Species transfer via topsoil translocation: lessons from two large Mediterranean restoration projects

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Author contributions

EB identified the article content, wrote the first version of the article and managed the manuscript preparation; all coauthors collected the data; RJ analyzed the data, produced all figures and participated in writing the discussion; all coauthors edited the various versions of the manuscript.

Abstract

Restoration success of species-rich grasslands is often limited by low seed dispersal. To reintroduce target species of local provenances, bulk topsoil transfer is performed by excavating soil and vegetation from donor sites and spreading these on receptor sites. Our first objective was to determine the most advantageous season for transferring topsoil in Mediterranean grasslands. The second objective was to assess which treatment - combination of season (spring or autumn) and transfer ratio (1:1 or 1:3) - performed best in restoring a Mediterranean grassland through bulk topsoil transfer after two large restoration projects. Just-in-time soil transfer (i.e. with no stockpiling) was implemented 1) on a former 3-ha orchard where topsoil was spread after removing trees and levelling soil, and 2) after a 5-ha oil pipeline leak where polluted soil was removed prior to treatment (soil horizons were reconstituted). A seed bank study showed that the summer seed bank contained higher seed densities, species richness and similarity to the reference site than the spring seed bank. Spring transfers gave better results than autumn transfers in terms of species richness and composition similarity with the reference site, while transfer ratios gave similar results. Long-term success was not driven by season or transfer ratio but by the underlying seed bank at receptor sites: the former orchard's weed-containing seed bank hampered topsoil transfer success. This study also suggests that restoration success cannot be deduced from seed bank studies alone, as species establishment is highly dependent on differences in growing conditions (including competition at receptor sites).

Keywords: bulk topsoil transfer; sod dumping; soil inoculation; seed bank; steppe

Implications for practice

- Mediterranean grassland summer seed bank (vs. spring seed bank) contains higher seed densities and species richness, indicating that summer is better than spring for transferring seed banks of Mediterranean grasslands; using spring topsoil transfer (vs. autumn transfer) to restore Mediterranean grasslands gives better results in terms of species richness and composition similarity with the reference site (summer transfer could not be tested here);
- Topsoil transfer success is not greatly impacted by transfer ratio: the tested ratios 1:1 and 1:3 yield identical species richness and composition similarity with the reference Mediterranean grassland;
- The underlying seed bank at the receptor sites is an important factor in long-term topsoil transfer success to restore Mediterranean grasslands.

Introduction

The restoration of species-rich grasslands is often limited by low seed dispersal (Buisson et al. 2006; Grman et al. 2015; Halassy et al. 2016), both in space and time (e.g. Römermann et al. 2005). Successful methods of reintroducing target species of local provenances commonly include sowing seed mixtures and transferring seed-containing hay or soil (Kiehl et al. 2010). Soil transfer can be accomplished through i) turf transfer, also called community translocation, or ii) bulk topsoil transfer, also called sod dumping (Bullock 1998; Kiehl et al. 2010; Jaunatre 2014a; Bulot et al. 2014, 2017). Soil transfer means that not only seeds are transferred, but also other propagules such as rhizomes, small shallow-rooted plants, and micro-organisms that may play an important role in the restoration of the plant community (Török et al. 2011; Wubs et al. 2016). Of the two techniques, bulk topsoil transfer is the cheapest (Good et al. 1999), especially if soil is spread at a ratio lower than 1:1, i.e. spread on an area larger than where it was gathered (e.g. ratio of 1:2, Good et al. 1999; ratio of 1:3, Jaunatre et al. 2014a).

While both soil transfer techniques are based on extracting soil from donor sites scheduled to be destroyed and thus are not sustainable, they are fundamentally different. Turf transfer consists in cutting and transferring turfs and mainly relies on the survival and colonization potential of the plants contained in the turfs (Bullock 1998; Kardol et al. 2009;

Vécrin & Muller 2003). Bulk topsoil transfer, the type explored in this study, consists in excavating soil and vegetation from donor sites and spreading these on receptor sites, often at a ratio of 1:1 (see Kiehl et al. 2010 for review); it relies mainly on the availability of viable soil-stored seeds and other propagules.

Prior to large-scale bulk topsoil transfer, seed bank studies are often carried out at the donor sites. First, restorationists need to evaluate the potential of implementing such a technique: topsoil transfer is worth implementing only if the topsoil contains seeds of target species, particularly in regions where seeds are not available commercially (Fowler et al. 2015). Using soil seed banks as a source of material for restoration of various plant communities (forests, marshes, grasslands, heathlands) has been widely explored *via* seed bank studies (e.g. the review by Bossuyt & Honnay 2008 of 102 studies and the case study by Kalamees et al. 2012). With the exception of some grasslands, heathlands and early successional communities, these 103 seed bank studies concluded that restoration cannot rely solely on seed banks. However, bulk topsoil transfer is still used in many countries and for the restoration of various types of ecosystems, such as roadsides, meadows, grasslands, forests, etc., with relative success (Kiehl et al. 2010; Fowler et al. 2015). While this discrepancy between the findings of seed bank studies and the continuing practice of bulk topsoil transfer (Fowler et al. 2015) remains insufficiently studied, both bulk topsoil transfer and seed bank studies continue to be performed.

The second reason for performing seed bank studies prior to large-scale bulk topsoil transfer is to determine the most advantageous time for transfer. Ideally, transfer should be carried out at a season when a maximum proportion of the target species are found as seeds in the soil (De Villiers et al. 2004; Hosogi & Kameyama 2004), typically at the end of the vegetation period and, in the Mediterranean region, before the onset of the autumn rainy season (Grillas et al. 2004; De Villiers et al. 2004). This is particularly important when aiming to restore plant communities containing many species with a transient seed bank (Buisson et al. 2006). The third objective of prior seed bank studies is to permit subsequent evaluation of restoration success. After topsoil transfer, monitoring is carried out to document vegetation establishment on the receptor sites; the composition of the vegetation is often compared with the seed bank and vegetation of the donor sites to evaluate restoration success (Vécrin & Muller 2003).

