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Running head: Substrates for grassland restoration in quarries

Julie Chenot-Lescure[1,2,3]*, Renaud Jaunatre[2], Elise Buisson[3], Hervé Ramone[3], Thierry Dutoit[3]

1. Société des Carrières de la Ménudelle, 13. BP 80011, 13551 Saint-Martin-de-Crau, France
2. Univ. Grenoble Alpes, INRAE, UR LESSEM, 2 rue de la Papeterie-BP 76, 38402 St-Martin-d'Hères, France
3. Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE), Avignon Université, UMR CNRS IRD Aix Marseille Université, IUT site Agroparc, BP 61207, 84911 Avignon cedex 09, France

*author corresponding: elise.buisson@univ-avignon.fr

Author contributions

JCL, EB, TD, RJ conceived and designed the research; JCL, EB, HR, TD conducted the vegetation survey; JCL, EB, RJ analyzed the data; JCL, EB wrote the manuscript; EB, RJ, TD corrected the manuscript.

Abstract

Quarry restoration is difficult to implement due to the scarcity of the original soil. The restoration of Mediterranean dry grasslands to mitigate similar ecosystem/habitat loss is thus still a developing process. So far, very few studies have created artificial substrates to address the lack of soil. In the *La Crau* plain (southeastern France), quarries were established in an ancient Mediterranean steppe-like dry grassland. Once the remaining grassland zones were protected in 2001, quarries were extended over former intensive orchards established on the grassland in the 1990s before it was protected. Now, restoration has to be done without the unaltered protected grassland soil but with the soil from orchards, which contains fertilizers spread during the orchard exploitation. To recreate a more suitable substrate, the orchard topsoil was mixed with poor substrate materials directly extracted from the quarry (0-30 mm pebbles with sandy matrix). Different substrate mixtures were tested with or without sowing the dominant species of the grassland, a perennial grass: *Brachypodium retusum* (and an annual grass *Brachypodium hybridum*). After five years of monitoring, our results show that raw substrate materials (100%) limit the establishment of all species, and that, at the opposite, orchard topsoil (100%) favors a significantly higher species richness of target and non-target species. The recommended substrate mixture to reach a compromise between high target cover and low non-target species cover is composed of 50% raw quarry material and 50% arable soil. Sowing *Brachypodium* reduces target species richness establishment but also limits non-target species richness and cover.

Keywords: Grassland, Raw substrate materials, Mediterranean pseudo-steppe, Artificial soil mixture, Soil restoration, Thero-Brachypodietea.

Implications for practice:

- Soil resources may be scarce for ecological restoration therefore creating substrates based on soil mixed with raw quarry material is an opportunity to increase available substrate volumes
- Substrate composed of 100% arable soil maximized both target and non-target species establishment
- Substrate mixtures composed of 50% arable soil and 50% raw quarry material are a good compromise to create more substrate than there is soil, favor target species and lower non-target species establishment
- Sowing *Brachypodium* lowers non-target species richness but also target species cover; it creates a physiognomy close to that of the reference grassland.

Introduction

Ecological restoration in former quarries is often difficult because of the definitive loss of the original soil resource (Castillejo & Castelló 2010; Jarvis & Walton 2012). Its replacement by low quality substrates (e.g. rubble sometimes mixed with inert waste: low nutrient and organic contents, low mycorrhize, poor soil structure, low water storage, etc.) is often insufficient for successful revegetation because it limits the establishment and growth of plants, which are also exposed to recurrent drought stress in the Mediterranean region (Oliveira et al. 2011). To date, very few studies have focused on the creation *in integrum* of a soil in ecological restoration projects, while this is known to be one of the key drivers of restoration success (Mchergui et al. 2014; Muñoz-Rojas 2018).

When quarrying is performed, soils can be discarded or stockpiled, altering their quality (Stahl et al. 2002). If quarrying occurs in pristine / undamaged ecosystems, immediately transferring the soil to a restoration site (just-in-time soil transfer) is a good way to restore high species-diversity grasslands in Southeastern Mediterranean France (Jaunatre et al. 2014; Bulot et al. 2017; Buisson et al. 2018). However, this technique is hardly applied because it is costly and need to be performed concomitantly with undesirable soil extraction on undamaged well preserved ecosystems. It is thus limited to cases where a reference site is planned to be destroyed (Bullock 1998).. Other sources of soils, such as from abandoned agricultural areas may also be available for restoration. However, fertilizers spread in the course of intensive exploitation (nitrogen, phosphorus and potassium) may have considerably increased the nutrient contents of these soils (Römermann et al. 2005; Wang et al. 2017; Helm et al. 2019). The use of the arable layer (i.e. topsoil) resulting from abandoned agricultural areas might therefore favor the establishment of undesirable species from the seedbank (Buisson et al. 2018), including ruderal and exotic invasive species, to the detriment of target stress-tolerant plant species (Castillejo & Castelló 2010; Ballesteros et al. 2012; Jaunatre et al. 2014). Thus, the use of such arable soil may not enable the restoration of nutrient poor grassland vegetation with high species-diversity, thus impeding the restoration of such ecosystems.

The selection of the materials at the outset of a project determines the success of the restoration process (Bradshaw 2000), and is particularly decisive for the recovery of the target vegetation associated with specific substrates (Ballesteros et al. 2014, 2012; Whiting et al. 2004). In order to recreate an adequate substrate starting with arable soil as a base, mixing it with natural materials (for example, originating from the geological substrate), not impacted by the chemical inputs of former agricultural practices, should allow approaching the oligotrophic characteristics of the soils of the reference communities.

The first aim of the present experimental setup was to test different mixtures of substrates in the context of a Mediterranean dry grassland (*La Crau*, Southern France) restoration. This will allow us to select the best substrate for initiating the successional trajectory of the vegetation towards the reference dry grassland (*Asphodeletum fistulosi* – Molinier & Tallon 1950). During the restoration process, species may not recolonize owing to a lack of seed dispersal. In that case, the recolonization process may be accelerated by sowing target species, or by sowing so-called 'nurse' species to encourage the establishment of other species (Padilla & Pugnaire 2006; Ren et al. 2008; López & Cavallero 2017). The second aim of this experimental setup is thus to test whether *Brachypodium retusum* (Poaceae) can act as a nurse species for grassland restoration in quarries. It is a perennial rhizomatous grass species which represents the most abundant species in biomass and cover in many north-western Mediterranean grasslands (Molinier & Tallon 1950; Luis et al. 2004; Buisson et al. 2021). It is common within its natural range (Contu 2013), where it plays an important role particularly in post-wildfire recovery (Caturla et al. 2000). *B. retusum* is one of the first species to reproduce after a fire (Vidaller et al. 2019), and to rapidly recolonize the environment. It plays a key role in protecting the soil against erosion, favoring the establishment of target shrub species and preventing the establishment of undesirable species (Caturla et al. 2000; Larchevêque et al. 2010; Raventós et al. 2012). Its rather tall and dense physiognomy can be expected to enable the capture of windborne seeds (Bullock & Moy 2004; Giladi et al. 2013) and the protection of seedlings from grazing. Consequently, *B. retusum* would be a good candidate to have an indirect positive impact on the establishment and survival of target species (defined here as the species found in the reference grassland).

The experimental setup was designed to test the effect of (1) the physical-chemical composition of the substrate, (2) *B. retusum* sowing and (3) their interaction, on the natural colonization of the regional species pool. We tested the following hypotheses: (1) The substrate mixtures, constituted exclusively of materials extracted from the quarry (pebbles of 0-30 mm with sandy matrix hereafter raw quarry material), are poor in nutrients and organic matter, and will limit the establishment of all species, i.e. undesirable ruderal species and target species; (2) Spontaneous colonization enables plant communities to tend towards the reference grassland, as long as i) they are not in competition with dense patches of ruderal species (like on the substrate mixtures with the highest proportion of arable soil), or ii) the abiotic conditions are not too harsh (like on the substrate mixtures with raw quarry material); and (3) Sowing *Brachypodium retusum* enables the rapid establishment of this species, and potentially facilitates the trapping of windborne seeds from the reference grassland. Sown plots will then have higher cover of *B. retusum*, increased richness of target species and greater similarity to the reference grassland.

Methods

Study area

Our study was carried out on a quarry in the *La Crau* plain, located in southeastern Mediterranean France (Bouches-du-Rhône). An area of the gravel quarry "*La Ménudelle*" was dedicated to this study. It was in operation from 2008 to 2011, impermeabilized in 2012 (using deposits of decantation sludge – clays and silt - presenting a strong water retention capability) and filled up in 2012-2013 (with inert materials that are non-fermentable, non-polluting and not likely to alter the physical and chemical properties of the soil down to 3.50m depth (BO-MEDDTL 2011; Figure 1). After compacting and levelling of the surface of the landfill, the site was then at an elevation of 40 cm below the original surface of the reference grassland and ready to receive the experimental setup.

The different substrate mixtures

Two types of substrates were used in the experiment for the different mixtures. 1) Arable soil from an orchard abandoned in 2015, composed of the topsoil (organo-mineral layer, 0-20 cm) and the subsoil (mineral layer, 20-40 cm) of cultivated Haplic Cambisol soil mixed together (Chenot et al. 2018). It was directly collected at the donor site at the same time as the different treatments were constituted to avoid any storage period of the soil and any enrichment in undesirable non-target species during storage (in particular windborne Asteraceae species) (Bordez 2015). 2) The raw quarry material was composed of sand and of alluvial gravel, sifted so as to keep only elements between 0 and 30 mm, also stored for less than 24h as in order to avoid enrichment with seeds of non-target species. Overall, five substrate mixtures with different proportions of each substrate were tested : 100% raw quarry material, 75% raw quarry material with 25% arable soil, 50% raw quarry material with 50% arable soil, 25% raw quarry material with 75% arable soil, and 100% arable soil (Table 1).

The various mixtures made it possible to obtain significant differences in substrate mixture physical-chemical properties (Supplement S1). The mixtures were spread in spring 2016 in the form of 30-40 cm thick 5m × 5m plots (similar thickness of soil to the reference ecosystem) using back hoes and hand tools. During the mixing of materials using backhoes, the soil was stirred, mixed and thus aired to ensure the growth of the plants (Jarvis & Walton 2012). Overall, 50 plots were installed at the site in February 2016 (Supplement S2).

Sowing the dominant species

Two sowing treatments were tested: (1) Each substrate mixture without sowing of any species (natural colonization); (2) Each substrate mixture with sowing of *Brachypodium* spp.. Commercially available non-certified seeds of non-local origin (Spain) were sown (germination rate: 73%) in March 2016 over 625m² of experimental plots (25m² × 25 plots), with a ratio of 10g of seeds/m² (3000 seeds/m²). While we purchased *Brachypodium retusum* seeds only, our seed mix turned out to be only 32% *B. retusum* and 68% *Brachypodium hybridum*, the latter being an annual *Brachypodium* which seems to have been confused during seed collection by the seed company. However this species is also present in the reference grassland with a lower plant cover (Pavon & Pires 2020). Throughout the remainder of this article, only the term *Brachypodium* was used for the sown seed mix. The experimental setup thus includes five replicates of each treatment (5 substrate mixtures × 2 sowing treatments × 5 replicates).

Experimental implementation

The different treatments were spread randomly over the site (Supplement S2). No material was spread in the paths between the plots. Once a year, paths were mowed using a grass trimmer to limit their colonization by plants and avoid their seeding, which might have influenced the establishment of species on the plots by edge effects. As the reference grassland is grazed (Saatkamp et al. 2010; Saatkamp et al. 2018), grazing by an itinerant flock of sheep, connecting the reference grassland and all the plots, was reintroduced after the experiment was set up (for a total of 3 days a year between February and May). Finally, a drainage outlet was dug at the end of the experimental plot to avoid the stagnation of water during rainy episodes.

Annual vegetation surveys

Monitoring of the vegetation was carried out every year from 2016 to 2018 and in 2020 to assess plant recolonization dynamics and to compare the different treatments. For that purpose, permanent 4m² quadrats situated at the center of each 25m² plot (to avoid any edge effect) were monitored in May 2016, May 2017, May 2018 and May 2020. The abundance of each plant species was estimated on the basis of percent cover (Gillet 2000) and total plant cover was also estimated. The vegetation developing on the quadrats was compared with the reference ecosystem after a survey of randomly placed 4m² quadrats on the reference grassland: ten quadrats in 2018 and five quadrats in 2020. Reference plant communities contained 52.2±2.2 species per 4m², and 109 species overall in these 15 quadrats.

