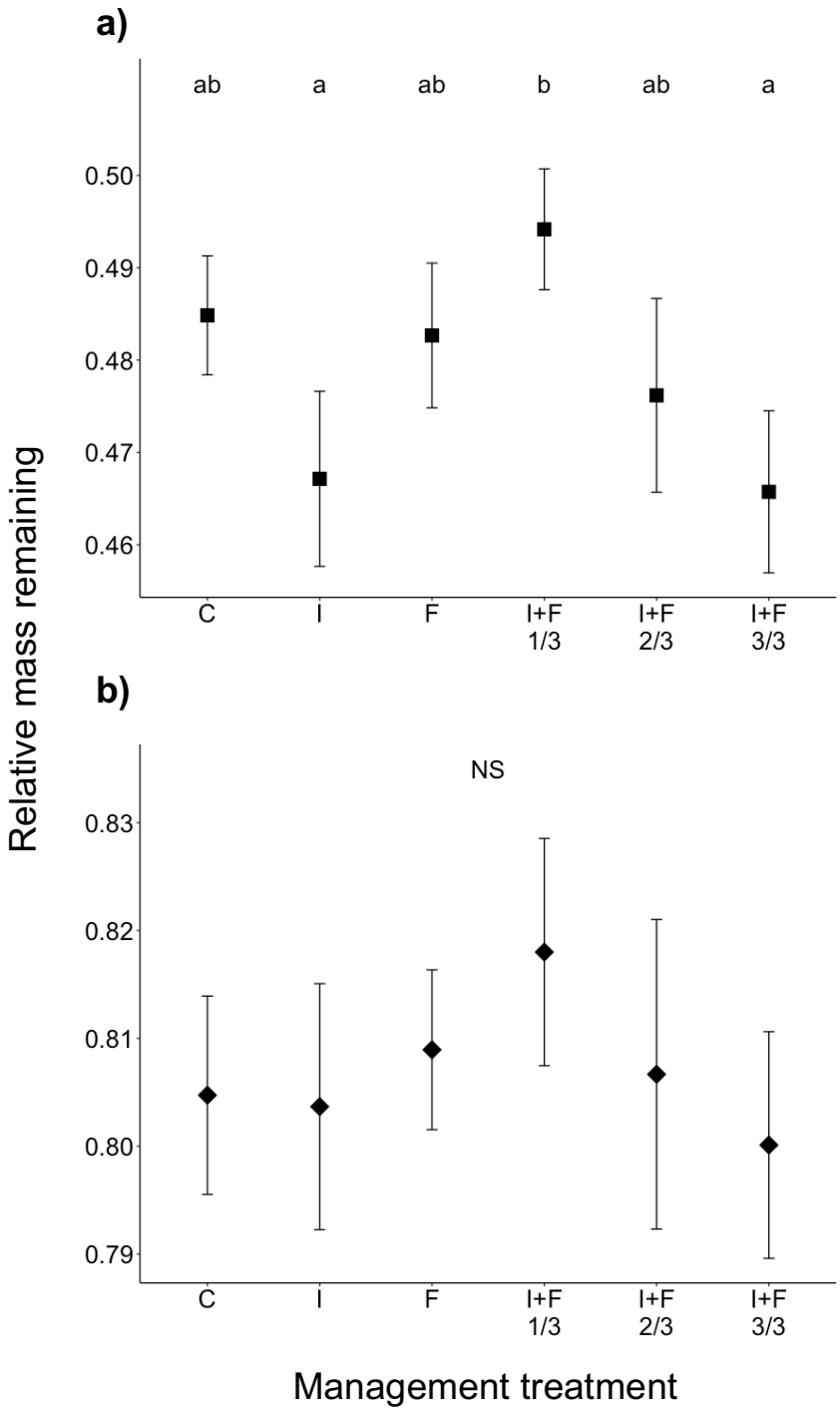

a)
ab
a
ab
b
ab
a
0.50
0.49
0.48
0.47
0.46
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
b)
NS
0.83
0.82
0.81
0.80
0.79
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
Relative mass remaining
Management treatment

Fig. S3.2. Decomposition rate *k* (a) and stabilisation factor *S* (b) after two months of burial in response to the different management treatments. For the treatment abbreviations see Fig. S3.1. Different letters indicate significant differences among treatments at an alpha rejection value set to 0.05. Mean values ± standard errors (SE) are presented.

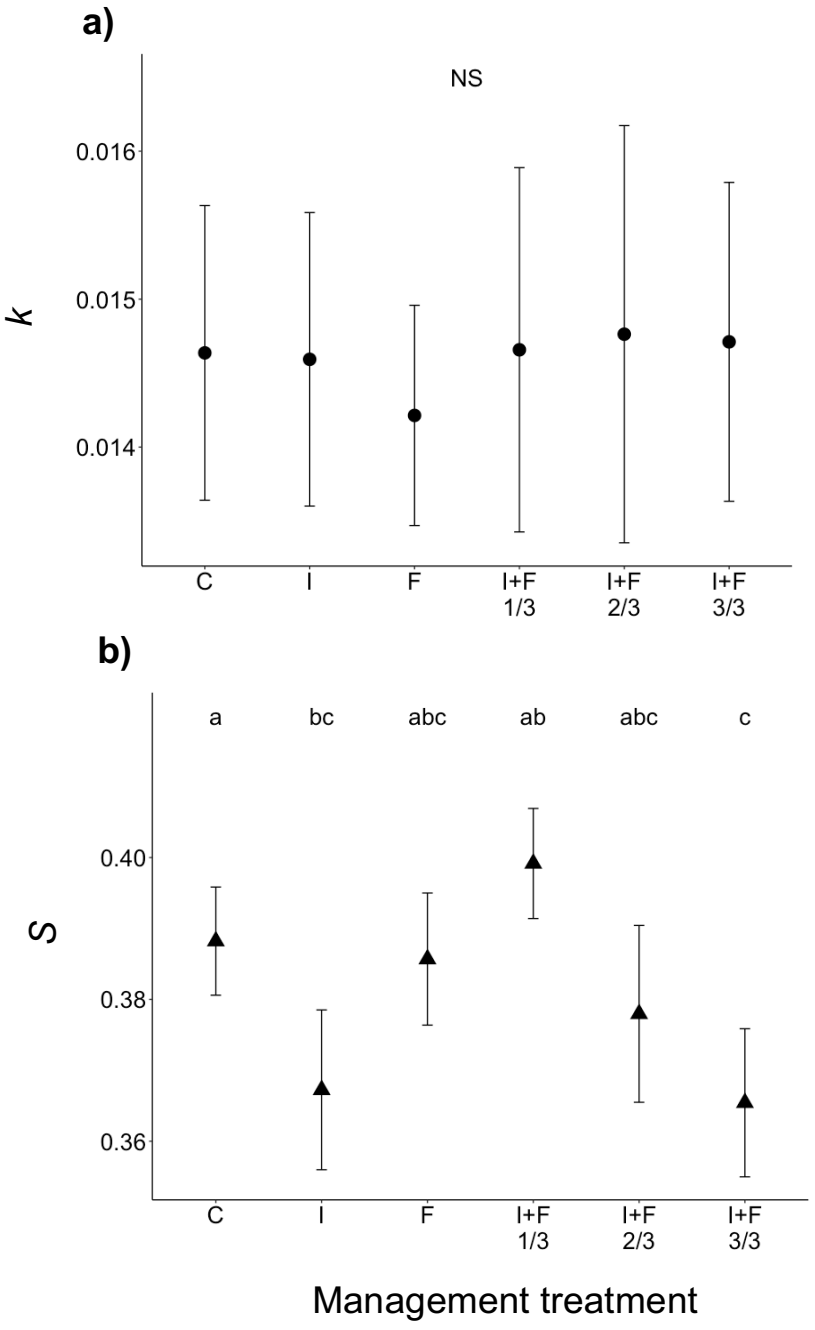


Table S3.1. Management treatments effects on relative mass remaining of green and rooibos tea after two respectively four months. Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. Significant p-values (*P*) are shown in bold. SE indicates standard errors. Analyses were performed with linear mixed-effects models where the management treatments were set as fixed effects and meadows as random intercept effects.