The objectives of this study are 1) using a seed bank study, to assess whether spring or summer is more advantageous for transferring topsoil to restore Mediterranean grasslands and 2) using a topsoil transfer experiment, to assess the best combination of season (spring or autumn) and bulk topsoil transfer ratio (1:1 or 1:3 hereafter termed topsoil transfer ratio) to restore a Mediterranean grassland plant community. The best transfer time should be summer, when topsoil contains both species that form a more persistent seed bank and species that form only a transient seed bank; however, this season could not be tested mainly due to constraints inherent to worksites. Soil transfers were implemented in autumn and spring. Our hypotheses are that i) the best transfer time between spring and autumn is spring, when topsoil contains the few species fruiting in late autumn, ii) the best topsoil transfer treatment is at a ratio of 1:1 (delivering a higher number of seeds per area unit).

Methods

Study sites

The study sites are situated in south-eastern France, in a pseudo-steppe located in the plain of La Crau which has recently undergone several large restoration projects, three of which included soil transfer. La Crau is the ancient delta of the Durance River, whose dry grassland vegetation once covered 60 000 ha. Dominated by perennial species and also including many annual species, this vegetation has been shaped by a dry and windy Mediterranean climate, particular soil conditions (i.e. shallow, relatively poor Haplic Cambisol (Calcaric) soil with silicaceous stones, covering about 50% of the ground) and many centuries of itinerant sheep grazing (Buisson & Dutoit 2006; Figure S1). This now fragmented grassland vegetation, of which only 11 500 ha remain, serves as a reference ecosystem and donor sites for soil transfers (Jaunatre et al. 2014b). The first restoration site is an area of 357 ha managed from 1987 to 2006 as an orchard, and rehabilitated in 2008 as part of the first mitigation banking experiment launched by the French government (Dutoit et al. 2013). Concurrently with this rehabilitation project, restoration trials were carried out on this orchard: direct topsoil transfer from La Crau dry Mediterranean grassland (with no stockpiling) was implemented on a few hectares in spring and autumn 2009 at ratios of 1:1 or 1:3 (partly described in Jaunatre et al. 2014a; Figure S1). Less than one kilometer away from this former orchard (Figure 1), the grassland soil and vegetation of a 5.5 ha grassland area were completely destroyed by an oil spill due to an underground pipeline leak in 2009 (second site hereafter referred to as pipeline leak site). Restoration consisted in excavating the polluted soil down to the bedrock (40 cm) followed by direct topsoil transfer from La Crau dry Mediterranean grassland, reconstituting soil horizons in spring 2011 (Bulot et al. 2017; Figure S1).

Seed bank study

To identify the season when the topsoil seed bank of the reference site has the highest seed content, ten replicate 2.5 L soil samples were taken at the site of Peau de Meau, in the center of the plain of La Crau, in April and August 2001 (Table 1; Römermann et al. 2005). These 20 samples were left to germinate following the protocol described hereafter. Each soil sample taken in the field consisted of 10 pooled subsamples, as recommended by Bakker et al. (1996) taken between 0 and 20 cm deep using a 4-cm diameter soil corer. The seedling emergence method (Ter Heerdt et al. 1996) was selected to qualify and quantify germinable seeds in the soil seed banks. Soil samples were concentrated by washing them with water on two different sieves (2 mm and 200 µm) to reduce bulk and clay. The concentrated soil was spread in a thin layer (0.5 cm) on plastic trays filled with 2 cm vermiculite topped with medical compresses (mesh size about 100 µm). The trays were covered with fine gauze to prevent contamination. All trays were watered frequently from below. Emerged seedlings were identified, counted and removed weekly. Unknown seedlings were grown for identification. After three months of cultivation (greenhouses), the samples were allowed to dry out. Following six weeks of cold stratification (5°C), the samples were cultivated for a further three months. Vegetation monitoring was carried out in ten replicate 4-m² quadrats in May 2001 (Table 1): we recorded the presence of each species.

Topsoil transfers and vegetation sampling

Direct topsoil transfer of the 20-cm upper soil layer was implemented on the former orchard in mid-March 2009 at a ratio of 1:3 on 1 ha (Table 1 and 2), and in late October 2009 both at a ratio of 1:3 on 3 ha (Jaunatre et al. 2014a) and at a ratio of 1:1 on 0.3 ha (Jaunatre et al. 2014a). Direct topsoil transfer was also implemented on the pipeline leak site in spring 2011 at a ratio of 1:1 on 5.5 ha (Table 1 and 2; Bulot et al. 2017). At the former orchard, topsoil was spread over the orchard soil (Jaunatre et al. 2014a). At the pipeline leak site, all soil horizons were reconstituted: i) altered bedrock (35–40 cm deep), ii) subsoil (20–35 cm deep) and iii) topsoil (0–20 cm deep) (Bulot et al. 2017, 2014). Vegetation monitoring was carried out in May 2010, May 2011 and May 2012 on the former orchard and in May 2012, May 2013 and May 2014 on the pipeline leak site. At each site, a minimum of three replicate 4-m² quadrats were sampled (Table 1): we recorded the cover of each species and the total percent cover of vegetation using a modified Braun-Blanquet scale (Braun-Blanquet et al. 1952): 0.5 for species covering less than 1% of the quadrat, 1 for those covering between 1% and 5%, 2 for those covering between 5% and 25%, 3 for those covering between 25% and 50%, 4 for those covering between 50% and 75% and 5 for species covering more than 75% of the quadrat.

Data analysis

To identify the best time to transfer topsoil to the former orchard and pipeline leak sites, we compared spring and summer seed bank characteristics. Differences in composition between seed banks were assessed using i) species richness and seed density per sample and ii) a redundancy analysis (RDA; matrix: 39 samples × 50 species) based on abundance data from the seed bank, after a Hellinger transformation (Legendre & Gallagher 2001). Differences in composition between seed banks with above-ground vegetation were assessed using normalized Community Structure Integrity Indices (CSIInorm) calculated on plant species presence / absence data (Jaunatre et al. 2013). To compare means between seasons, GLMs using a Poisson distribution were performed on species richness and seed abundance data as they are count data, and a t-test was performed on CSIInorm as they met parametric conditions.