Data analyses

All the data analyses were performed using the packages *vegan* (Oksanen et al. 2015), *car* (Fox et al. 2020), *multcomp* (Hothorn et al. 2020), *emmeans* (Lenth et al. 2020) and *lme4* (Bates et al. 2016) in R 4.0.3. (R Core Team 2020).

We evaluated the effects of substrate mixtures, sowing and time on species composition, richness and cover of plant communities. We also evaluated the effects of substrate mixtures and sowing five years after the experiment was set up, in 2020. This was done on species composition, richness and cover of plant communities, as well as on *Brachypodium retusum* and *Brachypodium hybridum* covers. In 2020, we also assessed the effects of treatments on plant species richness and plant cover for target and non-target species separately (target species being the species found on the reference grassland plots in 2020). For species composition, we used the data of all species but *Brachypodium* spp. as the aim was to assess the effect on the plant community independently of *Brachypodium* cover. As the sum of species cover usually largely exceed total species cover, we therefore used as cover without *Brachypodium* the product of the sum of species cover excluding *Brachypodium* spp. cover by the estimated total plant cover, divided by the sum of all species cover, hereafter called plant cover without *Brachypodium*.

Two indices were used to assess the state of the plant communities compared to the reference ecosystem. 1) The $CSII_{norm}$ Index (normalized Community Structure Integrity Index) measuring the proportion of the abundance of species of the reference community represented in the restored community (it shows the percentage of similarity in terms of composition and abundance of the communities studied compared to the reference community). 2) The HAI Index (Higher Abundance Index) measuring the proportion of the abundance of species in the restored community which is higher than in the reference community (it shows what is 'in excess', in terms of species and abundance in the studied communities studied compared to the reference community) (Jaunatre et al. 2013).

In order to characterize the composition of the plant communities sampled on the different plots, two Non-Metric Multidimensional Scaling (NMDS) based on Bray–Curtis similarity were performed (Borcard et al. 2011). One NMDS was run on all years (200 quadrats (5 mixtures × 2 sowing treatments × 5 replicates × 4 years (2016, 2017, 2018, 2020)) × 155 species) and one in 2020 (55 quadrats (5 mixtures × 2 treatments × 5 replicates + 5 quadrats in the reference grassland) × 135 species).

To test the effects of the substrate mixtures × *Brachypodium* sowing × time on plant species richness, plant cover, $CSII_{norm}$ and HAI, one Generalized Linear Mixed Model (GLMM) was performed on each of these variables. For each, we used substrate mixtures, *Brachypodium* sowing and years as fixed factors and quadrats as a random factor to take repeated measures into account. They were followed by pairwise contrast comparisons with a Tukey adjustment when significant.

To test for possible effects of the substrate mixtures × *Brachypodium* sowing on plant species richness, plant cover $CSII_{norm}$ and HAI in 2020, one Generalized Linear Model GLM was performed on each of these variables. They were followed by pairwise contrast comparisons with a Tukey adjustment when significant. All models were fitted with a poisson error distribution for plant species richness and a gamma error distribution with log link for the other variables.

Results

Effects of substrate mixtures, Brachypodium sowing and time on plant communities

All plant communities changed over time (Supplement S3). They all started in 2016 with arable weeds, such as *Diplotaxis tenuifolia* (L.) DC., *Polygonum aviculare* L., *Solanum nigrum* L. and *Veronica persica* Poir.. With time, they gained target species of the reference grassland, such as *Avena barbata* Pott ex Link, *Catapodium rigidum* (L.) C.E.Hubb., *Hordeum murinum* L., *Medicago truncatula* Gaertn., *Rostraria cristata* (L.) Tzvelev, *Trifolium campestre* Schreb., *Trifolium glomeratum* L. and *Trifolium scabrum* L..

Overall, species richness increased with time on all plots. It increased by 5 fold between 2016 and 2017 and only by 10% from 2017 to 2020 (Supplement S3). Percent covers of unsown species increased by two fold from 2016 to 2018, then decreased by 10% in 2020 (Supplement S3). Substrate mixtures with 100% or 75% of raw quarry material had significantly lower species richness and lower percent cover of unsown species than other substrate mixtures on all years. Plots where *Brachypodium* was not sown had significantly higher species richness and higher percent cover of unsown species (Supplement S3).

CSIInorm increased by ten fold between 2016 and 2017 (especially on unsown plots and on sown plots with the highest proportion of arable soil) and then remained relatively stable or underwent a 20% decrease by 2020 (Supplement S3). HAI on unsown plots were always high (close to 1). HAI on sown plots first increased by 3 fold and then decrease by the same order to go back below 0.6 in 2020 (Supplement S3).

Effects of substrate mixtures on Brachypodium retusum cover in 2020

Brachypodium retusum had a significantly higher cover on sown plots (χ^2=381.23, df=1 p<0.001***; Figure 2). It established very well on all sown plots (found on 25 plots out of 25 sown in 2020; average percent cover = 38.8%). It was found on two of the 25 unsown plots in 2020, but with a significantly lower percent cover on average (0.04%). *Brachypodium hybridum* had a significantly higher cover only on plots sown on the substrate mixture with 100% arable soil (χ^2=23.97, df=4, p<0.001; Figure 2). Its percent cover (8.8%) was much lower than that of *Brachypodium retusum* (35%).

Effects of substrate mixtures and Brachypodium sowing on plant communities in 2020

Brachypodium sowing and the substrate mixtures influenced plant community composition in 2020 (Figure 3). Axis 1 of the NMDS on plant community data showed two major groups: the quadrats where *Brachypodium* was sown on the left and those where *Brachypodium* was not sown on the right. The sown quadrats were characterized by target species such as *Andryala integrifolia* L., *Lobularia maritima* (L.) Desv. and *Medicago monspeliaca* (L.) Trautv.. In contrast, those without sowing of *Brachypodium* were characterized by *Dittrichia viscosa* (L.) Greuter, *Oloptum miliaceum* (L.) Röser & Hamasha and *Symphyotrichum squamatum* (Spreng.) G.L.Nesom. Axis 2 of the NMDS showed a slight gradient in the structure of the plant communities in relation to substrate mixtures, and the reference grassland (Figure 3): mixtures with higher proportions of arable soil sown with *Brachypodium* were slightly more similar to the reference grassland than mixtures with higher proportions of raw quarry material.

Substrate mixtures with a higher proportion of arable soil had a significantly higher species richness of both target and non-target species (respectively: χ^2=37.94, df=4, p<0.001 and χ^2=30.94, df=4, p<0.001, Figure 4). *Brachypodium* sowing did not influence target plant species richness (χ^2=0.05, df=1, p=0.825), and plots where *Brachypodium* was sown had lower richness of non-target species (χ^2=13.10, df=1, p<0.001, Figure 4). Substrate mixtures with a higher proportion of arable soil also had a higher plant cover of target species, although this was significant only on plots sown with *Brachypodium* (χ^2=38.57, df=4, p<0.001, Figure 5). The plant cover of non-target species was only significantly higher on unsown plots with a substrate mixture with 100% of arable soil compared with sown plots with the same substrate mixture (χ^2=13.62, df=4, p=0.009, Figure 5).

Similarity of plant communities obtained in the different soil mixtures to those of the reference grassland

In 2020, 60 species, out of the 85 species inventoried in the five reference grassland plots, were recorded on the experimental substrate mixtures (50 plots – total number of species in 2020 = 136). Among these 60 species, we found *Anisantha madritensis* (L.) Nevski, *Anisantha rubens* (L.) Nevski, *Catapodium rigidum* (L.) C.E.Hubb., *Crepis foetida* L., *Galactites tomentosus* Moench, *Medicago monspeliaca* (L.) Trautv., *Rostraria cristata* (L.) Tzvelev, *Sonchus oleraceus* L., *Trifolium campestre* Schreb., *Trifolium scabrum* L. and *Vulpia ciliata* Dumort. (Supplement S4, S5). The plant communities developing on newly created substrates remained highly different from the reference grassland five years after implementation (the best CSIInorm reached 18.15% for substrates composed of 100% arable soil; χ^2=31.08, df=4, p<0.001; Figure 6). The substrate mixtures with the poorest nutrient content (QM and QM_AS), presented the plant communities that were farthest from the reference grassland in terms of composition and abundance, in contrast to those with a high proportion of arable soil, such as AS and AS_QM (Figure 6). The CSIInorm restoration index also showed that plots where *Brachypodium* was not sown were more similar to the grassland than where *Brachypodium* was sown (χ^2=11.89, df=1, p<0.001; Figure 6).

Unsown plots contained species not found in the reference grassland nor on sown plots, such as *Artemisia verlotiorum* Lamotte, *Clinopodium nepeta* (L.) Kuntze and *Bituminaria bituminosa* (L.) C.H.Stirt.. Sown plots contained species not found in the reference grassland nor on unsown plots, such as *Hirschfeldia incana* (L.) Lagr.-Foss. and *Medicago orbicularis* (L.) Bartal. (Supplement S4, S5). Unsown plots contained 10 reference grassland species not found on sown plots, such as *Carduus nigrescens* Vill., *Eryngium campestre* L., *Linum strictum* L., *Neatostema apulum* (L.) I.M.Johnst., *Sideritis romana* L. and *Urospermum dalechampii* (L.) Scop. ex F.W.Schmidt. Sown plots contained 6 reference grassland species not found on unsown plots, such as *Aira cupaniana* Guss., *Logfia gallica* (L.) Coss. & Germ., *Petrorhagia prolifera* (L.) P.W.Ball & Heywood and *Plantago berlardii* All. (Supplement S4, S5). Most plots also contained high abundances (>5%) of species that are common in the reference grasslands but usually recorded there with lower abundances (<0.5%) (*A. madritensis, R. cristata, H. murinum, A. rubens, T. campestre, A. barbata, M. truncatula, C. foetida, Geranium molle*, etc.). These three sets of species explain the relatively high HAI index (86.4%; Figure 6; Supplement S4, S5). The higher HAI restoration index on the unsown plots showed that sowing *Brachypodium* limited the establishment of non-target species, but this was significant only on substrate with 100% raw quarry material (χ^2=11.89, df=1, p<0.001; Figure 6). There was no significant differences of HAI between substrate mixtures (χ^2=2.42, df=4, p=0.660; Figure 6).

Discussion

Despite the fact that plant communities are still distant from the reference grassland after five years, the tested substrate mixtures allow the rapid establishment of a grassland-like vegetation with numerous Poaceae species. The total number of target species (defined here as the species found in the reference grassland) increased with the age of succession in the majority of substrate mixtures as already demonstrated in other restoration experiments undertaken in the *La Crau* grasslands (Coiffait-Gombault et al. 2012; Bulot et al. 2014) or in other dry grasslands (Zobel et al. 1996). The first years after restoration are often characterized by weedy ruderal species (Vida et al. 2010). Our results confirmed this pattern with a strong dominance of ruderal species, such as *Diplotaxis tenuifolia*, *Polygonum aviculare*, etc. which then become rare or are replaced by a mix of ruderal-competitive species *sensu* Grime (1977) and some target species all of which naturally colonize the site from the available regional pool of species. The most common reference grassland species found on the experimental plots after five years are i) wind-dispersed Asteraceae (*Crepis foetida, Carduus pycnocephalus, Galactites tomentosa*), ii) small Poaceae

(*Anisantha madritensis, Anisantha rubens, Catapodium rigidum*) which are common in the short-term seed bank and can easily be transported by wind over small distances as well as by sheep and ants, and iii) Fabaceae (*Trifolium campestre, Trifolium glomeratum, Trifolium scabrum, Medicago truncatula*) which are found in the short-term and long-term seed banks and are transported by ants (Buisson et al. 2006; De Almeida et al. 2020). This finding is similar to that of Alday et al. (2011), where species that exhibited zoochory and anemochory were most abundant in the early stages of post-mining succession. Monitoring over the longest term possible is recommended as recovery rates and patterns of change in the plant communities may be slow (Jones et al. 2018). Recovery is particularly slow in the *La Crau* grasslands (Coiffait-Gombault et al. 2012; Bulot et al. 2014). In the long term, it is expected that the plant communities on the different substrate mixtures will keep developing in different directions and will never achieve the entire restoration of the reference grassland because of highly different starting conditions (substrate mixtures) (Prach et al. 2014). Such results have been already demonstrated for *La Crau* grasslands with former cultivation (Jaunatre et al. 2016) and sheep paddocks (Saatkamp et al. 2020) which still show different plant composition and diversity to the reference grassland several centuries or millennia after their abandonment (Römermann et al. 2005; Helm et al. 2018).