	After two months						After four months					
	Green tea			Rooibos tea			Green tea			Rooibos tea		
Management treatments	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***
I vs C	-0.018	0.010	0.084	-0.001	0.011	0.925	-0.028	0.013	**0.038**	-0.026	0.016	0.101
F vs C	-0.002	0.010	0.829	0.004	0.011	0.710	-0.015	0.013	0.258	-0.014	0.016	0.362
F+I 1/3 vs C	0.009	0.010	0.359	0.013	0.011	0.244	-0.003	0.013	0.805	-0.025	0.016	0.115
F+I 2/3 vs C	-0.009	0.010	0.393	0.002	0.011	0.864	-0.021	0.013	0.106	-0.040	0.016	**0.012**
F+I 3/3 vs C	-0.019	0.010	0.063	-0.005	0.011	0.683	-0.030	0.013	**0.026**	-0.053	0.016	**0.001**
F vs I	0.016	0.010	0.129	0.005	0.011	0.642	0.013	0.013	0.327	0.012	0.016	0.457
F+I 1/3 vs I	0.027	0.010	**0.010**	0.014	0.011	0.209	0.024	0.013	0.065	0.001	0.016	0.948
F+I 2/3 vs I	0.009	0.010	0.373	0.003	0.011	0.791	0.006	0.013	0.626	-0.014	0.016	0.359
F+I 3/3 vs I	-0.001	0.010	0.889	-0.004	0.011	0.753	-0.002	0.013	0.875	-0.027	0.016	0.094
F+I 1/3 vs F	0.012	0.010	0.258	0.009	0.011	0.425	0.012	0.013	0.374	-0.011	0.016	0.497
F+I 2/3 vs F	-0.006	0.010	0.522	-0.002	0.011	0.841	-0.006	0.013	0.620	-0.026	0.016	0.100
F+I 3/3 vs F	-0.017	0.010	0.098	-0.009	0.011	0.436	-0.015	0.013	0.256	-0.0383	0.016	**0.017**
F+I 2/3 vs F+I 1/3	-0.018	0.010	0.080	-0.011	0.011	0.319	-0.018	0.013	0.169	-0.015	0.016	0.326

F+I 3/3 vs F+I 1/3	-0.028	0.010	**0.007**	-0.018	0.011	0.118	-0.026	0.013	**0.046**	-0.028	0.016	0.082
F+I 3/3 vs F+I 2/3	-0.010	0.010	0.304	-0.007	0.011	0.563	-0.008	0.013	0.520	-0.012	0.016	0.437

Table S3.2. Management treatments effects on decomposition rates (*k*) and stabilisation factors (*S*) after two respectively four months. For treatment abbreviations see Table S3.1. Significant p-values (*P*) are shown in bold. SE indicates standard errors. Analyses were performed with linear mixed-effects models where the management treatments were set as fixed effects and meadows as random intercept effects.

	After two months						After four months					
	k			*S*			*k*			*S*		
Management treatments	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***
I vs C	< -0.001	0.001	0.974	-0.021	0.012	0.085	< 0.001	0.002	0.676	-0.033	0.015	**0.038**
F vs C	< -0.001	0.001	0.751	-0.003	0.012	0.833	< 0.001	0.002	0.709	-0.017	0.015	0.262
F+I 1/3 vs C	< 0.001	0.001	0.987	0.011	0.012	0.364	0.003	0.002	0.157	-0.004	0.015	0.811
F+I 2/3 vs C	< 0.001	0.001	0.924	-0.010	0.012	0.394	0.002	0.002	0.414	-0.025	0.015	0.109
F+I 3/3 vs C	< 0.001	0.001	0.955	-0.023	0.012	0.062	0.004	0.002	**0.028**	-0.035	0.015	**0.027**
F vs I	< -0.001	0.001	0.776	0.018	0.012	0.129	< -0.001	0.002	0.965	0.015	0.015	0.325
F+I 1/3 vs I	< 0.001	0.001	0.961	0.032	0.012	**0.010**	0.002	0.002	0.315	0.029	0.015	0.065
F+I 2/3 vs I	< 0.001	0.001	0.898	0.011	0.012	0.374	0.001	0.002	0.689	0.008	0.015	0.624
F+I 3/3 vs I	< 0.001	0.001	0.929	-0.002	0.012	0.129	0.004	0.002	0.069	-0.002	0.015	0.882
F+I 1/3 vs F	< 0.001	0.001	0.739	0.013	0.012	0.264	0.002	0.002	0.294	0.014	0.015	0.376
F+I 2/3 vs F	< 0.001	0.001	0.680	-0.008	0.012	0.521	0.001	0.002	0.657	-0.008	0.015	0.619
F+I 3/3 vs F	< 0.001	0.001	0.709	-0.020	0.012	0.096	0.004	0.002	0.063	-0.018	0.015	0.259
F+I 2/3 vs F+I 1/3	< 0.001	0.001	0.937	-0.021	0.012	0.082	-0.001	0.002	0.543	-0.021	0.015	0.170
F+I 3/3 vs F+I 1/3	< 0.001	0.001	0.968	-0.034	0.012	**0.007**	0.002	0.002	0.386	-0.031	0.015	**0.047**
F+I 3/3 vs F+I 2/3	< -0.001	0.001	0.969	0.008	-0.013	0.299	0.003	0.002	0.148	-0.010	0.015	0.524

Appendix 4. Soil parameters

Fig. S4.1. Experimental design. Each meadow harboured six different experimental plots of 20 m in diameter with a minimum of 5 m buffer zone between them. Treatments were randomly allocated. Five pooled samples were collected per plot at a distance of 7 m from the plot centre with a soil core sampler.

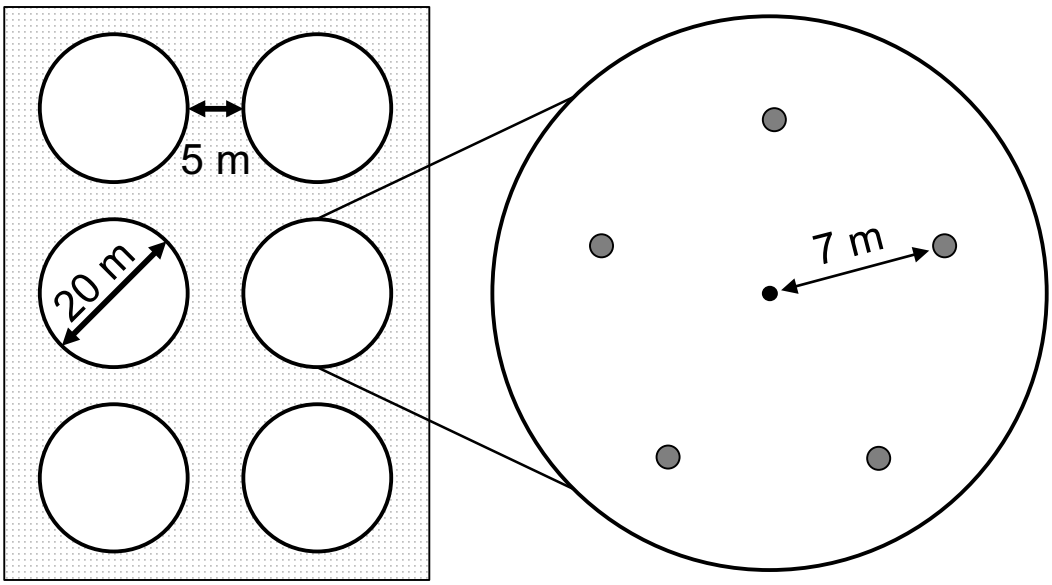


Fig. S4.2. Effect of management treatments soil parameters: (a) pH; (b) humus (%); (c) phosphorus (mg / kg dried soil); and (d) Total nitrogen in the soil in percentage (N_{tot}). Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. Mean values ± standard errors (SE) are presented. Different letters show significant differences among treatments, at an alpha rejection value set to 0.05. NS = not significant.