To compare topsoil transfers implemented to restore Mediterranean dry grassland at the former orchard and pipeline leak sites, and to test the effects of season and transfer ratio (treatments = ratio-season combination) on success, we compared plant species richness and composition between the restored sites. Here again, differences in composition were assessed using i) a normalized Community Structure Integrity Index (CSIInorm) calculated on plant species presence / absence data and ii) a non-metric multidimensional scaling (NMDS; matrix: 33 quadrats × 99 species) based on Bray–Curtis distance calculated from presence / absence data. As species richness data is count data, they were analyzed with a Generalized Linear Model (GLM) procedure using a Poisson distribution to compare means between restored sites. CSIInorm met parametric conditions; we therefore performed an ANOVA. Three pairwise comparisons, with Benjamini-Hochberg adjustment, were carried out: 1) to compare dates of transfer at the 1:3 ratio on the former orchard, 2) to compare autumn transfer ratios on the former orchard and 3) to compare the spring 1:3 transfer on the former orchard and the spring 1:1 transfer on the pipeline leak site. To determine the combined effect of year (1, 2 and 3 years after transfer), and treatments (ratio-season combination) on CSIInorm we performed two-way ANOVA on repeated measures, followed by Tukey honestly significant difference post-hoc tests.

All analyses were performed with R software 3.3.2 (R Core Team 2016) and its 'vegan' package (Oksanen et al. 2017).

Results

Seed bank

The soil seed bank collected in summer contained more species (15.2±0.6 species / sample) than that collected in early spring (5.4±1.0 species / sample) ($z = 45.08$, $p<0.001$; Figure 2a). The seed bank collected in summer also contained more seeds (101.1±8.3 seeds / sample) than that collected in early spring (8.7±1.8 seeds / sample) ($z = -852.86$, $p<0.001$; Figure 2b). Although they shared many species, the two seed bank communities were separated along the RDA first axis (Figure 3). Spring samples were differentiated from summer samples by the presence of *Sagina apetala*, absent from the summer seed bank. The summer seed bank samples were characterized by species like *Gastridium ventricosum, Vulpia* sp., *Aira cupaniana, Catapodium rigidum, Galium parisiense, Bromus hordeaceus* and *Melica ciliata* which were not, or only rarely, found in the spring seed bank. The samples collected in spring were more dispersed on the RDA ordination than those collected in summer. Most of the species found in the spring and summer seed bank were species found in the reference grassland (thus target species), although similarity was below 0.4 for both seasons (Figure 3; $t=-6.58$; $p<0.001$). Out of the 1089 germinations, we identified 50 species (14 in spring and 38 in summer), only 5 of which were non-target species. *Senecio vulgaris* (49 germinations), which mainly germinated outside the summer seed bank, is naturally and sporadically found in La Crau grasslands. The other four species, *Verbena officinalis* (65 germinations, 13 in spring and 52 in summer), *Kickxia elatine* (3 germinations, 1 in spring and 2 in summer) *Chenopodium album* (2 germinations, 1 in spring and 1 in summer) and *Portulaca oleracea* (2 germinations in spring) were not found in the above-ground grassland vegetation; they are arable weeds dispersed from formerly irrigated fields found around the study site (Römermann et al. 2005; Buisson et al. 2006).

Topsoil transfers

The year following topsoil transfer, species richness ($z = 106.59$; $p<0.001$) and similarity to the reference site (CSIInorm; $F = 14.35$; $p<0.001$) significantly differed between treatments (ratio-season combination): both these variables were higher for spring soil transfer than for autumn soil transfer on the former orchard (see results of post-hoc tests, Figure 4). Moreover, for the spring transfers, similarity to the reference site was slightly significantly higher with the 1:1 transfer ratio on the pipeline leak site than with the 1:3 transfer ratio on the former orchard (Figure 4). Transfer ratios did not affect species richness and similarity to the reference site for the former orchard. In spring, similarity was higher for the pipeline leak site transfer at a ratio of 1:1 than for the former orchard transfer at a ratio of 1:3 (Figure 4). Overall, the plant compositions of the different topsoil transfers were rather similar, with many species characteristic of the reference grassland such as *Carlina corymbosa*, *Thymus vulgaris* or *Trifolium stellatum* (Figure 5). However the topsoil transferred onto the pipeline leak site had a slightly different plant composition characterized by ruderal species like *Cardamine hirsuta* or *Lolium perenne*. The topsoil transferred onto the former orchard was characterized by ruderal species like *Bromus diandrus*, *Chenopodium album*, *Hirschfeldia incana* and *Lactuca serriola* (Figure 5).

From the second year after transfers, only the spring pipeline leak site transfer at a ratio of 1:1 retained high similarity to the reference site, above 80%. For all transfers implemented at the former orchard site, regardless of transfer season or ratio, similarity dropped to below 60% (Figure 6).

Discussion

Topsoil transfer timing

The La Crau Mediterranean dry grassland has a mainly transient seed bank (Buisson et al. 2006). Since most of the plants are annuals and spend the harsh season (summer) as seeds in the seed bank, the most advantageous season for topsoil transfer should clearly be summer. Moreover, seeds can be expected to germinate in autumn: in a Mediterranean climate, not only is germination triggered by temperature and light, but it also occurs only after a threshold amount of precipitation (Ne'eman & Goubitz 2000). Differences cannot simply be explained by dormancy cycles, as germination of annual Mediterranean species depends on the timing and amount of autumn precipitation (Espigares & Peco 1993, 1995). The results of our seed bank study support our first hypothesis, showing that species richness and seed density and similarity of species composition between seed bank and above-ground vegetation of the reference site are much higher in the summer than in the spring seed bank. The latter is more dispersed on the community ordination because only a few seeds germinated in each spring sample (Römermann et al. 2005), giving each species a higher weight than the species composing the more abundant summer seed bank community.

While summer appears to be the most appropriate season for transferring the seed bank, restoration work cannot always be implemented when it would be ideal in terms of resilience of the restored plant community. This is mainly due to constraints inherent to worksites and because restoration planning usually takes into account not only the phenology of plant communities but also that of endangered plant species and of animals, such as nesting birds (Buisson et al. 2017). Thus, topsoil transfer was never implemented in summer to restore our Mediterranean dry grassland.