While some target species colonized the restored areas, the high Higher Abundance Indices (HAI) show that some target species over develop (compared to their abundance on the reference grassland) and that non-target species are common. The latter may be due to the fact that the site is not directly surrounded by reference grassland areas, which impede possibilities of target species recolonization (Lanta et al. 2020). The neighboring areas are strongly impacted by quarry exploitation, former quarries, or former orchards where ruderal species predominate. Non-target species may thus easily find their way to the experimental plots, carried in the seed bank of the arable soil used for the experiment, by the wind or by itinerant grazing, sheep transporting indistinctively target and non-target species (Albert et al. 2015). The pool of plant species, that is, the source of seeds, of diaspores and the potential for colonization of species plays a major role in the restoration, by influencing the process of spontaneous succession (Řehounková & Prach 2008; Strykstra et al. 1998; Zobel et al. 1998). Therefore, the characteristics of the landscape should be properly taken into account for any restoration initiatives (Bakker et al. 1998; Prach et al. 2001; Son et al. 2020), and in our case, may have a higher success if adjacent to patches of the reference ecosystem (Buisson et al. 2006).

The substrates represent major variables determining the rhythm and direction of plant successions and should unavoidably be taken into account in restoration programs (Prach et al. 2001). The substrate mixtures poor in nutrients, i.e. with a higher percentage of raw quarry material, may not be the best as they have lower target species plant covers and lower species richness of both target and non-target plant species than the richer soils (with a higher percentage of arable soil). Survival of target species is indeed severely impeded by harsh abiotic conditions (Pueyo et al. 2009). Moreover, as they generally have a lower plant cover, these substrate mixtures may lead to higher soil erosion, the region having very strong winds blowing 334 days/year (Buisson & Dutoit 2004).

In the present study, fertilizers (N, P, K) applied during the phase of exploitation of the orchards (1983-2015) and thus found in the arable soil have certainly contributed to increasing plant cover on richer substrate mixtures, as it has already been demonstrated elsewhere (Norman et al. 2006). Higher plant species richness on nutrient-richer soils is likely due to the fact that arable soils contain a seed bank while raw material does not (Bulot et al. 2014). Substrate mixtures with 50% or less of arable soils have significantly less non-target species richness and substrate mixtures with 50% or more of arable soils have significantly more target species richness or cover. Therefore, in order to favor target species richness and cover and maintain non-target cover as low as possible, the best compromise would be the substrate mixture with 50% arable soil and 50% raw quarry material. This substrate mixture also has a similarity to the reference grassland plant community which is amongst the highest.

Sowing *Brachypodium* seeds allowed *Brachypodium retusum* to establish on all plots, although its cover was lower on the raw quarry material. In 2020, sown plots have a lower richness of non-target species, a lower cover of target species, and a lower similarity to the reference grassland (as well as a lower cover of non-target species on arable soils only). These results contrast with other studies where seeding increased the establishment of target species (Baasch et al. 2012; Norman et al. 2006). These experiments were however greatly different from this one: we sowed one dominant grassland species and they sowed species-rich mixtures or hay collected from a reference grassland. *B. retusum* therefore seems to compete with non-target species establishment and with target species growth: its clonal growth and ability to cover the ground, and its rhizome and root system make it a quite competitive species (Bonet 2004; Clary et al. 2004; Saatkamp et al. 2018). This seems to contradict Raventós et al. (2012) study which showed a facilitative effect of *B. retusum* during the establishment phase of target species after fire. However, that study was carried out on shrubby chamaephyte species (*Ulex parviflorus*

Pourr., *Cistus albidus* L., *Helianthemum marifolium* Mill. and *Ononis fruticosa* L.) and not on grassland plants as it is the case here. The reason for a greater competitive effect of *B. retusum* on target species than on non-target species cover most probably lays in the fact that target grassland species are stress tolerant and non-target species rather ruderal (e.g. *D. tenuifolia*) and competitive (e.g. *O. miliaceum*) species.

There was no significant effect of *B. retusum* on target species richness. We can therefore conclude that *B. retusum* impacts target species cover rather than target species establishment. In addition, these data suggest that *B. retusum* may not facilitate target species arrival by capturing windborne seeds as we initially hypothesized, although this could also be due to the fact that few target species seeds were wind-blown onto the experimental plots as there is no adjacent reference grassland nearby. While we show that *B. retusum* cover is much higher than *B. hybridum*, it is not possible to fully disentangle the specific effect of *B. retusum* from that of *B. hybridum*. However, so far, it is still not possible to obtain pure and certified seeds of *B. retusum* in Southern France. Future restoration projects in Southern France will therefore still use this mixture of *B. retusum* + *B. hybridum* from Spain, until a better option is available for purchase.

While plots with a substrate composed of 100% of arable soil and not sown with *Brachypodium* had the highest richness of target species and highest similarity of the plant communities to the reference grassland community, they also had the highest cover of non-target species. Moreover, using a substrate composed of 100% arable soil may limit opportunities for restoration activities due to insufficient substrate being available to restore more surface area than the area from which it was excavated. In order to increase the volume of substrate produced to restore wider areas than solely that of abandoned orchards (but also quarries), arable soil should be mixed / diluted with raw quarry material. Therefore, the next best option would be the substrate mixture with 50% arable soil and 50% quarry material. This substrate mixture does not differ significantly from the 100% arable soil mixtures in similarity to the reference grassland community and target species richness and cover. It is then a good compromise for increasing target and decreasing non-target species richness and cover.

Not sowing *Brachypodium* allows better growth and thus higher cover of target species; but also leads to higher non-target species richness and cover and some over-abundance of target species (*A. madritensis*, *R. cristata*, *H. murinum*, *A. rubens*, *T. campestre*, *A. barbata*, *M. truncatula*, *C. foetida*, *Geranium molle*, etc.). However, sowing *Brachypodium*, while not preventing the colonization of non-target species, does reduce their cover and richness and creates a physiognomy similar to that of the reference grassland with *B. retusum* as a dominant grass. *B. retusum* is known to be extremely slow at recolonizing areas with disturbed soils (Coiffait-Gombault et al. 2012). Hence, sowing *B. retusum* is a major step towards reintroducing it.

Further studies should look at enriching sown and unsown plots with a few target species to identify the potential key roles of *B. retusum* in interacting with grassland species establishment. A new experiment could be about sowing a more diverse seed mixture of target species (which is still a problem as most seeds of the target species are not available for purchase) and whether sowing of *B. retusum* and this seed mix should be simultaneous or sequential. Longer monitoring of these experimental plots may allow us to refine the best treatment to be applied, i.e. where non-target species decrease the most and where target species increase.

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Table 1: Composition of the different mixtures used for the experiment.

Code for figures	raw Quarry Material (QM)	Arable Soil (AS)	Color code for figures
QM	100%	0%	
QM_AS	75%	25%	
HALF	50%	50%	
AS_QM	25%	75%	
AS	0%	100%	

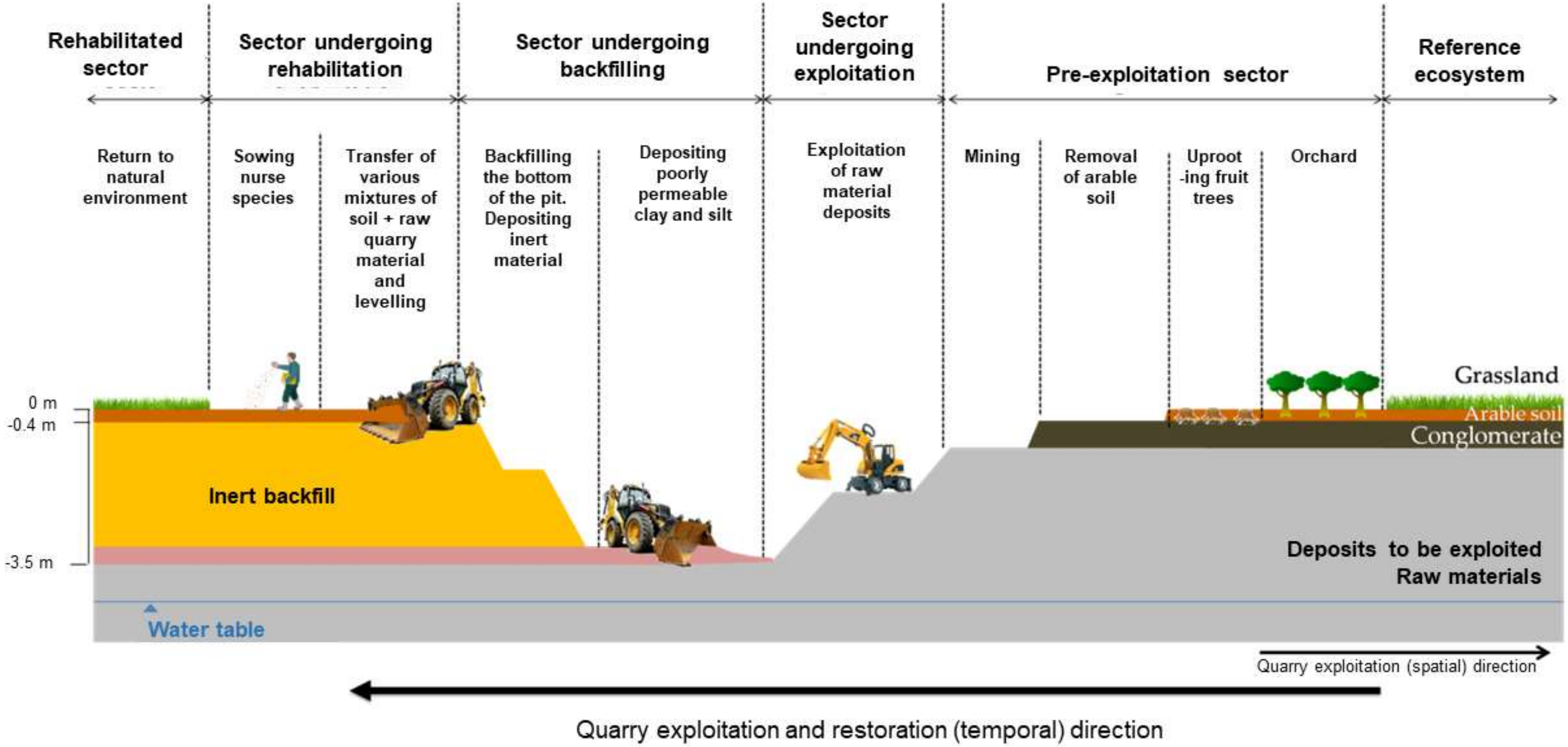

Figure 1. Diagram of the exploitation and restoration of the Ménudelle quarry in Southeastern France.