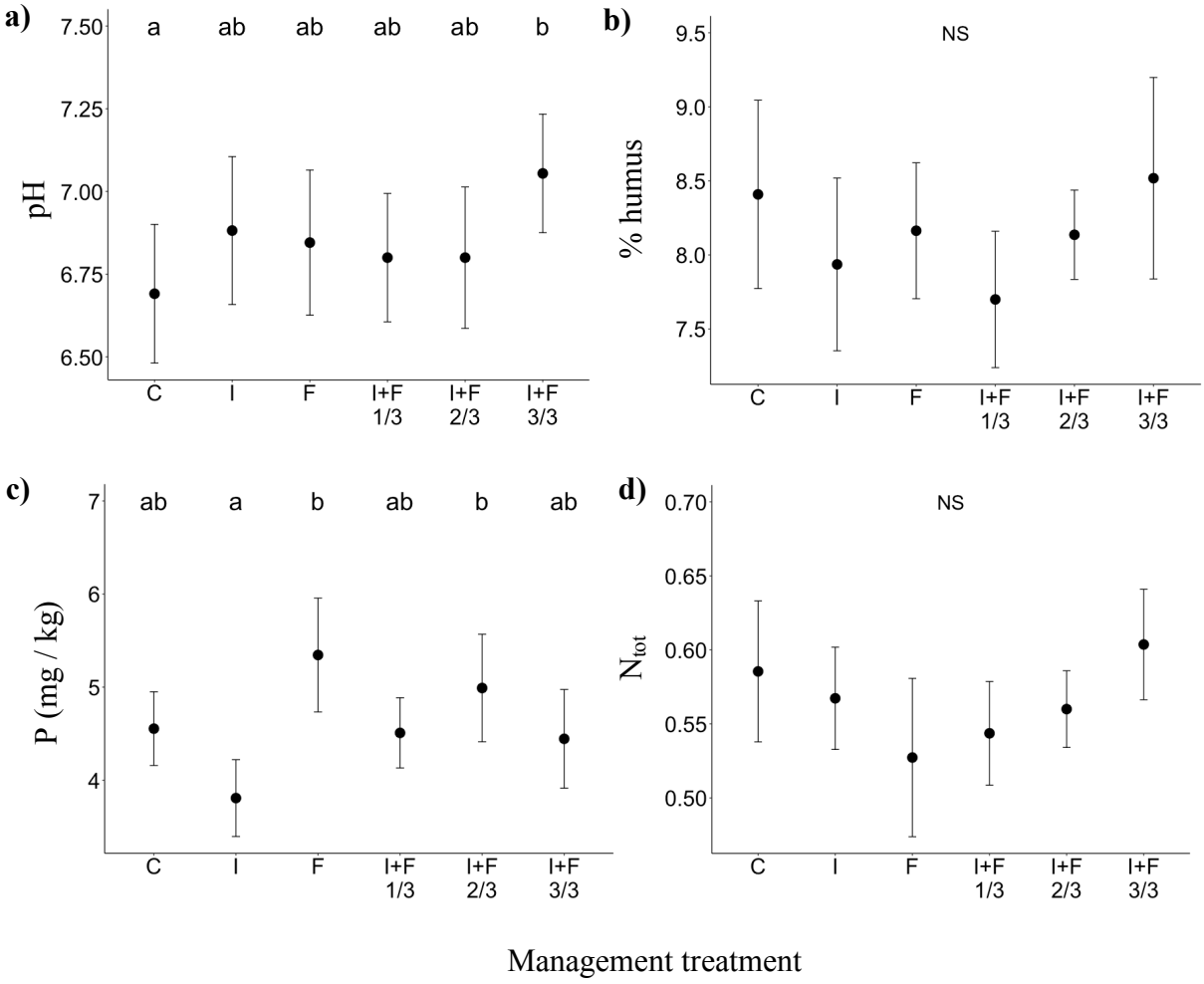


Fig. S4.3. We found a significant relationship (estimate = 0.325, SE = ± 0.068, *t* = 4.772, P = < 0.001) between the decomposition rate *k* and soil pH after four months of burial.

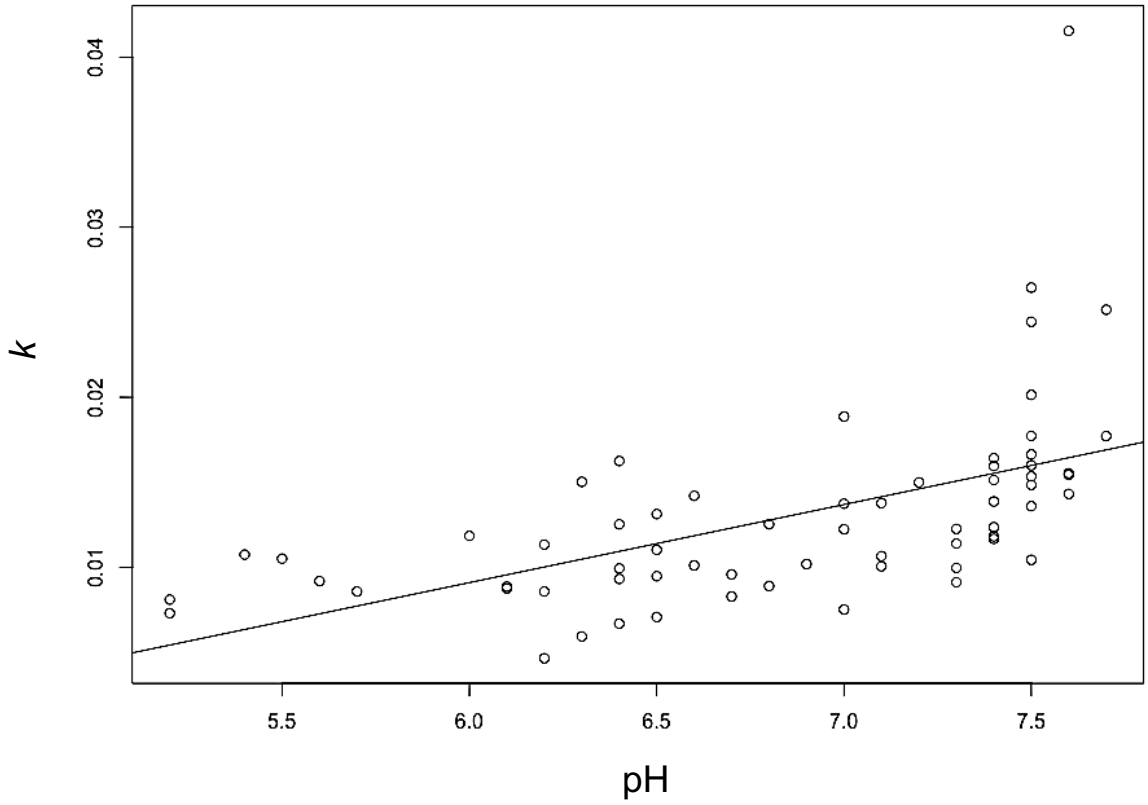


Table S4.1. Effect of management treatments on soil parameters: (a) pH; (b) humus content (%); (c) phosphorus (mg / kg dried soil); and (d) total nitrogen in the soil (N_{tot}). Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. Significant p-values (*P*) are shown in bold. SE indicates standard errors. Analyses were performed with linear mixed-effects models where the management treatments were set as fixed effects and meadows as random intercept effects.

Management treatments	a) pH			b) % humus			c) P (mg / kg)			d) N_{tot}		
	Estimate	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	SE	P
I vs C	0.191	0.139	0.176	-0.473	0.578	0.418	-0.745	0.499	0.142	-0.018	0.041	0.658
F vs C	0.155	0.139	0.272	-0.246	0.578	0.673	0.791	0.499	0.120	-0.058	0.041	0.160
F+I 1/3 vs C	0.109	0.139	0.436	-0.709	0.578	0.226	-0.045	0.499	0.928	-0.041	0.041	0.310
F+I 2/3 vs C	0.109	0.139	0.436	-0.273	0.578	0.639	0.436	0.499	0.386	-0.025	0.041	0.535
F+I 3/3 vs C	0.364	0.139	**0.012**	0.109	0.578	0.851	-0.109	0.499	0.828	-0.418	0.041	0.310
F vs I	-0.036	0.139	0.795	0.227	0.578	0.696	1.536	0.499	**0.003**	-0.040	0.041	0.332
F+I 1/3 vs I	-0.082	0.139	0.559	-0.236	0.578	0.684	0.700	0.499	0.167	-0.024	0.041	0.565
F+I 2/3 vs I	-0.082	0.139	0.559	0.200	0.578	0.731	1.182	0.499	**0.022**	-0.007	0.041	0.859
F+I 3/3 vs I	0.173	0.139	0.220	0.581	0.578	0.319	0.636	0.499	0.209	0.036	0.041	0.377

F+I 1/3 vs F	-0.045	0.139	0.745	-0.464	0.578	0.426	-0.836	0.499	0.100	0.016	0.041	0.690
F+I 2/3 vs F	-0.045	0.139	0.745	-0.027	0.578	0.963	-0.355	0.499	0.481	0.033	0.041	0.426
F+I 3/3 vs F	0.209	0.139	0.139	0.355	0.578	0.543	-0.900	0.499	0.078	0.076	0.041	0.067
F+I 2/3 vs F+I 1/3	0.000	0.139	1.000	0.436	0.578	0.454	0.482	0.499	0.339	0.016	0.041	0.690
F+I 3/3 vs F+I 1/3	0.255	0.139	0.073	0.818	0.578	0.163	-0.064	0.499	0.899	0.060	0.041	0.148
F+I 3/3 vs F+I 2/3	0.025	0.139	0.073	0.382	0.578	0.512	-0.546	0.499	0.280	0.044	0.041	0.290

Management intensification decreases arbuscular mycorrhizal fungi root colonisation in mountain meadows

Master thesis

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Summary

1. Arbuscular mycorrhizal fungi (AMF) play a fundamental role in ecosystem functioning, particularly in nutrient cycling. While negative effects of intensive agricultural management practices on AMF have been reported in previous studies, AMF response to increased fertiliser and water inputs in mountain grasslands remains poorly known.

2. Using a randomised block design, six management treatments were experimentally applied in traditionally managed hay meadows (n = 11) situated in Valais (SW Swiss Alps): 1) extensively managed control plot (no irrigation, no fertilisation); 2) plot only irrigated using sprinklers; 3) plot only fertilised with slurry (liquid organic manure); 4-6) as well as plots with low-, medium-, and high-input levels of water and fertiliser. AMF root colonisation levels were investigated in: 1) plant roots sampled directly in the field plots; and 2) colonised roots of *Plantago lanceolata*, *Trifolium pratense*, and *Lolium perenne* cultivated in soil inoculum collected in the field (AMF trap cultures).

3. Intensive management practices with higher fertiliser and water inputs negatively affected mean colonisation by AMF in the field, which was 22% lower in high-input compared to low-input plots. Irrigation or fertilisation treatments applied separately as well as low and medium inputs of a combination of both treatments had no effect on AMF colonisation in field samples.

4. In the trap cultures, colonisation levels depended on the functional plant species group and were lower in *Lolium perenne* (grass) compared to *Trifolium pratense* (legume) and *Plantago lanceolata* (forb). Moreover, lower colonisation was

detected under medium and high intensity for *Trifolium pratense* (minus 45% and 47 %, respectively, compared to control plots).