Season for Mediterranean dry grassland topsoil transfer

Topsoil transfer was not carried out in summer on the Mediterranean grassland due to site preparation delays (soil levelling on the former orchard and polluted soil excavation on the pipeline leak site; see Jaunatre et al. 2014a; Bulot et al. 2014; 2017 for details). As many annual species germinate with the first autumn rains, it was difficult to hypothesize that either autumn or spring would be the best transfer season. In the first year, spring topsoil transfer led to high species richness on the restoration site and high similarity with the reference site (ca. 80%) compared to autumn transfer (slightly over 40%). This may be partly due to the fact that a few species, such as *Bellis sylvestris*, *Lobularia maritima*, flower and produce seeds mainly in autumn and are thus added to the pool of potentially germinable species in spring. In this study, these two species germinated with both transfers but their frequency of occurrence was higher on the restoration sites where spring topsoil transfers were performed. We observed annual Fabaceae common in the reference grassland, such as *Medicago minima, Medicago monspeliaca, Medicago truncatula, Trifolium cherleri, Trifolium scabrum* and *Trifolium stellatum*, more frequently with the spring than with the autumn transfers. Van Assche et al. (2003) showed that some species congeneric to those cited here show increased germination efficiency with cold stratification. They would therefore germinate better after winter, explaining the results from spring transfers in our study. Surprisingly, numerous annual species known through seedling emergence studies to have a transient seed bank and accordingly not found in our spring seed bank study, were found under spring soil transfer. This may partly be due to the fact that species with low seed production and species whose germination requirements are narrow are less likely to be detected by seed bank studies (Saatkamp et al. 2009). Moreover, species whose seeds massively germinate with autumn rain are hard to detect in spring. It can thus be expected that a wider range of species will establish from spring topsoil transfer than predicted from seed bank studies (Kalamees & Zobel 2002). Here, the restoration following polluted soil excavation on the pipeline leak site, which involved a spring transfer of 41 000 tons of soil, gave better results than predicted *via* seed bank studies on a few kilos of soil (Bulot et al. 2014).

Perennial species of this Mediterranean dry grassland do produce seeds that can germinate in autumn in the field and form a seed bank (e.g. *Dactylis glomerata*; 64 germinations in the summer seed bank vs. 14 in the spring seed bank / 1089 germinations). Perennial plants also make it through summer drought thanks to the survival of the organs (meristematic tissues at the bases of leaves, roots, underground storage organs) from which they regrow once water is available (Volaire et al. 2009; Moreira et al. 2012). They store carbohydrates during the growing season, before the onset of drought, which allows them to maintain respiration demands during summer and to initiate resprouting (Volaire et al. 2009; Moreira et al. 2012). Thus, topsoil transfer in autumn may not have given them enough time to replenish their reserves to be resilient to a second stressful situation, such as translocation: *Brachypodium retusum* established better in spring than in autumn, as did *Cynodon dactylon* and *Bothriochloa ischaemum*, though to a lesser extent. An attempt to translocate perennial species in summer would show whether experiencing two simultaneous disturbances (drought and translocation), rather than one occurring after the other, provides a better chance of survival.

Differences between restored grassland sites

Topsoil transfer on the pipeline leak site yielded few ruderal species, and even those were ruderal species also found on the reference grassland, such as *Cardamine hirsuta* or *Lolium perenne*. Contrastingly, topsoil transfer on the former orchard led to communities dominated by ruderal species like *Bromus diandrus*, *Chenopodium album* and *Hirschfeldia incana*, absent from the reference grassland but common on the rehabilitated areas of the former orchard where topsoil transfer was not performed (Jaunatre et al 2014a). This major difference between the two restored sites is due to a difference in restoration process: on the pipeline leak site, the soil horizons were entirely reconstituted, while on the former orchard, topsoil was spread on top of orchard soil containing its own seed bank of weeds (Jaunatre et al 2014a). It is likely that the different restoration processes also largely explain the differences between transfer ratios in spring: the 1:1 ratio soil transfer implemented on the pipeline leak site produced results more similar to the reference one year after transfer than the 1:3 ratio soil transfer implemented on the former orchard.

Autumn soil transfer ratios on the former orchard did not affect species richness and similarity to the reference one year after transfer. The representation on the ordination of the autumn 1:3 transfer ratio on the former orchard is more dispersed, most probably due to the greater sampling effort.

In subsequent years, 80% similarity to the reference site was maintained with the spring 1:1 ratio topsoil transfer on the pipeline leak site, whereas the spring 1:3 ratio topsoil transfer on the former orchard led to similarity falling to below 50%. There are two possible explanations. First, the 1:3 ratio does not transfer enough seeds for the community to be maintained long-term. However, this is contradicted by the fact that similarity with the reference is identical with both ratios for the autumn transfers. The second likely explanation is the differences between sites and restoration processes. The seed bank and soil nutrient content are affected by cultivation legacy, and there can be long-term effects on plant communities (Cramer et al. 2008; Smits et al. 2008). On the former orchard, weed seed load in the seed bank and nutrients in the soil are therefore greater, resulting in high average vegetation height and cover (Jaunatre et al 2014a). This may have had a negative effect on the germination and growth of less competitive light-demanding grassland target species (Hautier et al. 2009).

Seed bank studies help acquire a better understanding of ecosystem functioning and thus help restorationists plan better transfer timing. However, they do not provide a clear prediction of restoration success, since they largely underestimate the true seed density in the seed bank due to detection probabilities, dormancy and germination niches (De Villiers et al. 2004; Fowler et al. 2005). The outcome for bulk soil transfers in Mediterranean ecosystem restoration depends on several factors: transferred soil depth, whether the soil is stockpiled and whether soil horizons are reconstituted (Koch 2007; Coiffait-Gombault et al. 2011; Bulot et al. 2014), the proportion of annual species in the donor site seed bank, and the fertility and seed bank of the receptor site (Jaunatre et al 2014a; Fowler et al. 2015). Restoration success is also highly dependent on appropriate scheduling of the various tasks, although in practice timing is also governed by worksite imperatives. More importantly, restoration implies taking into account a set of environmental parameters not at play in seed bank studies. The patterns of some of these parameters can partly be foreseen on worksites (e.g. sites scheduled for restoration may be colonized by arable weeds germinating from the seed bank dating back to former agricultural activities; Buisson et al. 2006; Jaunatre et al. 2014a). However, this does not necessarily mean that they can be dealt with in advance. Ecological restoration will therefore partly remain a trial and error process and restored sites will require subsequent adaptive management.