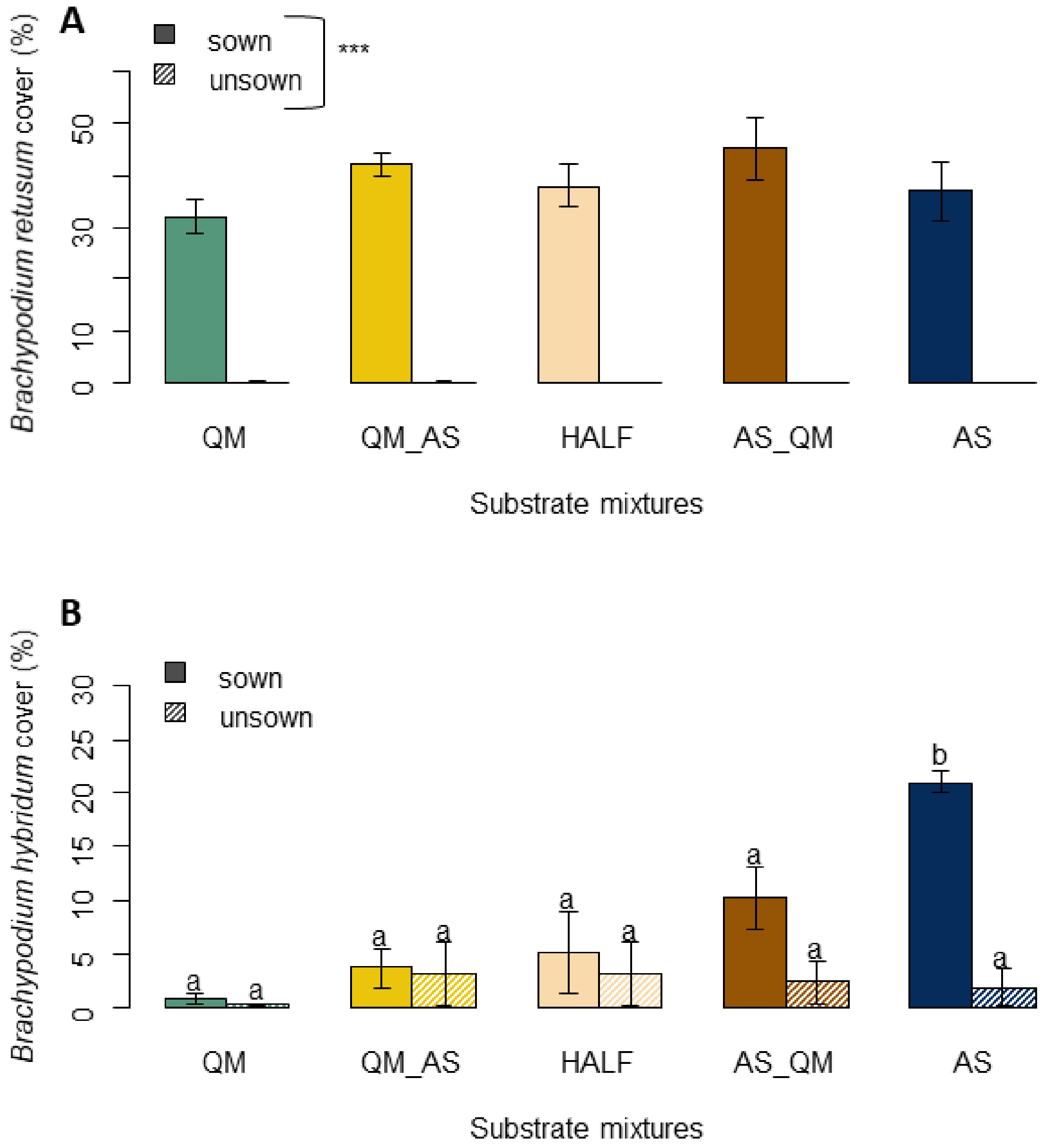

Figure 2. *Brachypodium* cover on experimental plots in 2020 (mean cover ± standard error). Plots were sown (dark grey) or not (light grey) with *Brachypodium*. Substrate mixtures included QM – Quarry raw Material or AS – Arable Soil (see Table 1 for mixture codes). A. *Brachypodium retusum* (mean cover = 38.8%): Substrate mixtures χ^2=4.98, df=4, p=0.290 n.s.; *Brachypodium* sowing χ^2=381.23, df=1, p<0.001*** (Substrate mixtures × *Brachypodium* sowing χ^2=5.05, df=4, p=0.282 n.s.). B. *Brachypodium hybridum* (mean cover = 8.2%): Substrate mixtures × *Brachypodium* sowing χ^2=23.97, df=4, p<0.001*** (Substrate mixtures χ^2=25.07, df=4, p<0.001***; *Brachypodium* sowing χ^2=17.44, df=1, p<0.001***).

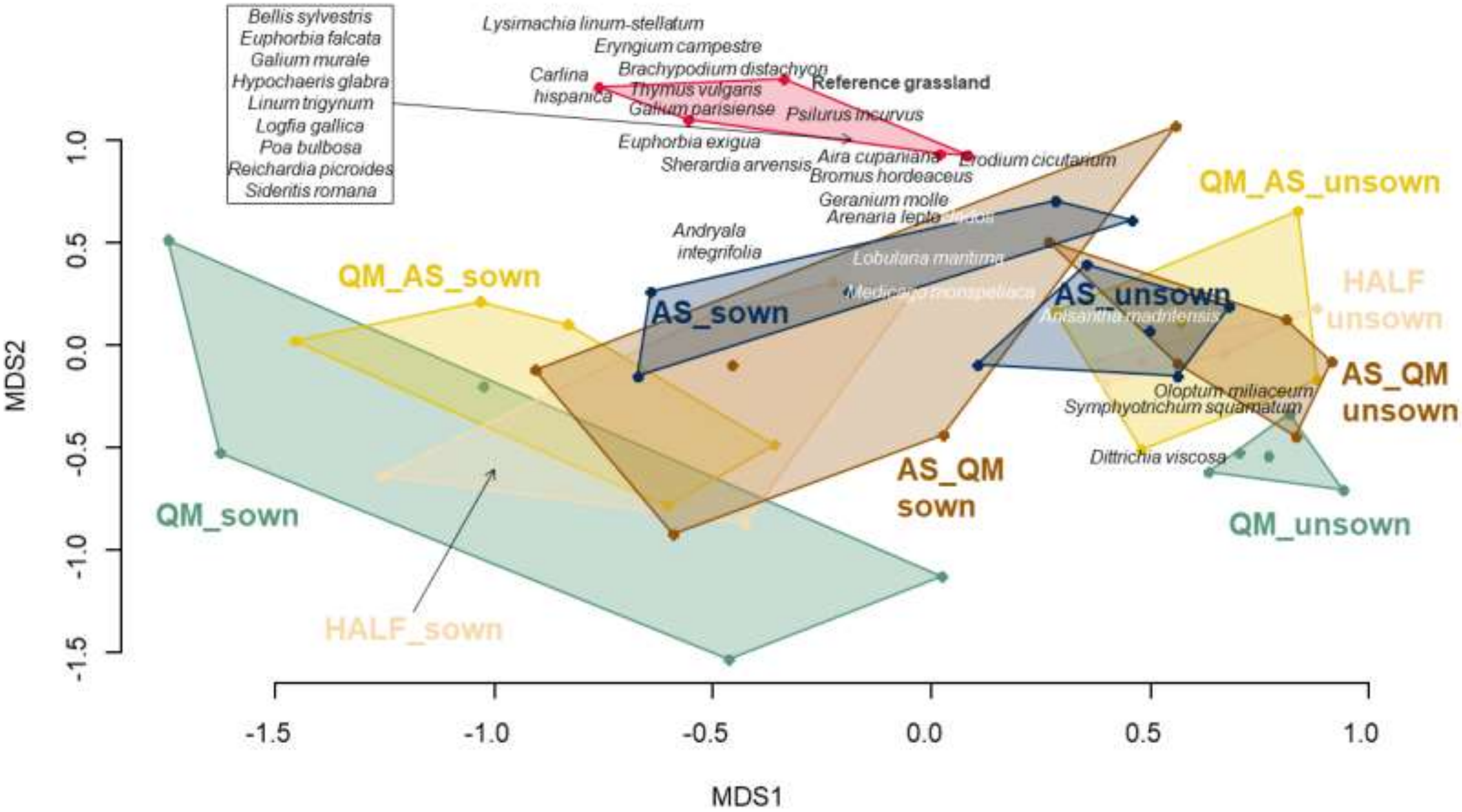

Figure 3. Plant species composition in 2020 using NMDS. Polygons indicate of plots with the same treatment (NMDS stress: 0.21). Plots were sown (sown) or not (unsown) with *Brachypodium*. Substrate mixtures included QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil (see Table 1 for mixture codes).

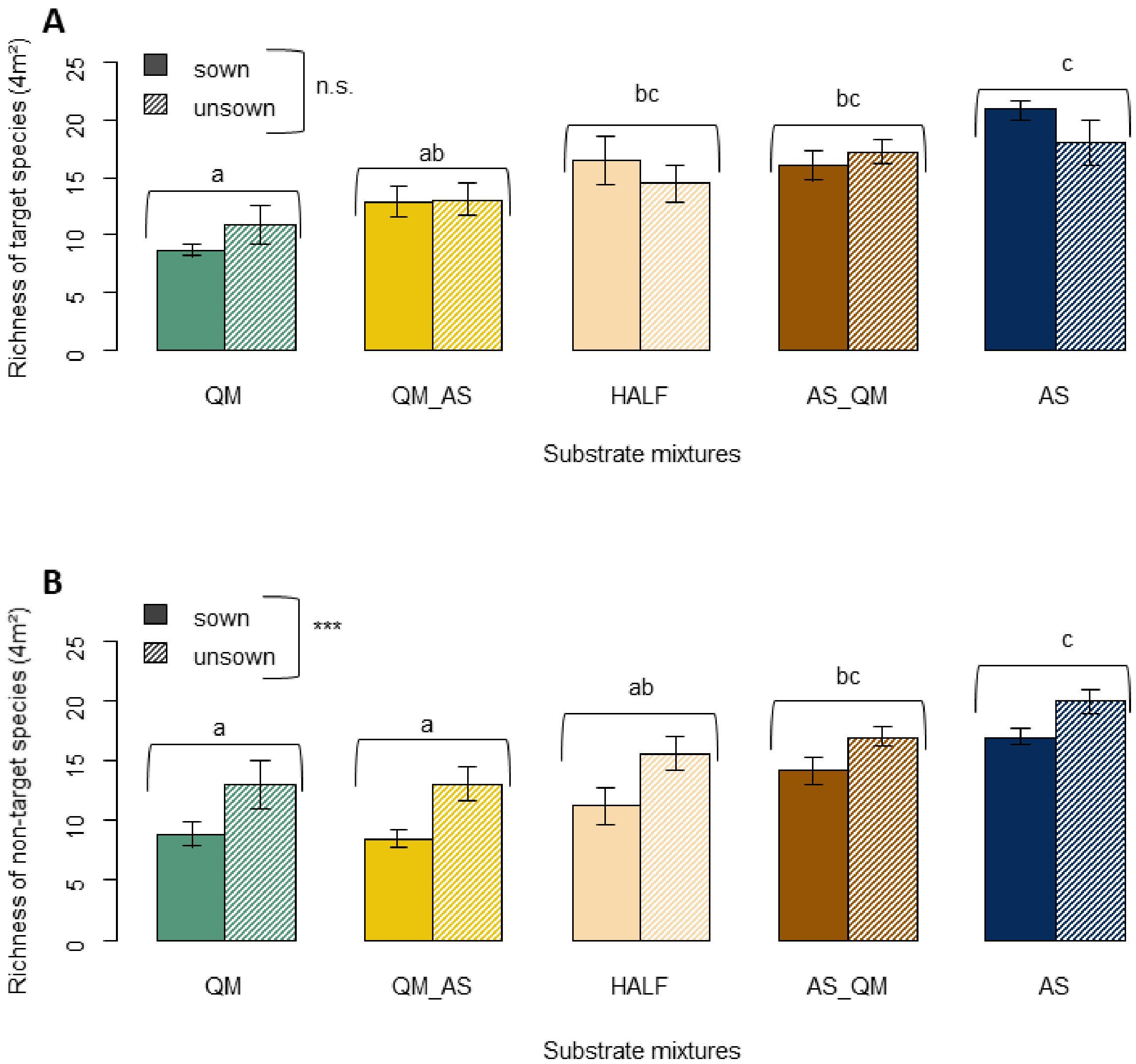

Figure 4. Mean species richness (± standard error) of A. target species and B. non-target species on experimental plots in 2020. Plots were sown (dark grey) or not (light grey) with *Brachypodium*. Substrate mixtures included QM – Quarry raw Material or AS – Arable Soil (see Table 1 for mixture codes). A. Substrate mixtures χ^2=37.94, df=4, p<0.001***; *Brachypodium* sowing χ^2=0.05, df=1, p=0.825 n.s. (Substrate mixtures × *Brachypodium* sowing χ^2=3.09, df=4, p=0.543 n.s.). B. Substrate mixtures χ^2=30.94, df=4, p<0.001***; *Brachypodium* sowing χ^2=13.10, df=1, p<0.001*** (Substrate mixtures × *Brachypodium* sowing χ^2=2.06, df=4, p=0.726 n.s.).