5. *Synthesis and applications*. Our results demonstrate that high agricultural inputs are decreasing plant root colonisation by AMF. Possible negative consequences are expected in nutrient transfer to plants, plant health as well as soil structure. Ultimately, our results suggest promoting low-input management strategies in alpine hay meadows that do not negatively affect AMF colonisation levels of plant roots and thus support soil functioning as well as beneficial plant-soil interactions.

Keywords: agriculture, subalpine grasslands, belowground, fertilisation, irrigation, soil

Introduction

Belowground organisms are crucial for ecosystem functioning (Bardgett & van der Putten 2014) and for sustainable agriculture (Barrios 2007) while the soil community composition is essential (Brussaard, de Ruiter & Brown 2007; Wagg *et al.* 2014). Within this community, arbuscular mycorrhizal fungi (AMF) are regarded as "keystone mutualists" because of their critical role in nutrient cycling. AMF create a symbiotic relationship with the majority of plant species, where plant sugars are exchanged against nutrients, e.g. phosphorus and nitrogen. In the symbiosis, the AMF colonises plant roots and its mycelium enhances water and nutrient uptake for the plant (López-García, Azcón-Aguilar & Barea 2014). Important ecosystem processes along with nutrient cycling (Kowalchuk & Stephen 2001) depend on AMF, such as crop productivity (Brussaard *et al.* 1997; van der Heijden *et al.* 1998), carbon cycling (Högberg *et al.* 2001), soil structure (Rillig & Mummey 2006), plant biodiversity (van der Heijden et al. 1998) and -health (Berendsen, Pieterse & Bakker 2012). Hence, understanding the interactions of land use and AMF, which represent ecological linkages between aboveground and belowground biota, is crucial for efficient conservation actions (Wardle *et al.* 2004).

During the last decades, grassland management in subalpine and montane regions has been highly intensified (Tasser & Tappeiner 2002; Bowman *et al.* 2006; Marini *et al.* 2008; Tonn & Briemle 2010; Riedener, Rusterholz & Baur 2013). The application of slurry instead of solid manure (Sommer & Hutchings 2001; Velthof, Kuikman & Oenema 2003) and the progressive replacement of traditional ground irrigation with water channels by aerial irrigation with sprinklers (Crook & Jones 1999; Leibundgut 2004) are two examples of novel practices. Previous studies

observed considerable impacts of agricultural intensification on the belowground communities in permanent grasslands (Wardle *et al.* 2004). In particular, intensified management practices lead to reduced diversity and abundance of belowground species, incl. AMF (van der Heijden 2010; de Vries *et al.* 2013; Geisseler & Scow 2014). This is because high inputs of nutrients, especially phosphorus, may hinder plants to form a symbiosis with AMF and invest carbohydrates as they can already get enough nutrients from the soil (Schubert & Hayman 1986). If there is a nitrogen or phosphorus deficiency in turn, plants seem to stimulate AMF colonisation. By doing this, their nitrogen uptake is increased up to 40% (Mäder *et al.* 2000). For phosphorus, this effect is even more pronounced: plants can receive up to 90% of their phosphorus through the symbiosis with AMF (Oehl *et al.* 2011). Further, the importance of water availability on the formation of mycorrhizal symbiosis has been demonstrated. Plants appear to better cope with drought stress and increase their water use efficiency when colonised with AMF (Farahani *et al.* 2008). Mycorrhizae help plants under drought stress not only by improved phosphorus uptake (Nikolaou, Angelopoulos & Karagiannidis 2003), but also through osmotic changes in the roots, adjusted hormone synthesis or transport, less oxidative damage and improved soil water access (Augé 2001; Ruiz-Lozano 2003). In addition, plants are able to compensate for the lower production of fine roots under dry conditions by promoting AMF colonisation (Schreiner, Tarara & Smithyman 2007). In contrast, roots of well-watered plants demonstrate lower AMF colonisation than plant roots under dry conditions (Augé 2001). However, the irrigation effect seems to be not completely clear, as Kumar & Garampalli (2013) observed a positive effect of irrigation on AMF colonisation. Importantly, their

study also highlighted the difficulty to differentiate between factors explaining root colonisation under natural conditions.

The impact of fertilisation and irrigation on plant and invertebrate communities in mountain grasslands has already been investigated (Riedener, Rusterholz & Baur 2013; Andrey *et al.* 2014; Melliger *et al.* 2014; Riedener *et al.* 2015; Andrey, Humbert & Arlettaz 2016; Humbert *et al.* 2016) while belowground responses remain largely unknown (Wardle *et al.* 2004). There is a lack of experimental studies investigating soil organisms, belowground processes as well as its link to ecosystem services under field conditions. Specifically, there is a need to study agricultural intensification gradients and different land use types in order to understand the influence of agriculture on soil systems. Land use and soil management strategies should be targeted to find optimal trade-offs between productivity and preservation of ecosystem services as pointed out in the review of Barrios (2007). Finally, experimental research is needed to understand soil processes as well as to evaluate the biological status of soils for designing sustainable agricultural production in the long-term (Lemanceau *et al.* 2015). In doing so, AMF are particularly suitable as soil-bioindicators and are useful to characterise the status of agriculturally used area (Oehl *et al.* 2011). However, there is a lack of profound knowledge in order to optimally promote AMF and profit from their ecological services, i.e. efficient plant nutrition (Oehl *et al.* 2011).

Here, we tested the response of AMF root colonisation to fertilisation and irrigation applied separately, as well as to an intensification gradient that combines both treatments using two different approaches: 1) analysing roots from plants in semi-natural meadows dedicated to hay production and 2) analysing roots from plants grown in a trap culture experiment, i.e. in sterilised soil and soil inoculum

from the field, to control for the host plant identity. We hypothesised decreasing colonisation levels with intensification but also negative effects of fertiliser and irrigation inputs applied separately (Schubert & Hayman 1986; Augé 2001; Nikolaou *et al.* 2003; Schreiner, Tarara & Smithyman 2007; van der Heijden 2010; de Vries *et al.* 2013). With this study, we contribute to understand the impact of intensified management on AMF and provide management recommendations for farmers to conserve important services provided by AMF in mountain grasslands.

Material and methods

STUDY SITES

In 2010, eleven montane or subalpine hay meadows were selected in Valais, SW Switzerland (Appendix 1). Central Valais, the location of our study sites, is characterised by a continental climate with cold winters and dry as well as hot summers. The selected meadows were extensively managed for at least the ten years before the study started with no or very low fertiliser and water applications and only one mowing event per year. The meadows were situated along an altitudinal gradient between 790-1740 m above sea level. For details on meadow selection see Andrey et al. (2016).

STUDY DESIGN

A randomized block design was used in which every meadow (the block, n = 11) harboured six experimental plots of 20 m diameter (314 m^2) with a buffer zone of at

least 5 m between plots. Six different management treatments (= intensity levels of irrigation and/or fertilisation) were randomly assigned to the plots: 1) control plot (C): extensively managed without fertilisation and irrigation; 2) plot only irrigated (I); 3) plot only fertilised (F); as well as plots that were irrigated and fertilised at low (I+F 1/3), medium (I+F 2/3), and high (I+F 3/3) intensity levels. C-plots were mown once a year according to local standards of traditional extensively managed hay meadows while all the other plots were mown twice a year. Irrigated plots were watered weekly with sprinklers from mid-May to the beginning of September, unless it was raining strongly. Slurry, consisting of dried organic manure NPK pellets and mineral potassium oxide (K_2O) dissolved in water, was applied in May and August. The maximum input of slurry (I+F 3/3) was calculated according to the theoretical hay production potential of each meadow to reach maximum productivity. The applied doses of slurry represented a site-adapted intensification gradient ranging from extensively (C) to intensive (I+F 3/3) management and are listed in the study of Andrey et al. (2016). Plots only fertilised (F) received medium inputs of slurry, thus the same amount as I+F 2/3 plots. In contrast, irrigation treatments (I plots) were not site-adapted but equal in all plots of a specific treatment. Similarly to the F treatment, I plots received medium inputs of water, thus the same amount as I+F 2/3 plots.

SOIL SAMPLING

To investigate general plant root colonisation levels by AMF in the field, soil samples were collected in August and September 2015. Two samples per plot were randomly taken between 3 and 7 meters form the plot centre (Appendix 1) at spots

representative for the vegetation with a soil core sampler (10 cm deep, Ø 8 cm). Samples were pooled per plot and stored in a cooling room at 2.3 °C.

To inoculate roots of plants in trap cultures, 16 soil samples were collected at a distance of 0.5 m along a transect across all plots between 2.5 and 6 m from the plot centre (Appendix 1) with a soil core sampler (5 – 10 cm deep, Ø 1.8 cm) in July 2015. Samples were pooled per plot and stored in a cooling room at 2.3 °C.