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Table 1. Dry grassland seed bank study and topsoil transfer details, showing protocols as well as subsequent vegetation monitoring. FO stands for former orchard and PL for pipeline leak site.

<table>
<tr><th></th><th>reference vegetation</th><th>seed bank and season</th><th>topsoil transfer monitoring</th><th>objectives</th></tr>
<tr><td>sites</td><td>3 sites of dry grassland</td><td>1 site of dry grassland (Peau de Meau; 43°34'01.85''N, 4°50'07.12''E)</td><td>2 sites (FO, PL), see Table 1 for details</td><td>to determine the best time to transfer topsoil in Mediterranean grasslands</td></tr>
<tr><td rowspan="5">monitoring</td><td rowspan="4">6 × 4m² plots / site</td><td rowspan="4">Seed bank: 10 2.5-L samples (pooled subsamples) taken between 0 and 20 cm deep, 2 seasons (April, August) Vegetation: 10 × 4m² plots</td><td>Aut. 1:1 - FO: transfer from Ménudelle 1:1 (3 × 0.1 ha) in autumn: three 4-m² plots</td><td rowspan="5">to determine the best topsoil transfer treatment (ratio-season combination)</td></tr>
<tr><td>Aut. 1:3 - FO: transfer from Ménudelle 1:3 (3 × 1 ha) in autumn: eighteen 4-m² plots</td></tr>
<tr><td>Spring 1:3 - FO: transfer from Massilia 1:3 (1 × 1 ha) in spring: three 4-m² plots</td></tr>
<tr><td>Spring 1:1 - PL (1 × 5.5 ha) in spring: nine 4-m² plots</td></tr>
<tr><td>vegetation sampling, BB index (total = 18 plots)</td><td>germination identification and count, vegetation sampling (presence / absence)</td><td>vegetation sampling, BB index</td></tr>
<tr><td>references</td><td>Jaunatre et al. 2014a</td><td>Römermann et al. 2005 and unpublished</td><td>Jaunatre et al. 2014a,b; Bulot et al. 2017 and unpublished</td><td></td></tr>
</table>

Table 2. Topsoil transfer data used to determine the best topsoil transfer treatment (ratio-season combination). Coordinates of and distance between donor and restored (receptor) sites. Area covered (ha), ratio and season are indicated for each transfer.

donor sites	**coordinates**	**restored (receptor) sites**	**coordinates**	**distance (km) between donor and restored sites**	**Surface area (ha) covered by topsoil transfer**	**ratio**	**season**	**month**
Massilia	43°29'03.61"N, 4°53'16.56"E	Former orchard	43°31'07.38"N, 4°51'35.05"E	3.5	1 × 1 ha	1:3	spring	March 2009
Ménudelle	43°30'30.22"N, 4°54'03.14"E				3 × 1 ha	1:3	autumn	Oct. 2009
					3 × 0.1 ha	1:1	autumn	Oct. 2009
		Pipeline leak site	43°31′36.77″N, 4°53′04.50″E	2.5	1 × 5.5 ha	1:1	spring	March-April 2011

Figure 1. Map of the donor and restored (receptor) sites and as well as of the seed bank study site. FO = former orchard and PL = pipeline leak site where dry grassland topsoil transfers were implemented. See Table 1 for details.

Figure 2. a. Species richness, b. seed density density and c. normalized Community structure Integrity Index of the soil seed bank of Peau de Meau, Mediterranean dry grassland (reference grassland), at two seasons (spring: early spring and summer: end of summer).

Figure 3. Soil seed bank ordination of samples from Peau de Meau, Mediterranean dry grassland (reference grassland) gathered at two seasons (spring: early spring and summer: end of summer) based on RDA of plant community composition. For clarity, only the 16 most correlated species are shown (out of 38).

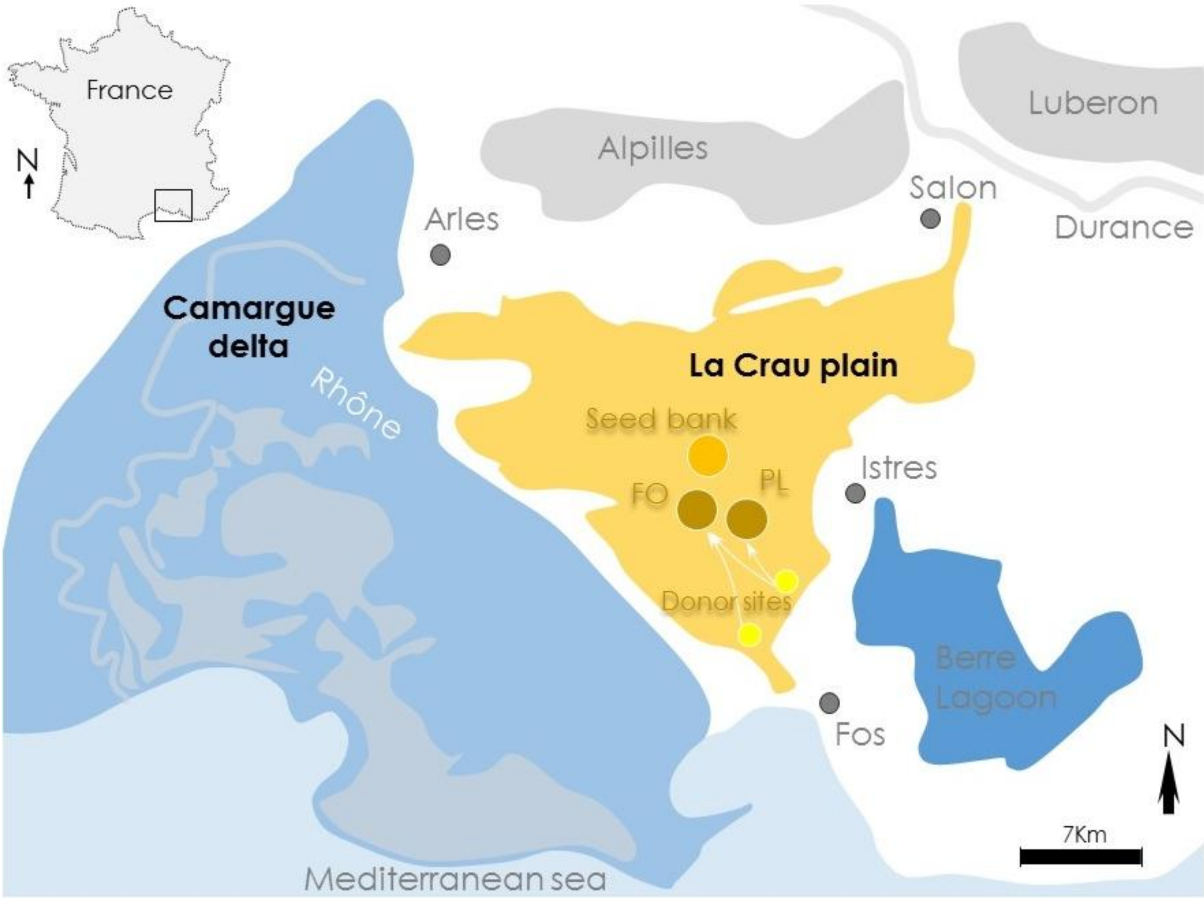
Figure 4. a. Species richness of the areas restored using topsoil transfers from Mediterranean dry grassland and; b. CSIInorm, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with restored communities. Soil gathering season (spring in pink; Aut.: autumn in green), transfer ratio (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site). Error bars represent ±SE, horizontal bars represent pairwise comparisons (NS: $p>0.05$; *: $p<0.05$; ***: $p<0.001$).