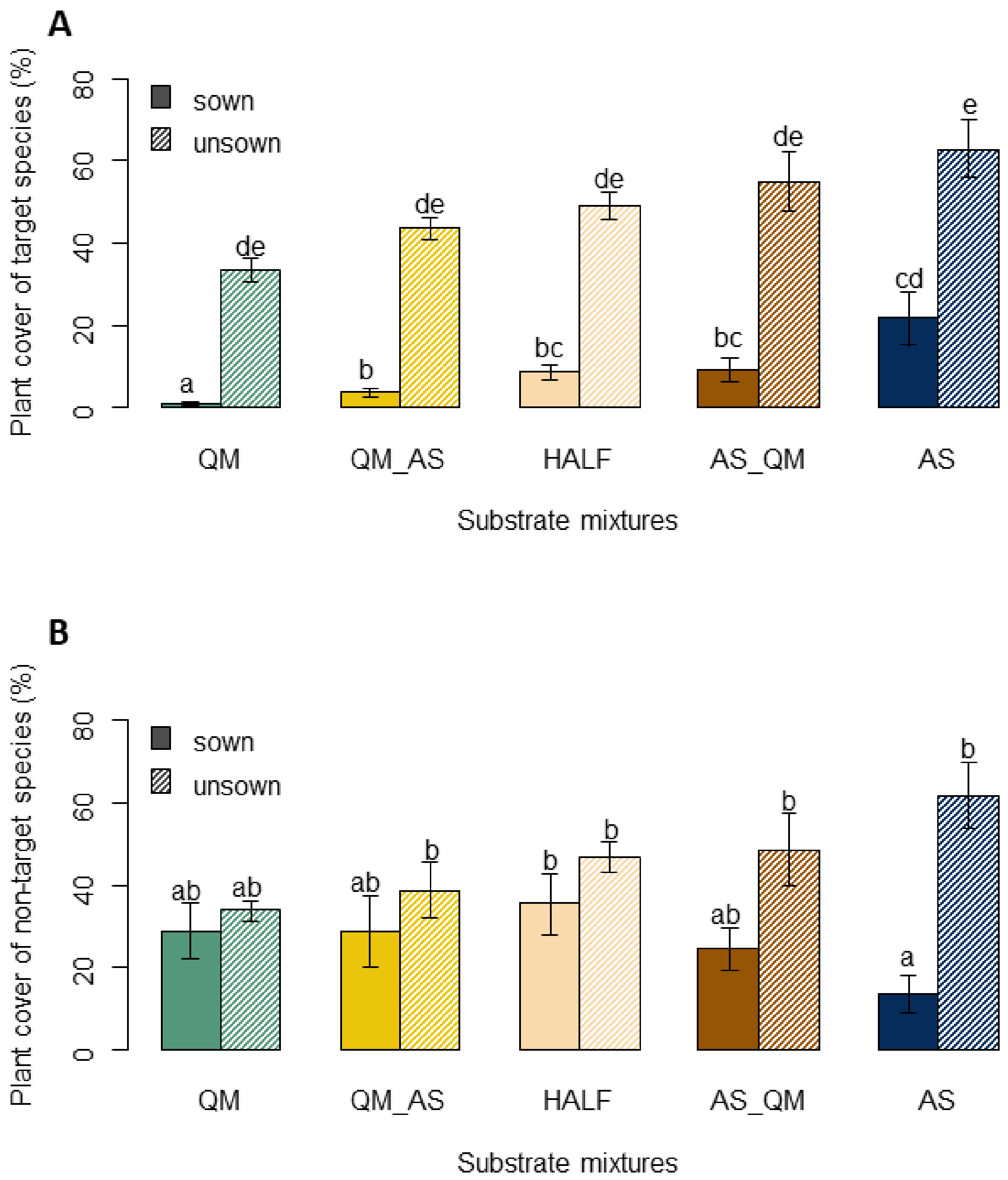

Figure 5. Mean plant cover (± standard error) (without *Brachypodium*) of A. target species and B. non-target species on experimental plots in 2020. Plots were sown (dark grey) or not (light grey) with *Brachypodium*. Substrate mixtures included QM – Quarry raw Material or AS – Arable Soil (see Table 1 for mixture codes). A. Substrate mixtures × *Brachypodium* sowing χ^2=38.57, df=4, p<0.001*** (Substrate mixtures χ^2=61.00, df=4, p<0.001***; *Brachypodium* sowing χ^2=174.19, df=1, p<0.001***). B. Substrate mixtures × *Brachypodium* sowing χ^2=13.62, df=4, p=0.009** (Substrate mixtures χ^2=2.01, df=4, p=0.735 n.s.; *Brachypodium* sowing χ^2=17.81, df=1, p<0.001***).

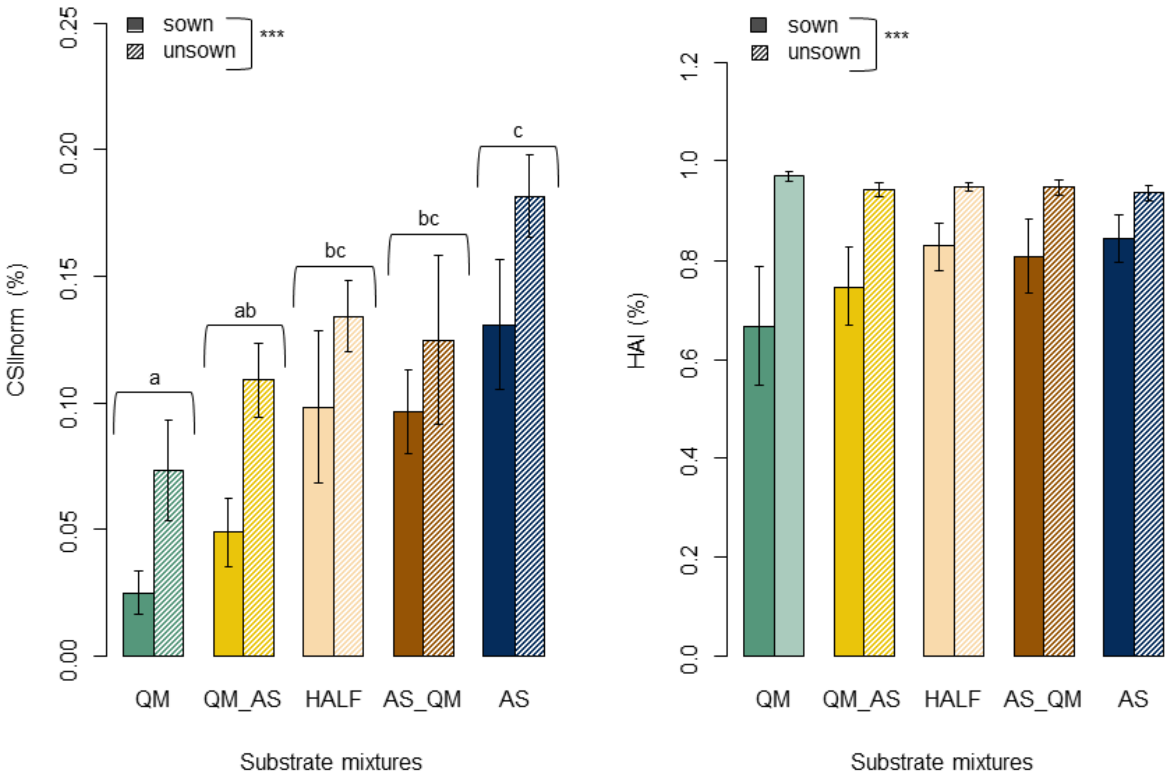

Figure 6. Mean CSII and HAI (± standard error) on experimental plots in 2020. A. CSII: Substrate mixtures χ^2=31.08, df=4, p<0.001***; *Brachypodium* sowing χ^2=11.89, df=1, p<0.001*** (Substrate mixtures × *Brachypodium* sowing χ^2=0.815, df=4, p=0. 936 n.s.). B. HAI: Substrate mixtures χ^2=2.42, df=4, p=0.660; *Brachypodium* sowing χ^2=19.98, df=1, p<0.001*** (Substrate mixtures × *Brachypodium* sowing χ^2=4.14, df=4, p=0.387 n.s.).

Supplement S1: Physical-chemical properties of mixtures tested at the Ménudelle quarry in the Crau plain

Soil parameter sampling

The physical and chemical analyses of the soil were performed twice: 1) in 2016 before sowing (35 samples, 6 replicates on each of the 5 substrate mixtures + 5 on the reference grassland) and 2) in 2018 (50 samples, 5 replicates on each of the 5 substrate mixtures * sowing/no sowing). Each 200-g sample of soil was collected by extracting the first ten centimeters of the soil layer outside the vegetation survey quadrats. Each sample was dried and sieved with a 2 mm mesh sieve. The physical and chemical analyses were performed by the soil analysis laboratory at INRA (*Institut National de la Recherche Agronomique*) in Arras, France. The reference(s) for the method used for each parameter is detailed here: pH water ratio 1:5 (Thomas 1996), $CaCO_3$ using a Bernard calcimètre (Sparks et al. 1996), organic C (Allison 1965), total N by dry combustion (Bremner 1996; Dumas 1831), C:N ratio, Cation Exchange Capacity (CEC) and exchangeable K^+ by Metson (Metson 1956; Ciesielski et al. 1997), available P (Olsen 1954). Particle-size distribution was studied only in 2016 in 5 fractions following the Robinson method without prior decarbonisation (clay (<0.002 mm), fine silt (0.002-0.02 mm), coarse silt (0.2-2 mm), fine sand (0.05-0.2 mm) and coarse sand (0.2-2 mm)).

Data analyses of physical-chemical properties of soils

In order to compare 2016 soil physical and chemical parameter values between substrate mixtures, univariate analyses were performed. When the parametric conditions were met, an ANOVA test, followed by a post-hoc Tukey Honestly Significant Difference test, were performed whenever significant differences were encountered (Stoll 2017). When the parametric conditions were not met, Generalized Linear Models (GLM) under gamma distribution were run. These analyses were followed by pairwise contrast comparisons with a Tukey adjustment. To study the changes in the chemical parameters over years (comparing the 2016 analyses with those of 2018), a principal component analysis (Dray et al. 2020) was run on the chemical data of both years, including that of the reference grassland, to show potentially differences between years and mixtures.

Patterns of change in the physical and chemical parameters of the soils

The mixture of arable soil with raw quarry material reduced the fertility of the soil (Figure S1). The concentrations of nitrogen, phosphorus and potassium were reduced by a factor of five between the substrates composed entirely of arable soil (Figure S1) and those composed exclusively of raw quarry material. The proportion of sand (fine and coarse) was much greater in the raw quarry material. In contrast, clay was found in greater quantities in the mixtures with arable soil (Figure S1). The raw quarry material was characterized by higher $CaCO_3$ and Ca content, whereas the arable soil contained significantly more organic matter and organic carbon (Figure S1). Finally, a gradient in the Cationic Exchange Capacity was observed, with greater quantities for the arable soil compared to the raw quarry material (Figure S1).
The PCA (Figure S2) opposed the reference grassland from all experimental plots. Substrate mixtures with higher levels of arable soils had higher phosphorus, nitrogen, potassium and Cationic Exchange Capacity, while substrate mixtures with higher levels of raw quarry materials had higher $CaCO_3$ and Ca content and higher pH.