TRAP CULTURE EXPERIMENT

Previous studies suggested that AMF growth is host specific (Bever *et al.* 2001) meaning that root colonisation might depend on the host plant identity. When analysing roots directly from field samples, plant identity was unknown because only soil samples were taken and consequently colonisation levels may not be comparable. Growing plants in the greenhouse using sterilised soil and soil inoculum from the field is a common approach to test differences in the microbial community under controlled conditions (van der Heijden & Wagg 2013). For this reason and to control for the host plant identity, we established trap cultures with *Lolium perenne*, *Plantago lanceolata* and *Trifolimum pratense* and evaluated initial root colonisation after two months (Oehl *et al.* 2003). In the trap culture experiment, AMF from the field were "trapped": amplified in pots by growing them on specific host plants under the same conditions (Bever *et al.* 2001). The colonisation of plant roots by the AMF could be analysed and compared between the different management treatments without plant species bias. Additionally, results from trap cultures could be compared to results from field conditions to see whether they matched.

For each plot (n= 66), one trap culture pot was filled with 3 kg of an autoclaved mixture: 10% sand (oven dried, 0 – 4 mm, Bauhag), 30% Loess (from a construction site in Buchrain LU) and 60% oil dry (Sorbix Universal Oelbinder Typ III, purchased from: http://www.maagtechnic.ch). A dried weight of 20 g of AMF inoculum from the field was added as a layer and covered with 1 kg of the autoclaved mixture. In order to grow three common grassland plant species with two replicates each per pot, pots were divided into six compartments with separation fleece to prevent competition between roots of different plant individuals. Seeds of *Lolium perenne* (grass), *Plantago lanceolata* (forb) and *Trifolium pratense* (legume) were randomly allocated to one compartment. These plant species were chosen because they are well colonized by AMF and represent different functional groups (Oehl *et al.* 2003). Four control pots with plant individuals growing in autoclaved soil mixture without AMF inoculum (n=2) and with autoclaved inoculum (n=2) were additionally established, thus making a total of 70 pots for the trap culture experiment.

Plants were grown outdoors for two months (from mid-August to mid-October 2015) under a net (PEHD, mesh size 3 x 7 mm, netzteam.ch) to protect them from hail, birds and slugs. Harvested plants were stored in a cooling room at 4 °C until further processing.

WASHING, CLEARING AND STAINING OF ROOTS

Fine roots from the field soil samples were washed under running water with a sieve. Roots from the trap culture plants were washed under running water likewise

and the roots were cut off from the plants. All roots were then processed according to the protocol of Vierheilig *et al*. (1998).

Roots were cut in pieces of ca. 1.5 cm and stored in 50% EtOH in falcon tubes (15 ml). Roots had to be cleared first because only transparent roots can be successfully stained (Vierheilig *et al.* 1998; Vierheilig, Schweiger & Brundrett 2005). Before doing so, the EtOH was rinsed off the roots with deionised water. 10% KOH was added and roots were incubated in a water bath at 80 °C for 60 min for clearing. After rinsing with deionised water, roots were stained in a 5% ink-vinegar solution in a water bath at 80 °C for 60 min. Ink and vinegar is a simple, non-toxic, low-cost and reliable method to stain AMF structures in roots (Vierheilig *et al.* 1998). Roots were finally rinsed with deionised water and stored in 50% glycerine in falcon tubes (15 ml).

MEASUREMENT OF AMF COLONISATION: INTERSECTION COUNTING

Roots were aligned parallel to the long axis of a microscopy slide (one per treatment and plot for the field samples (n = 66) and one per treatment, plot and plant species (n = 198) for the trap cultures) covered with 50% glycerine and a cover glass. Colonisation was measured using a light microscope at a magnification of x 200. A virtual transect was laid on the slide on which hundred counts were made (Appendix 2). For every count (n = 100 per microscopy slide), we observed whether the vertical crosshair of the eyepiece cuts any AMF structure or not. The observations were classified by the following categories: arbuscules, vesicles, arbuscules and vesicles, hyphae or none of them (Appendix 2). As 100 counts were

examined, AMF colonisation was directly calculated as % of non-negative intersections:

$$\text{AMF colonisation } (\%) = 100 - counts\ "none" \qquad \text{eqn 1}$$

STATISTICAL ANALYSIS

Colonisation levels were analysed with generalised linear mixed-effects models (GLMMs) using the glmer function with a binomial distribution from the lme4 R-package (Bates et al. 2015). Field samples and trap cultures were analysed separately. For the trap cultures, we analysed mean colonisation over all three plant species but also for each species separately. When analysing the trap cultures including all three species, the mean was calculated for the three replicates of a given plot to avoid pseudoreplication; finally resulting in one value per plot and meadow (n = 66). The response variable was AMF colonisation (%). Management treatment effects were included in the model as fixed effects and meadows as a random effect to account for the variation between sampling sites. We accounted for overdispersion by including an observation-level random effect to deal with the greater variance of the data than predicted by the binomial model (Harrison 2015). The *relevel* function in R, which allows changing the reference level of the fixed effects in the GLMM, was used to examine pairwise differences among treatments. All statistical analyses were performed using R version 3.2.2 (R Development Core Team 2015).

Results

FIELD SAMPLES

Mean AMF root colonisation in the field samples was significantly lower in intensively management plots (I+F 3/3) compared to only irrigated (I), only fertilised (F) and low-input (I+F 1/3) plots (Fig. 1). No differences were found between the other treatments.

TRAP CULTURES

No significant differences were detected in the analyses performed with means of the three host plant species but two main effects were detected when analysing each trap plant species separately (Fig. 2b). For *Trifolium pratense*, we found lower AMF colonisation under medium intensity and (I+F 2/3) and high intensity (I+F 3/3) management compared to control plots (C). Moreover, *Lolium perenne* showed significantly lower colonisation levels compared to *Plantago lanceolata* (estimate = -1.647, SE= ± 0.195, z= 8.434, $P < 0.001$) and *Trifolium pratense* (estimate = -1.478, SE= ± 0.193, z= 7.642, $P < 0.001$) while *Trifolium pratense* did not differ from *Plantago lanceolata* (estimate = -1.169, SE= ± 0.190, z = -0.890, P = 0.374). Control trap cultures that received no or autoclaved inoculum never showed more than 3% colonisation.

Discussion

This study experimentally tested whether plant root colonisation by AMF responds to grassland management intensification in the Swiss Alps. Our results demonstrated that neither low intensity management (combining slurry and sprinkler irrigation) nor fertilisation or irrigation applied separately (with medium inputs) hinders AMF colonisation. However, high water and fertiliser inputs combined decreased AMF colonisation in our field samples and in *Trifolium pratense* from the trap culture experiment. The analysis of colonisation of the trap cultures including all species, revealed the same pattern but it was not statistically significant. When analysing the plant species of the trap cultures separately, we additionally found a negative effect of medium inputs on root colonisation of *Trifolium pratense.*

RESPONSE TO MANAGEMENT INTENSIFICATION GRADIENT

After five years of treatment application, AMF colonisation levels in the field samples as well as in trap cultures with *Trifolium pratense* were lower in high-input plots (I+F 3/3) respectively than in low input plots (I+F 1/3) and extensively managed control plots (C), confirming the conclusions of previous studies that high inputs do affect AMF negatively (Schubert & Hayman 1986; Augé 2001; Nikolaou *et al.* 2003; Schreiner, Tarara & Smithyman 2007; van der Heijden 2010; de Vries *et al.* 2013; Geisseler & Scow 2014). We found the same pattern for the mean colonisation level with pooled data set of the trap cultures from the three plant species, but the effect was not statistically significant, probably because of lower

absolute colonisation levels and more variance in the trap culture data. Additionally, medium-input treatments had a significant negative effect on the colonisation of *Trifolium pratense* whereas low intensity management had no negative effect on AMF colonisation in our study. In brief, AMF colonisation was not affected under low but decreased under high management. In addition, medium management decreased AMF colonisation of *Trifolium pratense*. Our results illustrate a negative effect of intensification on AMF colonisation, although we expected clearer responses of the trap cultures.

In order to avoid losing beneficial services provided by AMF, we propose to limit water and fertiliser inputs and to support low-intensity management. Even though this recommendation is not only based on field samples, in which plant identity cannot be controlled, but also on the outcome of one specific plant species of alpine grasslands (*Trifolium pratense*) our findings should be considered with caution. Despite the fact that the negative response of AMF colonisation to intensification in field samples is confirmed within *Trifolium pratense*, there was no effect on *Plantago lanceolata* and *Lolium perenne* and neither on the mean over all three plant species. We hence recommend testing various plant species in trap cultures to better assess whether field samples or trap cultures are more appropriate to evaluate root colonisation.

SEPARATE EFFECTS OF IRRIGATION AND FERTILISATION

Surprisingly and contrarily to what has been shown before (Schubert & Hayman 1986; Augé 2001; Nikolaou *et al.* 2003; Schreiner, Tarara & Smithyman 2007; van der Heijden 2010; de Vries *et al.* 2013; Geisseler & Scow 2014), irrigation (I) and

fertilisation (F) applied separately did not significantly affect AMF colonisation in our study. A weakness of our experimental design is that irrigated and fertilised plots received only medium quantities of inputs, the treatments are thus not fully factorial. We interpret this outcome that medium inputs of either irrigation or fertilisation applied separately are not detrimental to root colonisation by AMF. Irrigation and/or fertilisation might thus still have an impact on AMF colonisation with higher inputs than in our study. A full factorial design, in which irrigation and fertilisation treatments are not only tested in all three intensity levels (low, medium and high input) when combined but also when applied separately would be needed to finally evaluate whether our findings reflect a true absence of an effect of I- and F treatments or whether medium inputs are just not high enough to influence AMF colonisation.