Figure 5. Plant community ordination one year after different topsoil transfers from Mediterranean dry grassland for different soil gathering seasons (spring in pink; autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site) based on NMDS of plant community composition, final stress=0.16. For clarity, only the 19 most correlated species are shown (out of 99).

Figure 6. CSIInorm, normalized Community Structure Integrity Index with reference grassland vegetation monitoring as reference compared with communities restored using topsoil transfers from Mediterranean dry grassland and interactions with years (1 to 3 years after transfers, from dark to light shades). Soil gathering seasons

(spring in pink; autumn in green), transfer ratios (1:3 light colors; 1:1 dark colors) and receptor sites (Former Orchard; Pipeline Leak site). Error bars represent ±SE, bars having a common letter are not significantly different (Tukey honest significant differences post-hoc tests after ANOVA on repeated measures; $p>0.05$).

Figure 1.



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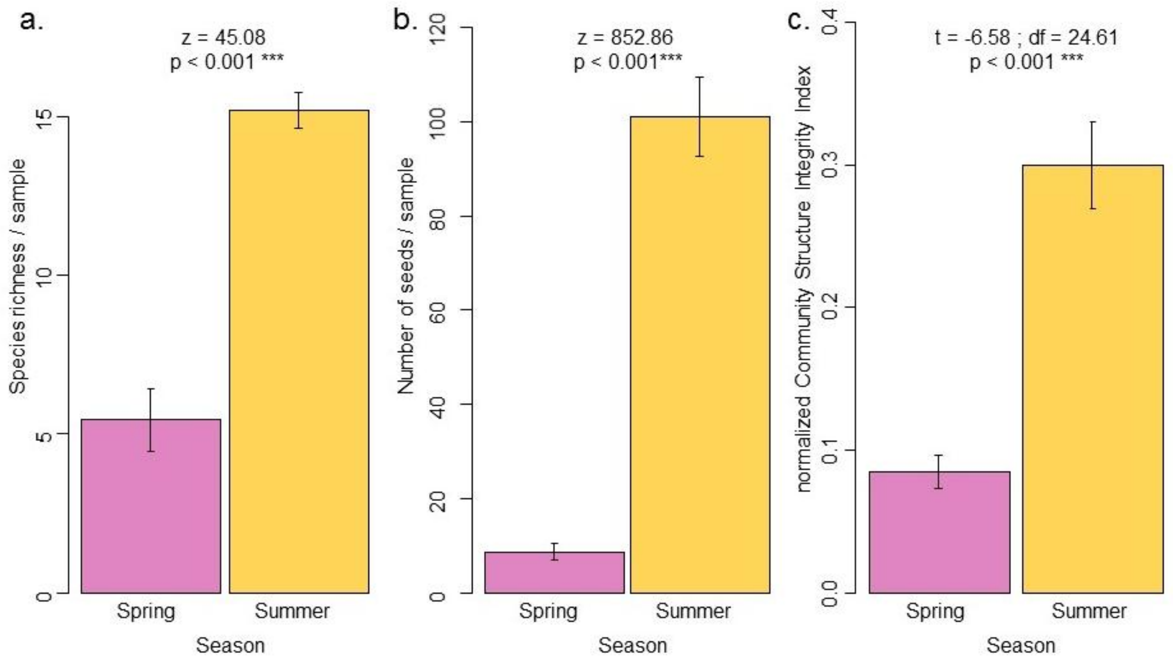