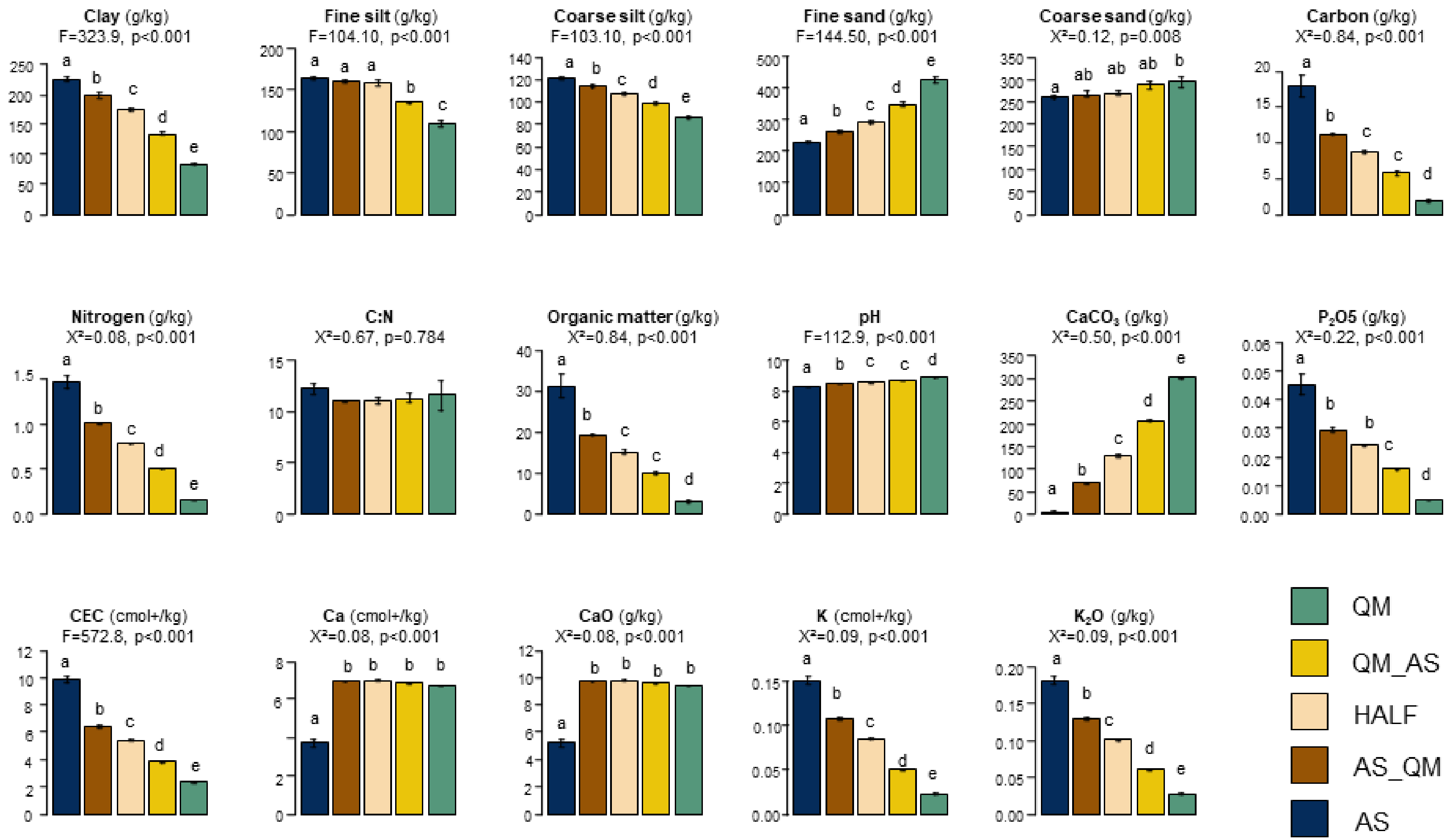

Figure A. The bars represent the mean ± standard error (QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil). These analyses were run on 2016 data

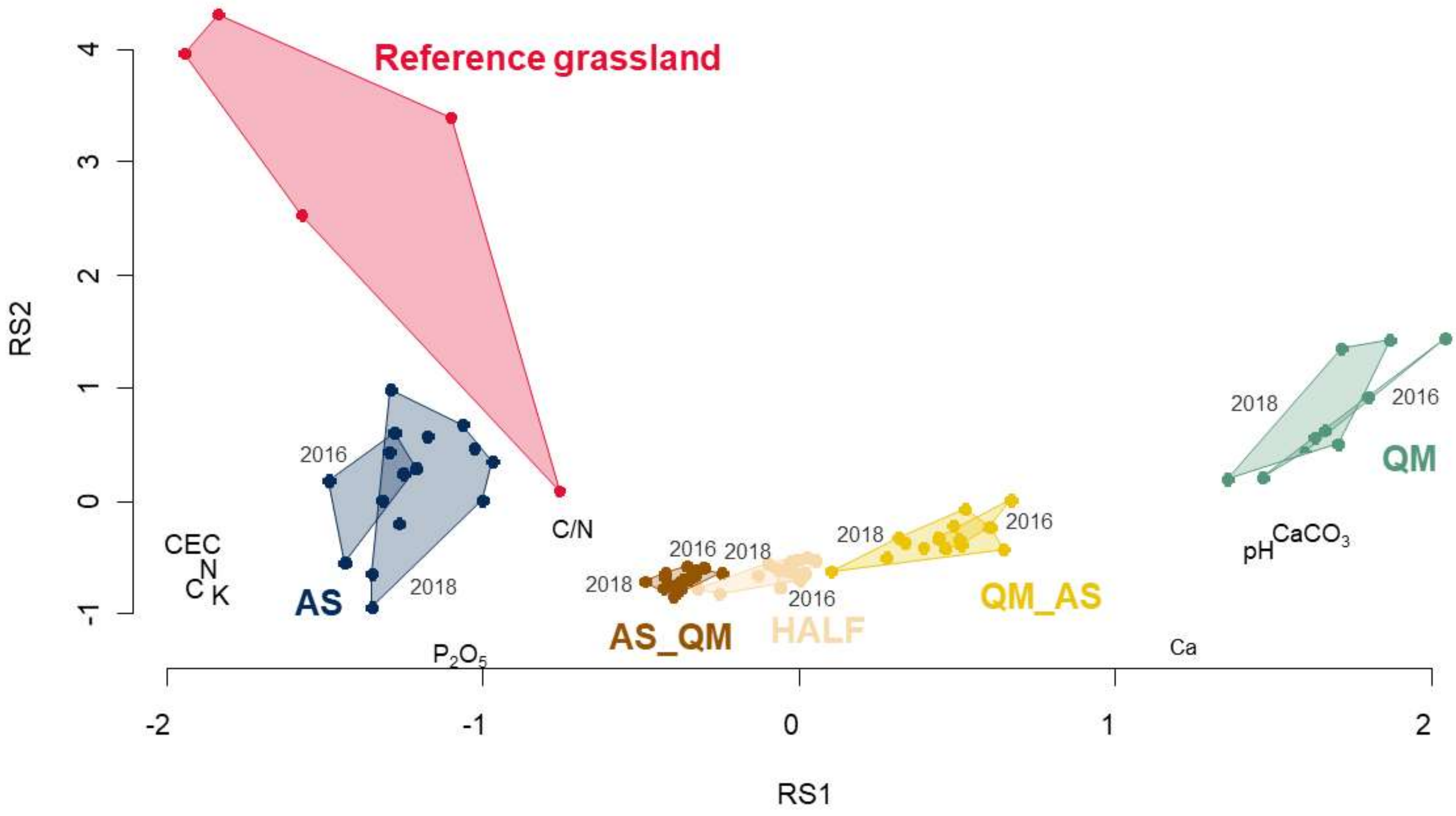

Figure B. Principal Component Analysis of chemical soil parameters on the reference grassland and on the experimental plots in 2016 and 2018. QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

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Supplement S2. Experimental plots set up in the Ménudelle quarry in Southeastern France.

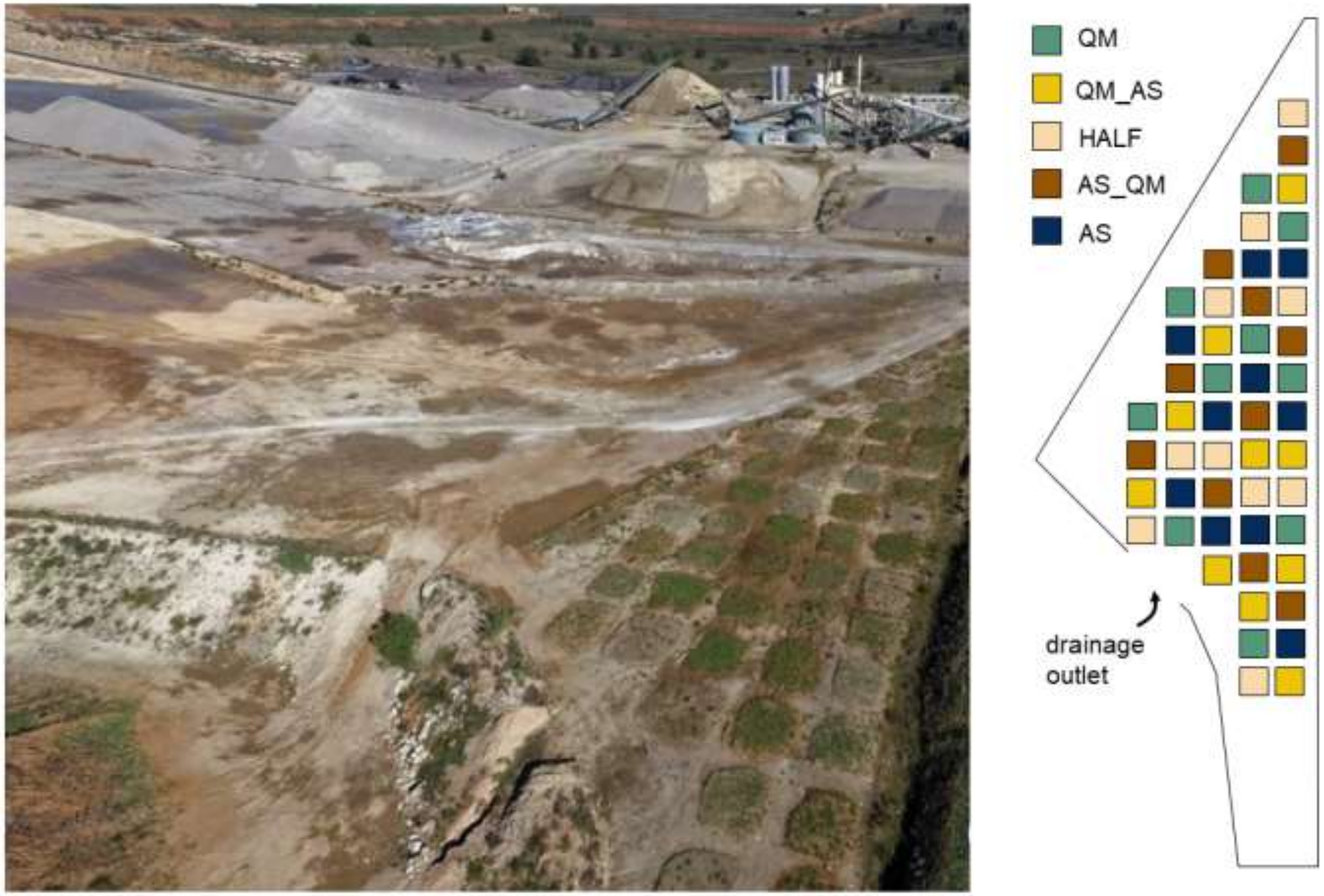


Figure C. Experimental plots set up in the Ménudelle quarry in 2016 (50 plots × 5 mixtures (QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil) × 2 sowing treatments + a drainage outlet) Photography: SCLM

Supplement S3: Influence of substrate mixtures, *Brachypodium* sowing and time on plant communities