FIELD SAMPLES COMPARED TO TRAP CULTURES

Analysing roots from the field directly and growing plants in trap culture to control for host plant identity allowed comparing whether trap cultures represent field colonisation levels. Trap cultures showed a similar pattern as roots from the field but the trend was not so striking. First of all, standard errors were bigger in trap cultures compared to field samples, contributing to statistically non-significant differences. Even though it looks like intensive management was decreasing AMF, we do not know whether our experiment fails to demonstrate an effect (e.g. due to a too short growth time of plants, environmental conditions during growth or too low inputs of water and fertiliser compared to real life) or there is a true absence of an effect. Another outcome of our experiment is that the two methods differed in terms

of absolute colonisation levels: roots from the field were colonised to much higher levels compared to roots from the trap cultures. This could be explained by the fact that we measured initial colonisation levels after only two months of plant growth in the trap cultures, which is a very short time compared to the time that AMF had to colonise plant roots in the field. We would like to point out that the plants analysed from the field were not from a certain age and compared to trap culture plants. They were all harvested as plantlets to analyse colonisation levels, so a lower level of colonisation was expected. In our study, the negative effect of high-input management was detected in the field samples but not when analysing the colonisation mean over the three plant species. We thus recommend trapping AMF for two, four, six and eight months as it has been done to study spore abundance and AMF species diversity (Oehl *et al.* 2003). We think this approach is needed to define the optimal growth time for host plants that best represents treatment effects on AMF colonisation in the field. Additionally, we advise testing AMF colonisation of specific plant species, i.e. *Trifolium pratense* because in our study, the negative effect of medium-input management on AMF colonisation could only be detected within this specific plant species but was levelled-off when analysing field samples or the mean over the trap culture species. This plant species is especially useful to study AMF colonisation due to its biology and is therefore discussed in the next section.

TRAP CULTURE HOST PLANT SPECIES

Absolute colonisation levels were much lower in *Lolium perenne* (grass) than in *Plantago lanceolata* (forb) and *Trifolium pratense* (legume). This finding may

illustrate that general colonisation levels are species-specific, meaning that not all species depend on the symbiosis with the fungi to the same extent. This would be in line with previous studies showing that legumes do depend more on mycorrhizae than grasses because of high phosphorus demands of the former (Oehl *et al.* 2011; Yang *et al.* 2016). Additionally, high phosphorus demands could explain why we only detected treatment effects on *Trifolium pratense* in the trap culture experiment. Legumes seem to profit from the symbiosis with AMF when phosphorus availability is low (C; I+F 1/3), but invest less in AMF symbiosis with high inputs of phosphorus (Oehl *et al.* 2011). In contrast, grasses (i.e. *Lolium perenne*) are colonised to a much lower level compared to forbs and legumes and measuring initial colonisation is therefore not optimal to evaluate treatment affects. Still, when using grasses in further studies, we therefore recommend a longer growth time. In contrast, forbs (i.e. *Plantago lanceolata*) and legumes (i.e. *Trifolium pratense*) show high enough absolute colonisation levels by AMF to compare treatment effects.

For further grassland studies, we finally recommend to use *Trifolium pratense* as an indicator AMF host plant species. Last but not least because it is a typical grassland species and commonly used in trap culture experiments (Oehl *et al.* 2003). Still, trap culture results should be taken with caution, as the outcomes might only be species specific, not reflecting grassland species in general. A negative effect of mid-intensive and intensive management therefore might be only true for this particular plant species but there is no effect on colonisation levels in general as true for *Plantago lanceolata* and *Lolium perenne* in our study.

CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

Beneficial services provided by AMF are possibly under threat due to intensified management practices in alpine grasslands. In our study, high inputs of slurry combined with sprinkler irrigation hindered mean root colonisation by arbuscular mycorrhizal fungi in field samples and in *Trifolium pratense* from our trap culture experiment. Possible consequences are expected in nutrient cycling, i.e. increased nutrient losses (Kowalchuk & Stephen 2001; Oehl *et al.* 2011), but also in plant biodiversity (van der Heijden et al. 1998), and -health (Berendsen, Pieterse & Bakker 2012) as well as soil structure (Rillig & Mummey 2006). Additionally, decreased AMF colonisation levels could lead to lower carbon sequestration as the latter is increased with higher proportions of AMF in the soil (Six *et al.* 2006). Such consequences are especially concerning in the face of the still ongoing intensification of mountain grasslands in European (Tasser & Tappeiner 2002). Based on our findings combined with previous findings of our long-term project (Andrey *et al.* 2014; Andrey, Humbert & Arlettaz 2016), we recommend to promote low-input management in hay meadows, represented by I+F 1/3 treatments in this study, with applications of one third of the doses of fertilisation and irrigation that would be needed to result maximum theoretical hay yield. Our conclusions are in line with the reviews of Isselstein, Jeangros & Pavlu (2005) and Humbert *et al.* (2016), which highlight the importance of preserving or even restoring mountain grasslands by means of sustainable management. Here, we point out the need for further studies, to find the most appropriate method for measuring AMF colonisation (e.g. host plants and growth time for trap cultures and reliability of field samples). Ultimately, belowground findings of this study contribute to

understanding aboveground-belowground linkage to finally target management recommendations to farmers in order to promote sustainable management practices that allow decent yield while preserving functional diversity.

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Table 1: Management treatment effects on AMF root colonisation in the field samples. Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. SE = standard errors. Significant p-values (*P*) are shown in bold. Analyses were performed with generalised linear mixed-effects models where the management treatments were set as fixed effects and meadows as random intercept effects.

	Field samples		
Management treatments	**Estimate**	**SE**	***P***
I vs C	0.039	0.250	0.875
F vs C	0.02631	0.250	0.916
F+I 1/3 vs C	0.102	0.251	0.684
F+I 2/3 vs C	-0.017	0.250	0.945
F+I 3/3 vs C	-0.470	0.250	0.061
F vs I	-0.013	0.250	0.958
F+I 1/3 vs I	0.063	0.250	0.802
F+I 2/3 vs I	-0.057	0.250	0.821
F+I 3/3 vs I	-0.509	0.250	**0.042**
F+I 1/3 vs F	0.759	0.251	0.762
F+I 2/3 vs F	-0.044	0.250	0.862
F+I 3/3 vs F	-0.496	0.250	**0.047**
F+I 2/3 vs F+I 1/3	-0.119	0.250	0.634
F+I 3/3 vs F+I 1/3	-0.572	0.250	**0.023**
F+I 3/3 vs F+I 2/3	-0.452	0.250	0.070

Table 2: Management treatment effects on AMF root colonisation in the trap cultures. For the treatment abbreviations see Table 1. Significant p-values (*P*) are shown in bold. SE indicates standard errors. Analyses were performed with generalised linear mixed-effects models where the management treatments were set as fixed effects and meadows as random intercept effects.

	Mean			*Lolium perenne*			*Plantago lanceolata*			*Trifolium pratense*		
Management treatments	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***	**Estimate**	**SE**	***P***
I vs C	-0.111	0.293	0.705	-0.527	0.459	0.251	0.356	0.491	0.486	-0.371	0.423	0.381
F vs C	0.019	0.292	0.948	-0.231	0.452	0.610	0.231	0.466	0.619	-0.195	0.422	0.644
F+I 1/3 vs C	-0.222	0.294	0.450	-0.513	0.458	0.263	-0.339	0.482	0.482	-0.136	0.422	0.747
F+I 2/3 vs C	-0.237	0.295	0.420	-0.228	0.452	0.613	0.163	0.468	0.727	-0.855	0.428	**0.046**
F+I 3/3 vs C	-0.368	0.295	0.212	-0.118	0.451	0.793	-0.242	0.479	0.614	-0.969	0.456	**0.034**
F vs I	0.130	0.293	0.657	0.296	0.464	0.524	-0.125	0.480	0.794	0.176	0.424	0.679
F+I 1/3 vs I	-0.111	0.296	0.707	0.014	0.470	0.976	-0.695	0.495	0.160	0.234	0.424	0.581
F+I 2/3 vs I	-0.126	0.296	0.669	0.298	0.464	0.520	-0.193	0.481	0.688	-0.484	0.430	0.260
F+I 3/3 vs I	-0.257	0.297	0.386	0.409	0.463	0.377	-0.598	0.493	0.225	-0.598	0.458	0.191
F+I 1/3 vs F	-0.241	0.294	0.412	-0.282	0.463	0.543	-0.570	0.470	0.225	0.059	0.423	0.890
F+I 2/3 vs F	-0.256	0.294	0.384	0.002	0.457	0.996	-0.068	0.456	0.881	-0.660	0.429	0.124
F+I 3/3 vs F	-0.387	0.295	0.189	0.112	0.456	0.805	-0.473	0.467	0.311	-0.774	0.457	0.090
F+I 2/3 vs F+I 1/3	-0.015	0.297	0.959	0.284	0.463	0.540	0.502	0.472	0.287	-0.718	0.429	0.094
F+I 3/3 vs F+I 1/3	-0.146	0.298	0.623	0.394	0.462	0.393	0.097	0.483	0.841	-0.833	0.457	0.069
F+I 3/3 vs F+I 2/3	-0.131	0.298	0.384	0.110	0.456	0.809	-0.405	0.469	0.388	-0.114	0.462	0.804

Figure legends

Fig. 1. AMF colonisation in the field. Management treatment abbreviations are: C = control; F = fertilised; I = irrigated; and F+I 1/3, F+I 2/3 and F+I 3/3 state for treatments fertilised and irrigated at respectively 1/3 (low), 2/3 (medium) and 3/3 (high) of the dose that would be necessary to achieve the maximum theoretical local hay yield. Different letters indicate significant differences among treatments at an alpha rejection value set to 0.05. Mean values ± standard errors (SE) are presented.