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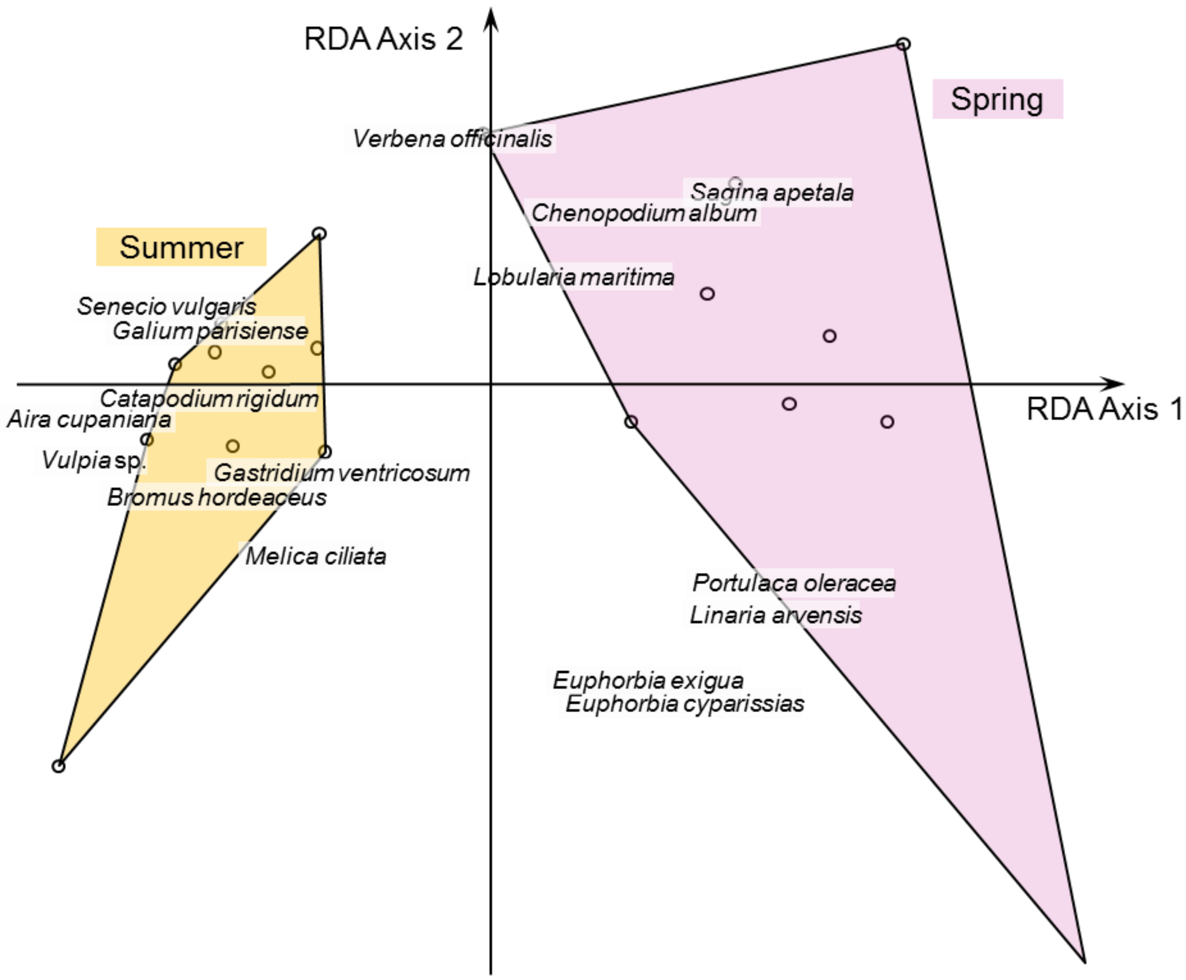
Figure 3.



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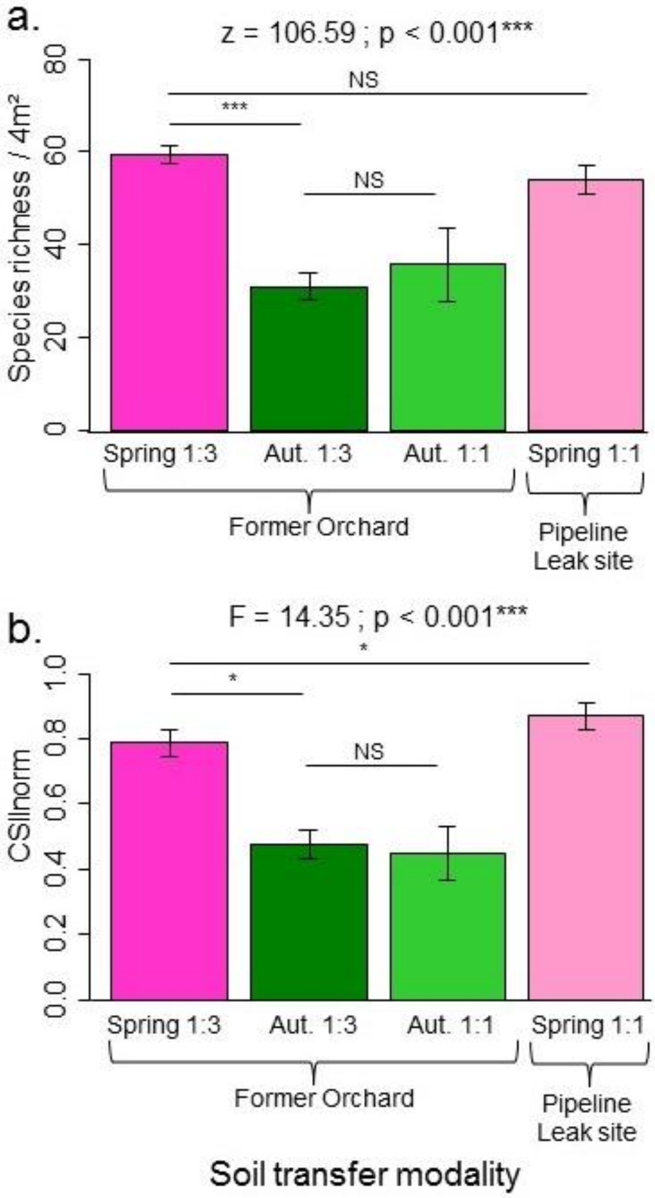