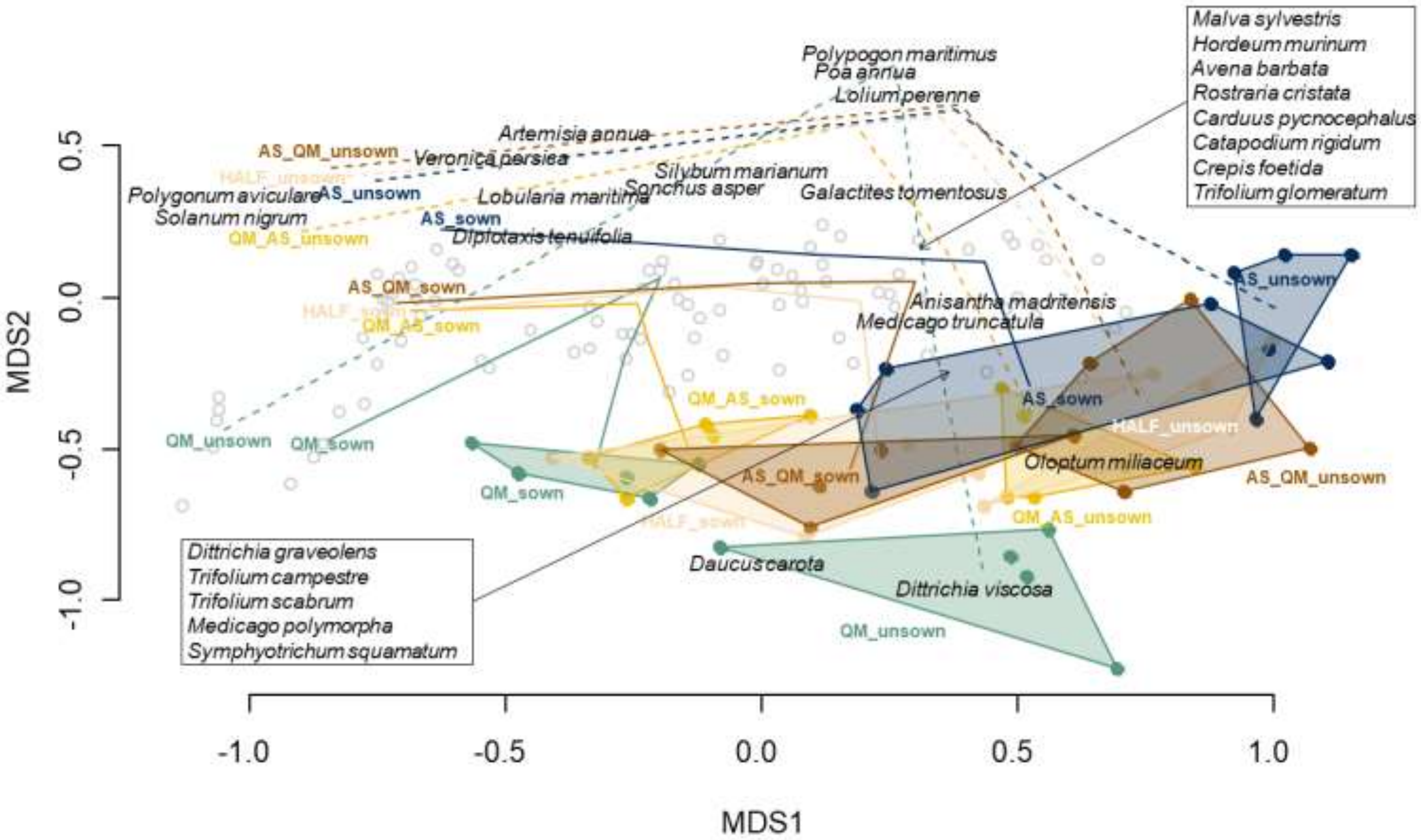


Figure D. Plant species composition using NMDS. Polygons indicate of plots with the same treatment in 2020 and lines the trajectory in 2016, 2017, 2018 and 2020 (NMDS stress: 0.21). Plots were sown (sown) or not (unsown) with *Brachypodium*. Substrate mixtures included QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

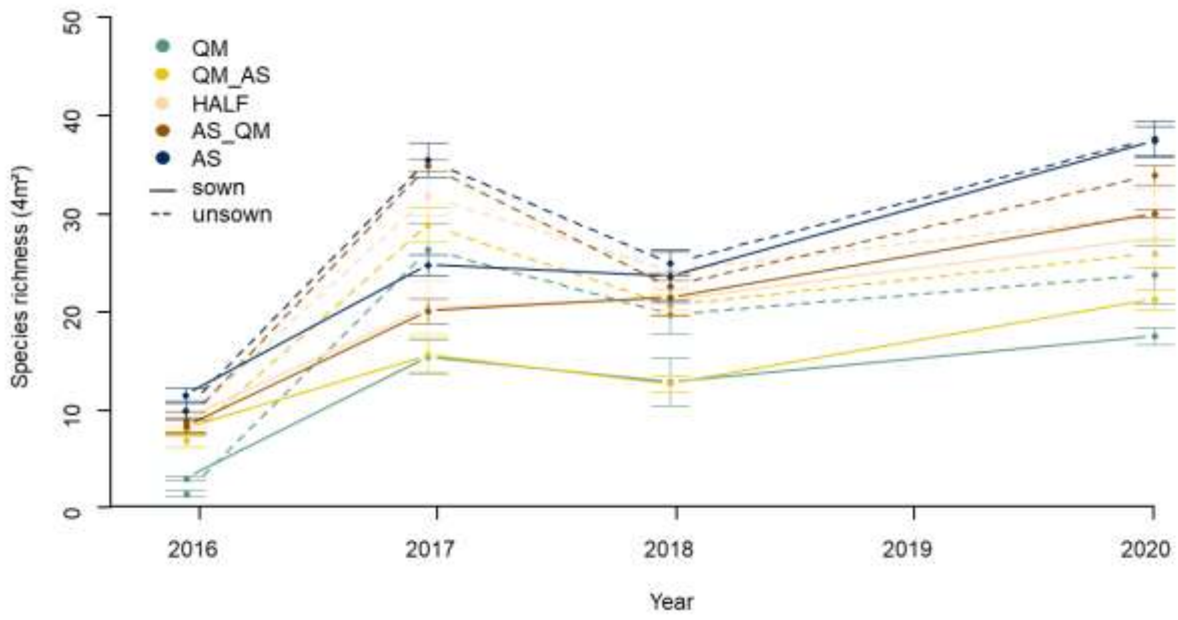


Figure E. Mean species richness on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – raw Quarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures × *Brachypodium* sowing χ^2=9.40, p=0.052; Substrate mixtures × Years χ^2=27.49, p=0.007**; *Brachypodium* sowing × Years χ^2=34.75, p<0.001***. QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

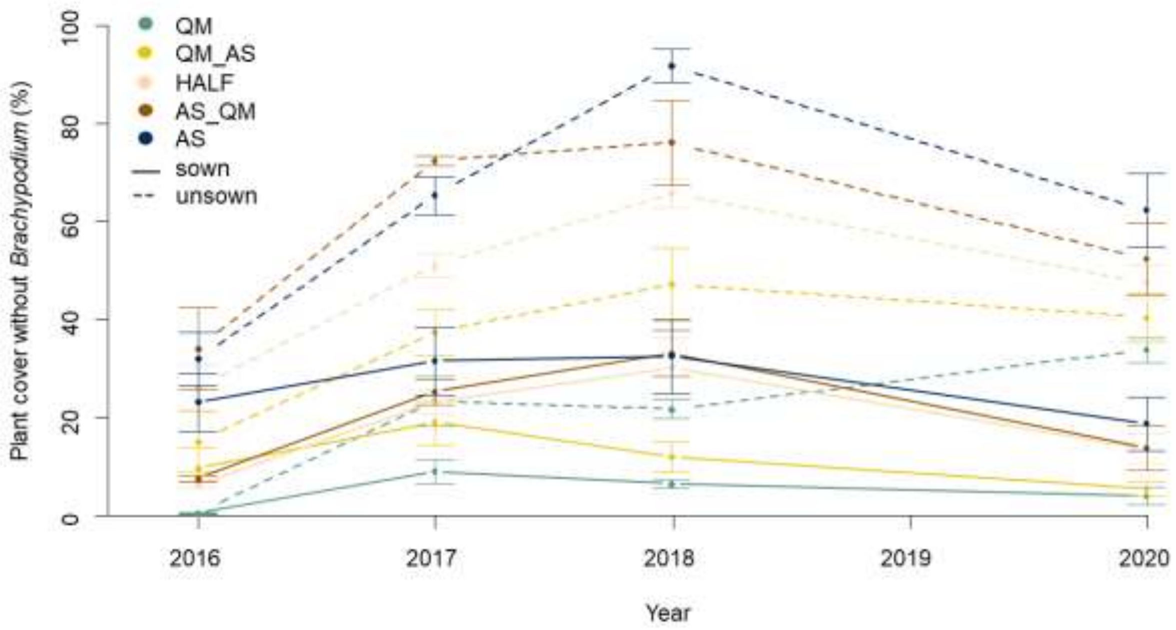


Figure F. Mean plant cover (without *Brachypodium*) on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – raw Quarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures × *Brachypodium* sowing χ^2=152.13, p<0.001***; Substrate mixtures × Years χ^2=49.96, p<0.001***; *Brachypodium* sowing × Years χ^2=47.98, p<0.001***. QM: 100% raw quarry material; QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

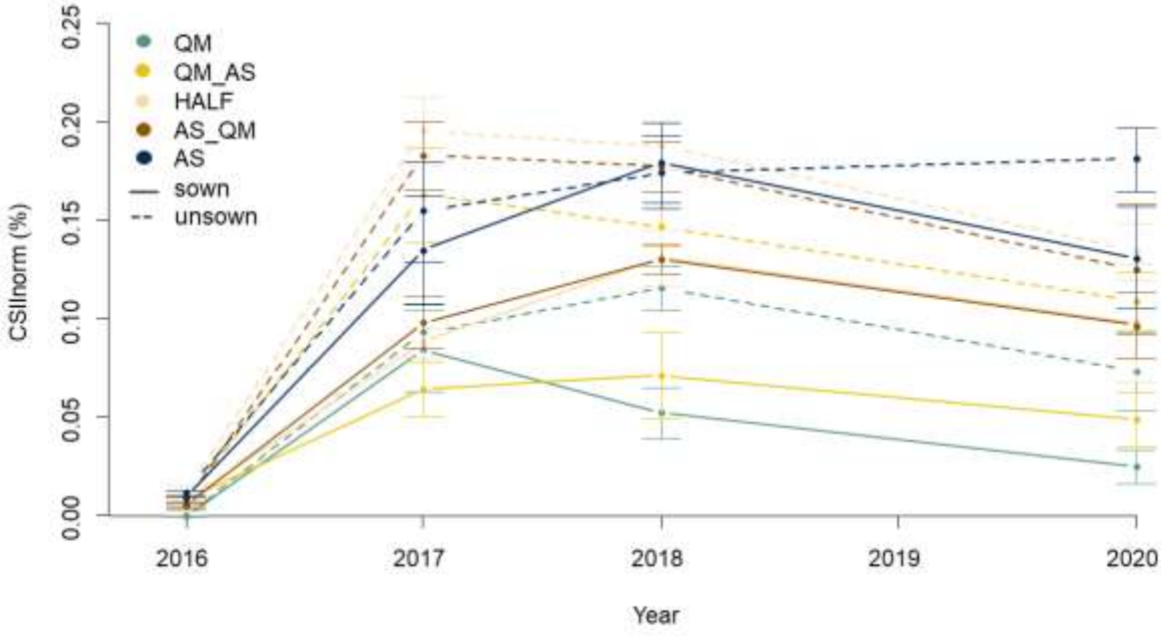


Figure G. Mean CSII on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – rawQuarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures × *Brachypodium* sowing × Years χ^2=26.24, p=0.010*. QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