Fig. 2. AMF root colonisation of the trap culture plants after two month of growth: (a) mean of all three species host plant species; and (b) per plant species. For the treatment abbreviations see Fig. 1. Different letters show significant differences among treatments, at an alpha rejection value set to 0.05. Mean values ± standard errors (SE) are presented.

Fig. 1.

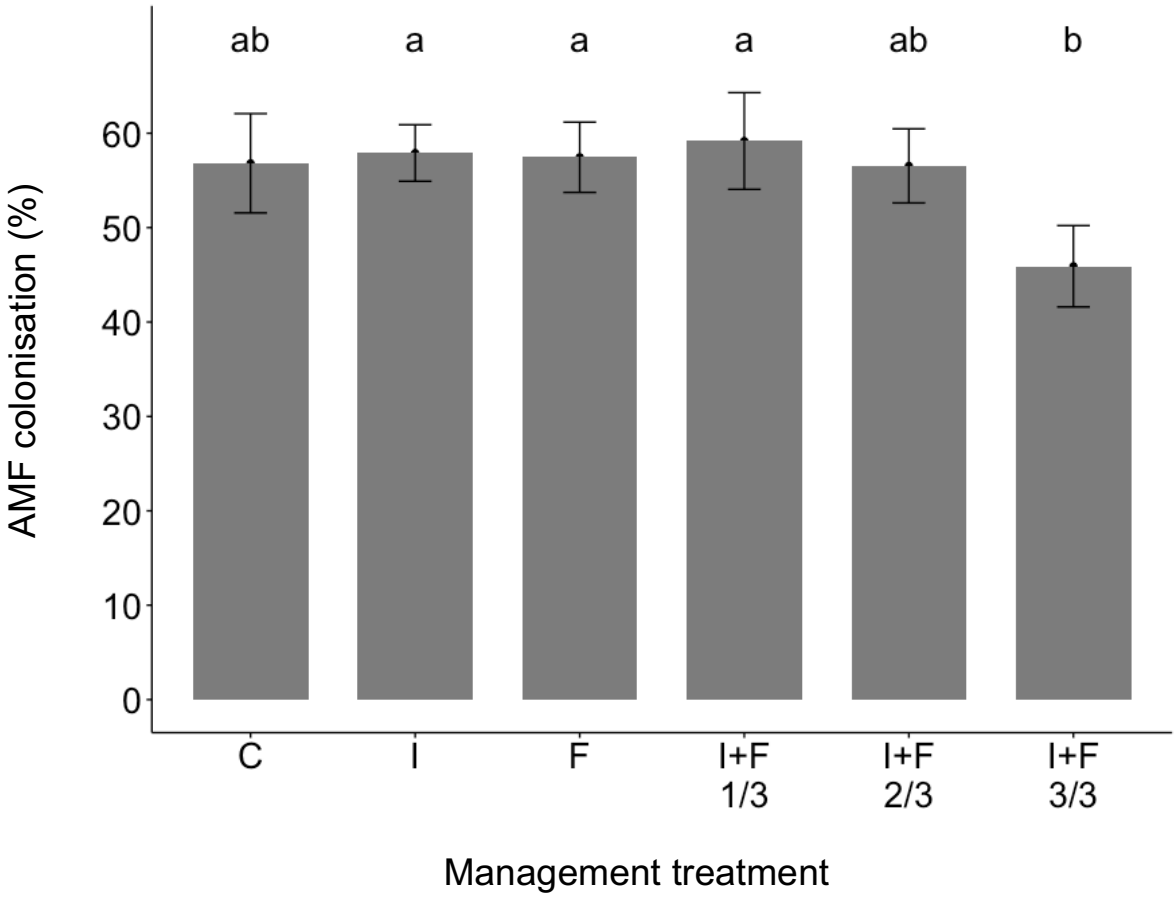
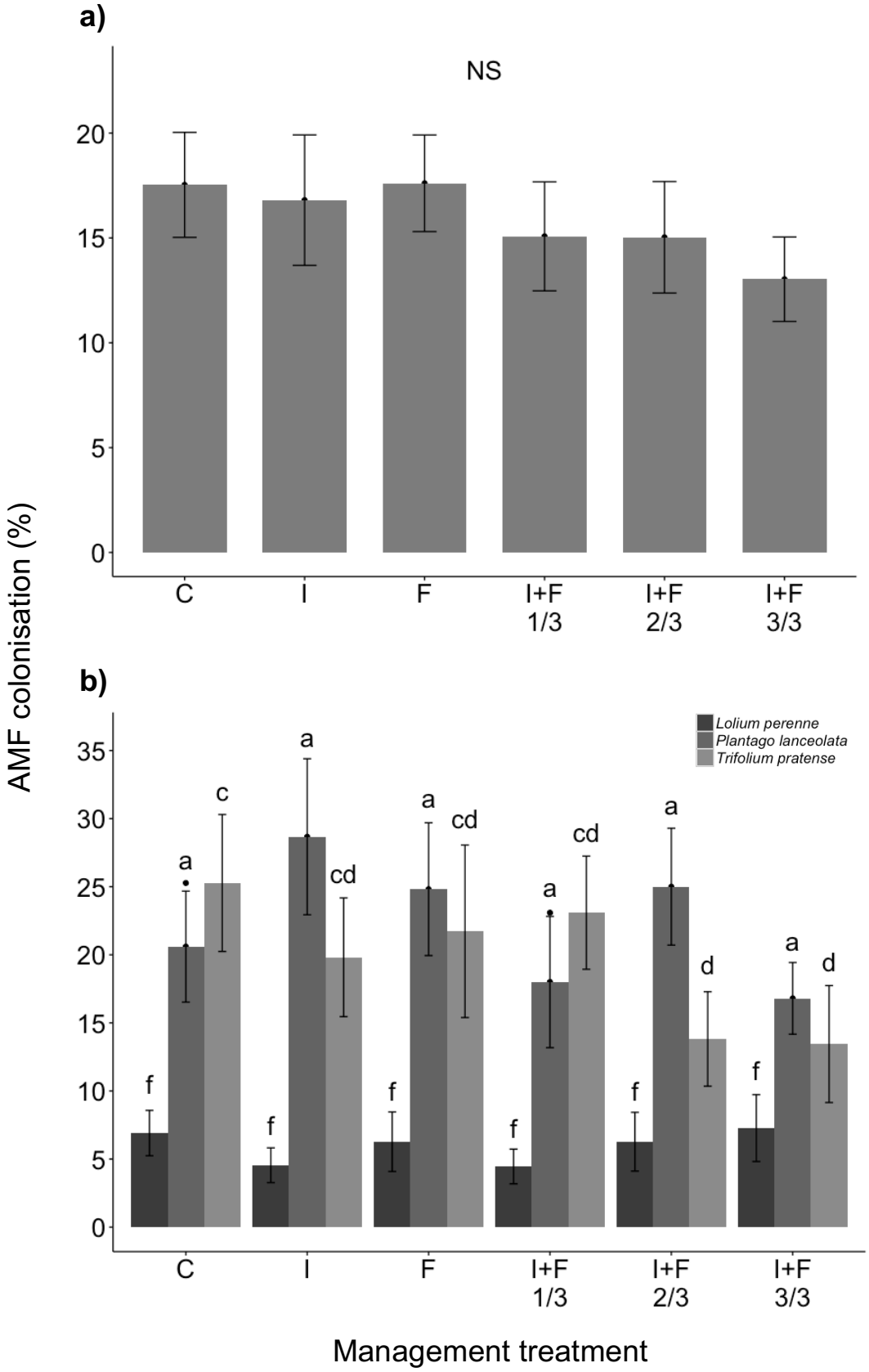

ab
a
a
a
ab
b
60
50
40
30
20
10
0
AMF colonisation (%)
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
Management treatment

Fig. 2.


a)
NS
20
15
10
5
0
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
b)
Lolium perenne
Plantago lanceolata
Trifolium pratense
35
30
25
20
15
10
5
0
a
c
a
cd
a
cd
a
cd
a
d
a
d
f
f
f
f
f
f
C
I
F
I+F 1/3
I+F 2/3
I+F 3/3
AMF colonisation (%)
Management treatment

Supporting Information

Appendix 1. Location of the different study sites (n = 11 replicates) and study design

Fig. S1. Map of Switzerland with the eleven study sites (white dots) in the canton of Valais (black region).