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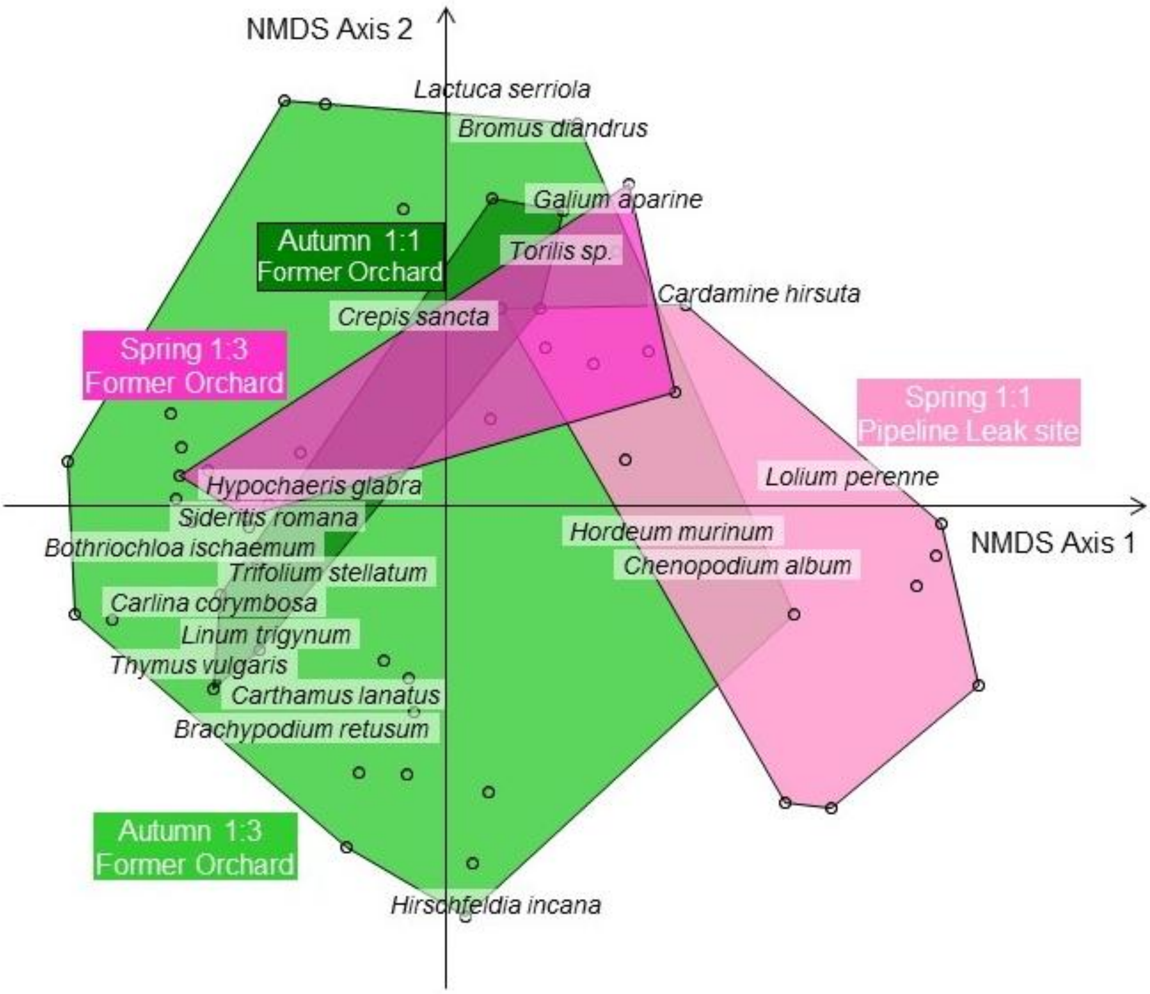

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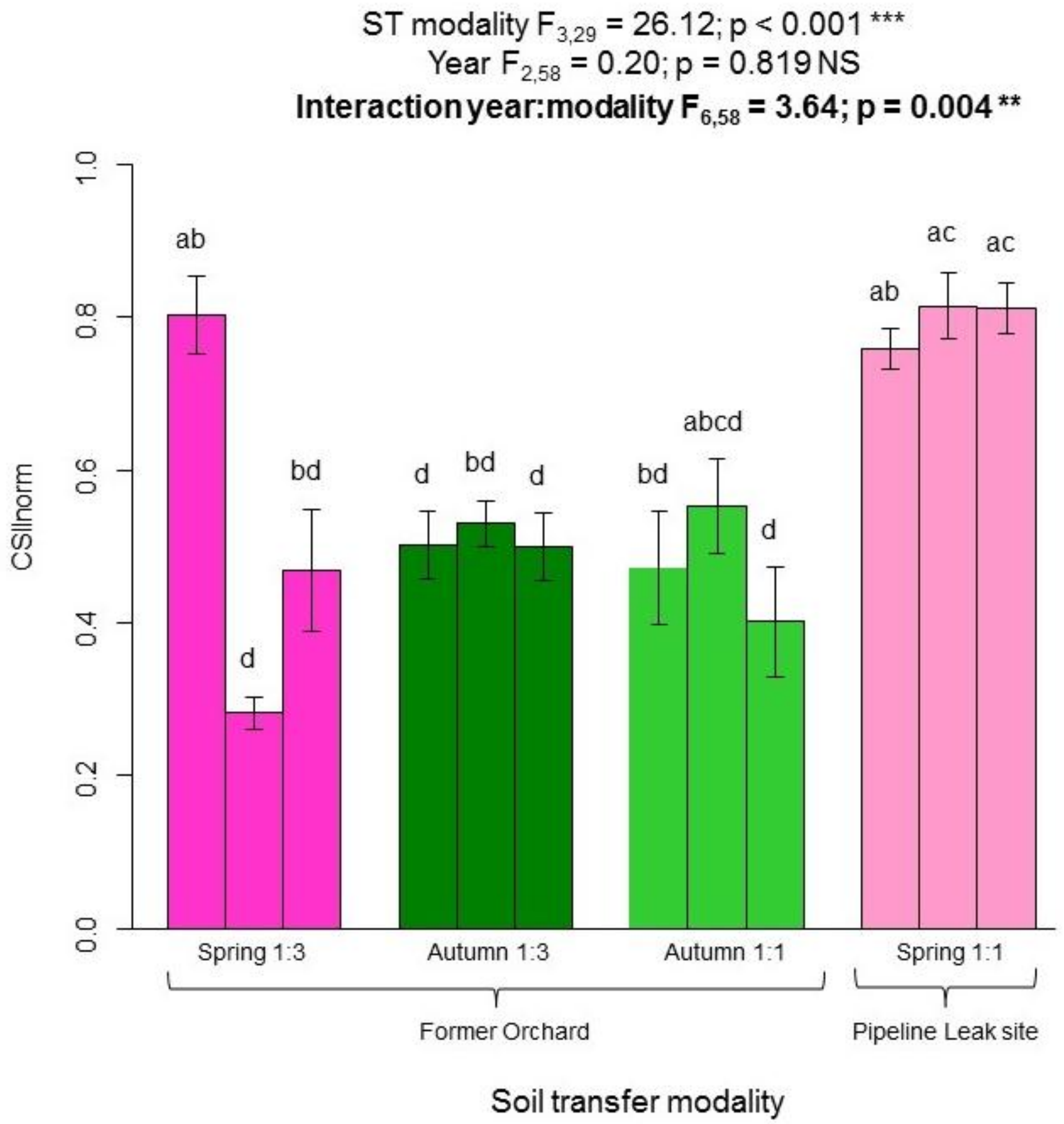

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