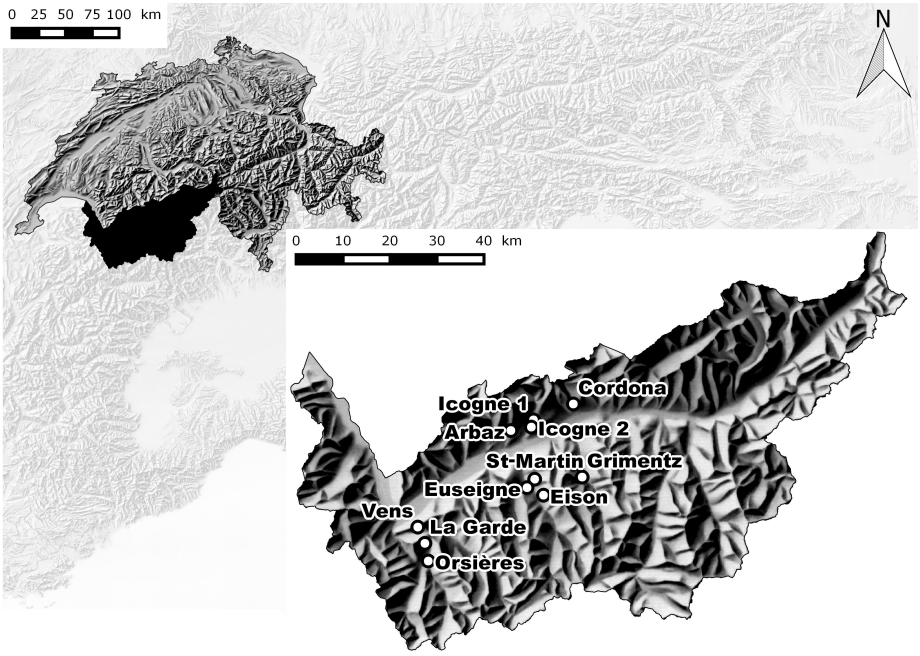
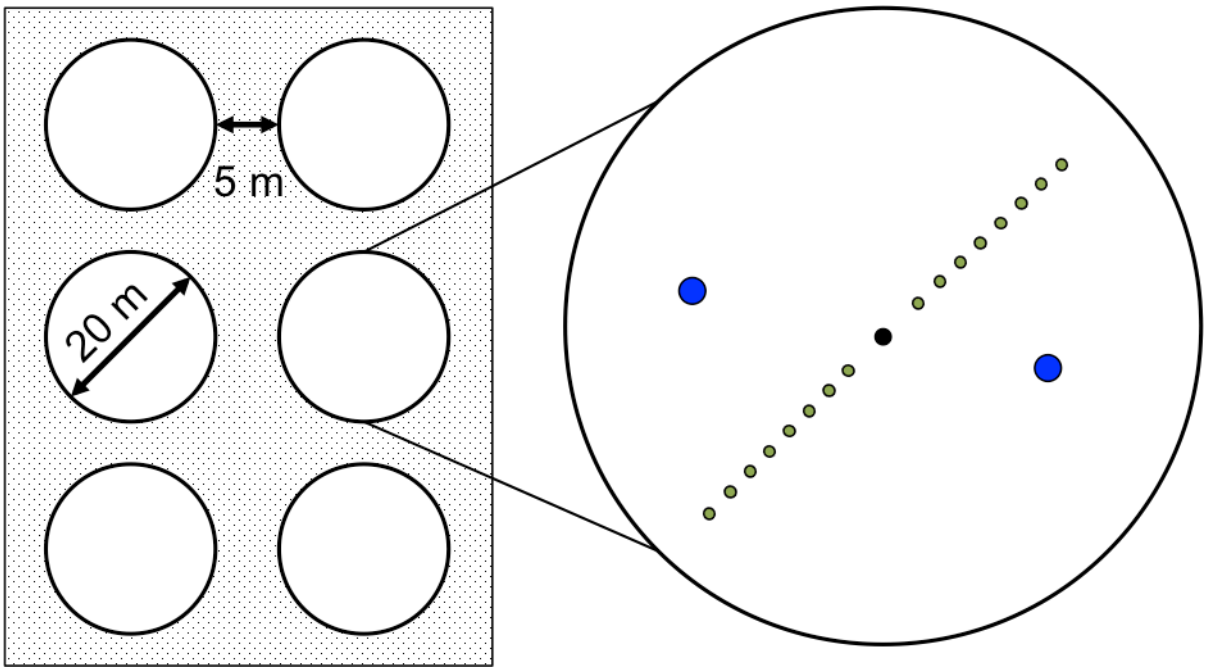


Table S1.1. The eleven study sites with altitude and geographical coordinates.

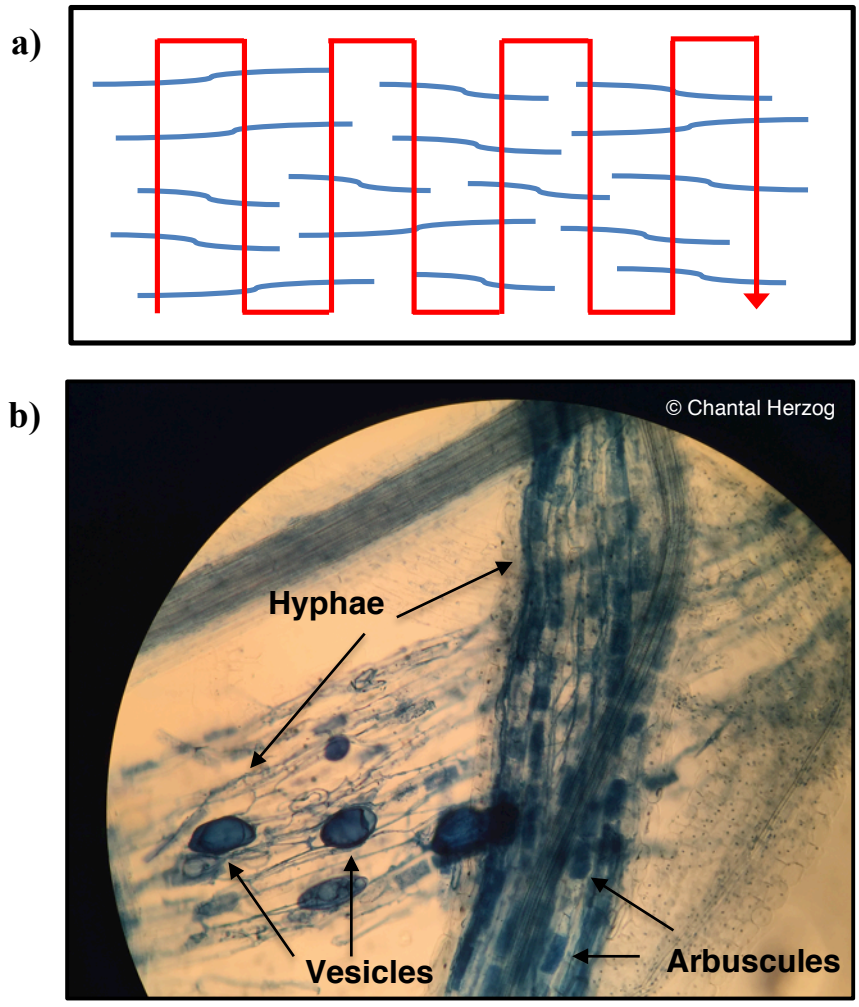
			Coordinates	
Sites	**Name**	**Altitude [m]**	**Latitude**	**Longitude**
1	Orsières	1022	46°1′44″N	7°9′8″E
2	Euseigne	1028	46°10′9″N	7°25′27″E
3	Icogne 2	880	46°16′42″N	7°26′10″E
4	La Garde	980	46°3′45″N	7°8′35″E
5	Vens	1373	46°5′7″N	7°7′24″E
6	Arbaz	1270	46°16′42″N	7°22′47″E
7	Icogne 1	1200	46°17′56″N	7°26′31″E
8	Cordona	1153	46°19′45″N	7°33′8″E
9	Eison	1768	46°9′18″N	7°28′10″E
10	Saint-Martin	1589	46°11′8″N	7°26′43″E
11	Grimentz	1738	46°11′22″N	7°34′35″E

Fig. S1.2. Experimental design. Each meadow harbours six different experimental plots of 20 m in diameter with a minimum of 5 m buffer zone between them. Treatments were randomly allocated. Soil cores were taken in each plot to measure AMF cultures in the field samples (blue dots) and to inoculate trap cultures (green dots).



Appendix 2. Intersection counting for measuring AMF colonisation levels

Fig. S2.1. (a) Stained roots were aligned on a microscopic slide. AMF colonisation was measured by intersection counting. (b) For each count (n = 100 per slide) we observed whether the vertical crosshair cut an AMF structure: arbuscules, vesicles, arbuscules and vesicles, hyphae only or none of them.



Declaration of consent

on the basis of Article 28 para. 2 of the RSL05 phil.-nat.

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Bachelor ☐ Master ◉ Dissertation ☐

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Supervisor: Prof. R. Arlettaz
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I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 para. 1 lit. r of the University Act of 5 September, 1996 is authorised to revoke the title awarded on the basis of this thesis. I allow herewith inspection in this thesis.

Bern, 24th August 2016

Place/Date

Signature