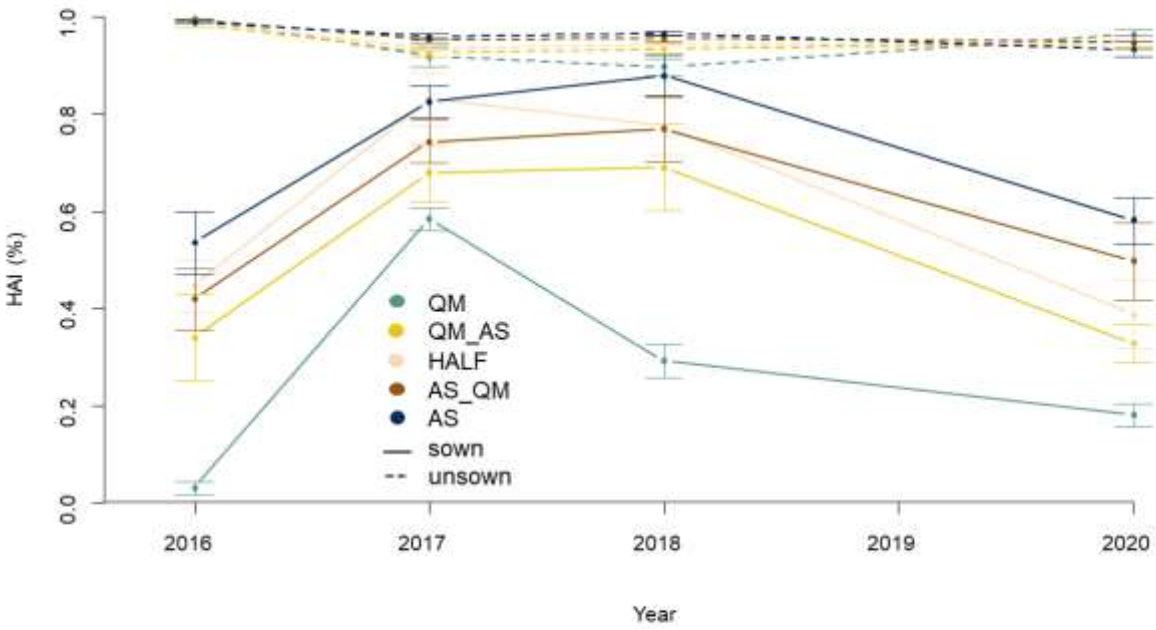
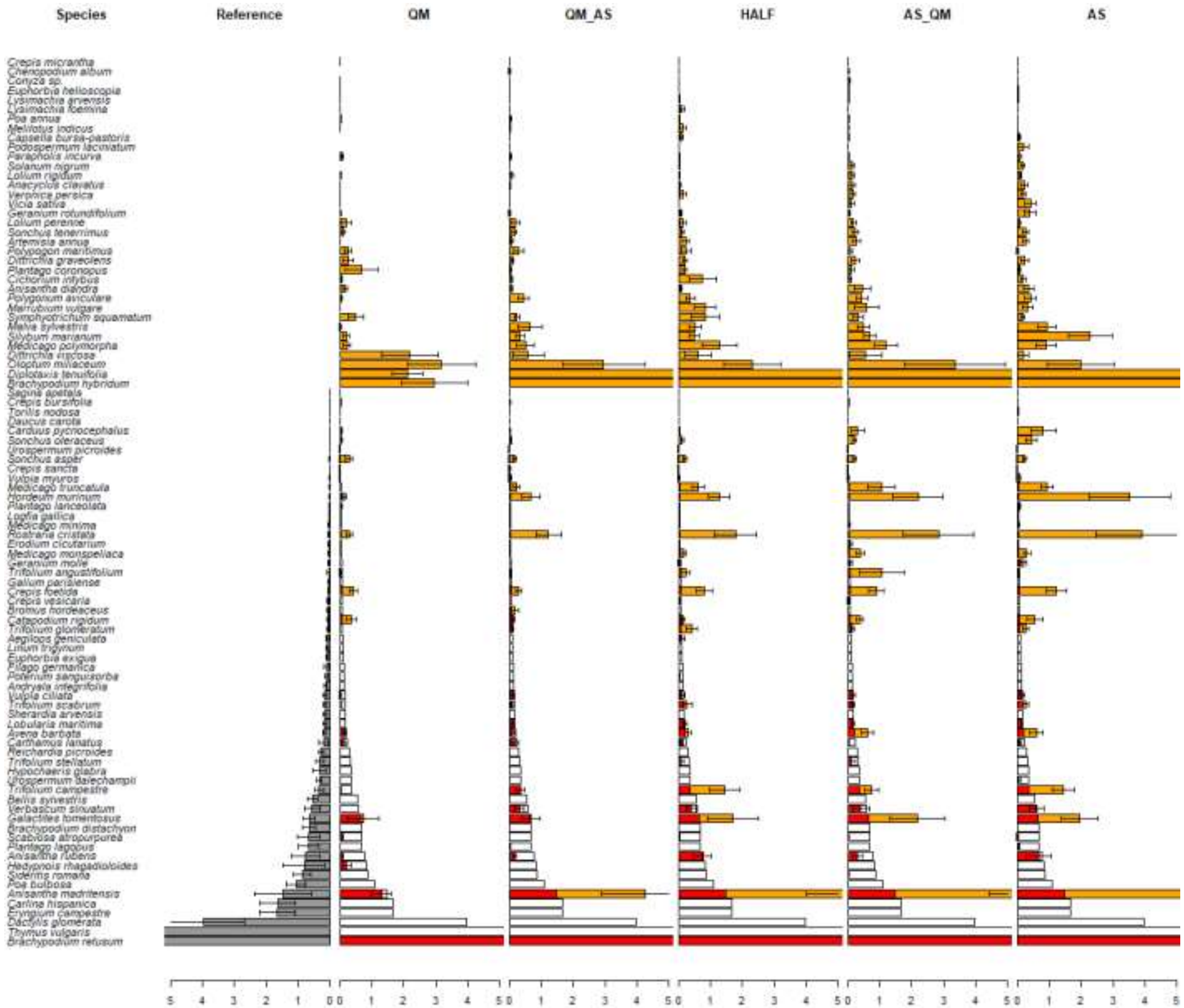


Figure H. Mean HAI on experimental plots in 2016, 2017, 2018 and 2020. Plots were sown (solid lines) or not (dashed lines) with *Brachypodium*. Substrate mixtures included QM – rawQuarry Material or AS – Arable Soil (see Table 1 for mixture codes). Substrate mixtures × *Brachypodium* sowing χ^2=152.13, p<0.001***; Substrate mixtures × *Brachypodium* sowing × Years χ^2=105.95, p<0.001***. QM: 100% raw quarry material; QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

Supplement S4: Structures of plant communities recorded on the different substrate mixtures tested against the reference grassland plant community (grey: mean abundance of species recorded in the reference; white: abundance lacking in relation to the reference; orange: abundance of target species present in the community assessed; red: excess abundance of species present in the communities assessed and not in the reference). (QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil)


Species
Reference
QM
QM_AS
HALF
AS_QM
AS
Crepis micrantha
Chenopodium album
Conyza sp.
Euphorbia helioscopia
Lysimachia arvensis
Lysimachia foemina
Poa annua
Melilotus indicus
Capsella bursa-pastoris
Podospermum laciniatum
Parapholis incurva
Solanum nigrum
Lolium rigidum
Anacyclus clavatus
Veronica persica
Vicia sativa
Geranium rotundifolium
Lolium perenne
Sonchus tenerrimus
Artemisia annua
Polypogon maritimus
Dittrichia graveolens
Plantago coronopus
Cichorium intybus
Anisantha diandra
Polygonum aviculare
Marrubium vulgare
Symphyotrichum squamatum
Malva sylvestris
Silybum marianum
Medicago polymorpha
Dittrichia viscosa
Oloptum miliaceum
Diplotaxis tenuifolia
Brachypodium hybridum
Sagina apetala
Crepis bursifolia
Torilis nodosa
Daucus carota
Carduus pycnocephalus
Sonchus oleraceus
Urospermum picroides
Sonchus asper
Crepis sancta
Vulpia myuros
Medicago truncatula
Hordeum murinum
Plantago lanceolata
Logfia gallica
Medicago minima
Rostraria cristata
Erodium cicutarium
Medicago monspeliaca
Geranium molle
Trifolium angustifolium
Galium parisiense
Crepis foetida
Crepis vesicaria
Bromus hordeaceus
Catapodium rigidum
Trifolium glomeratum
Aegilops geniculata
Linum trigynum
Euphorbia exigua
Filago germanica
Poterium sanguisorba
Andryala integrifolia
Vulpia ciliata
Trifolium scabrum
Sherardia arvensis
Lobularia maritima
Avena barbata
Carthamus lanatus
Reichardia picroides
Trifolium stellatum
Hypochaeris glabra
Urospermum dalechampii
Trifolium campestre
Bellis sylvestris
Verbascum sinuatum
Galactites tomentosus
Brachypodium distachyon
Scabiosa atropurpurea
Plantago lagopus
Anisantha rubens
Hedypnois rhagadioloides
Sideritis romana
Poa bulbosa
Anisantha madritensis
Carlina hispanica
Eryngium campestre
Dactylis glomerata
Thymus vulgaris
Brachypodium retusum
5 4 3 2 1 0
0 1 2 3 4 5

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Supplement S5: Species recorded in 2020 on the different substrate mixtures for the two sowing treatments (with or without sowing of *Brachypodium*) and the reference grassland. QM: 100% of raw Quarry Material; QM_AS: 75% of raw Quarry Material and 25% of Arable Soil; HALF: 50% of each; AS_QM: 75% of Arable Soil and 25% of raw Quarry Material; AS: 100% of Arable Soil

		QM		QM_AS		HALF		AS_QM		AS		Grassland
Genus	*species*	sown	unsown	sown	unsown	sown	unsown	sown	unsown	sown	unsown	
Aegilops	*geniculata*				x	x	x	x	x	x	x	x
Aira	*cupaniana*									x		x
Ajuga	*iva*					x						
Anacyclus	*clavatus*	x			x	x	x	x	x	x	x	
Andryala	*integrifolia*					x		x		x	x	x
Anisantha	*diandra*	x	x		x							
Anisantha	*madritensis*	x	x	x	x	x	x	x	x	x	x	x
Anisantha	*rubens*	x	x	x	x	x	x	x	x	x	x	x
Arenaria	*leptoclados*					x			x			x
Artemisia	*annua*						x	x	x	x	x	
Artemisia	*verlotiorum*						x				x	
Asphodelus	*ayardii*											x
Avena	*barbata*		x	x	x	x	x	x	x	x	x	x
Bellis	*sylvestris*											x
Bituminaria	*bituminosa*		x				x					
Bothriochloa	*ischaemum*											x
Brachypodium	*distachyon*											x
Brachypodium	*hybridum*	x	x	x	x	x	x	x	x	x	x	
Brachypodium	*retusum*	x	x	x	x	x		x		x		x
Bromus	*hordeaceus*	x			x	x	x			x	x	x
Bupleurum	*semicompositum*						x	x			x	
Carduus	*nigrescens*				x							x
Carduus	*pycnocephalus*	x			x	x	x	x	x	x	x	x
Carduus	*tenuiflorus*						x			x		

Carlina	*hispanica*											x
Carlina	*lanata*						x	x	x		x	x
Carthamus	*lanatus*		x	x	x		x	x	x	x	x	
Catapodium	*rigidum*	x	x	x	x	x	x	x	x	x	x	x
Centaurium	*erythraea*											x
Centaurea	*melitensis*											x
Cerastium	*glomeratum*	x									x	
Cerastium	*pumilum*		x				x		x			x
Chondrilla	*juncea*		x				x					
Cichorium	*intybus*					x					x	
Clinopodium	*nepeta*		x									
Convolvulus	*arvensis*						x	x	x	x		
Convolvulus	*cantabrica*			x	x				x	x	x	
Conyza	*sp.*		x		x			x	x	x	x	
Crepis	*bursifolia*		x	x	x			x	x	x		
Crepis	*foetida*	x	x	x	x	x	x	x	x	x	x	x
Crepis	*micrantha*		x	x		x	x	x		x		
Crepis	*sancta*			x	x	x		x	x	x	x	x
Crepis	*vesicaria*		x	x		x	x	x	x	x	x	x
Cynodon	*dactylon*	x				x				x	x	
Cynosurus	*echinatus*						x					x
Dactylis	*glomerata*				x	x	x		x			x
Daucus	*carota*	x	x	x	x	x	x	x	x	x		
Diplotaxis	*tenuifolia*	x	x	x	x	x	x		x	x	x	
Dittrichia	*graveolens*	x	x	x	x	x	x	x	x	x	x	
Dittrichia	*viscosa*	x	x	x	x	x	x	x	x	x	x	
Echium	*vulgare*		x		x		x		x		x	
Erodium	*cicutarium*							x	x		x	x
Erodium	*malacoides*						x		x	x	x	

Erodium	*moschatum*	x		x		x			x			
Eryngium	*campestre*						x					x
Euphorbia	*cyparissias*					x						x
Euphorbia	*exigua*											x
Euphorbia	*falcata*											x
Euphorbia	*helioscopia*		x						x	x	x	
Euphorbia	*segetalis*								x			
Euphorbia	*serrata*		x									
Filago	*germanica*		x	x	x	x	x			x	x	x
Filago	*pygmaea*		x							x		x
Filago	*pyramidata*		x									
Foeniculum	*vulgare*		x		x		x	x				
Galactites	*tomentosus*	x	x	x	x	x	x	x	x	x	x	x
Galium	*murale*											x
Galium	*parisiense*	x			x				x			x
Geranium	*molle*			x	x	x	x			x	x	x
Geranium	*rotundifolium*	x	x			x	x		x	x	x	
Hedypnois	*rhagadioloides*		x					x		x		x
Hippocrepis	*ciliata*							x				
Hirschfeldia	*incana*					x		x		x		
Hordeum	*murinum*	x	x	x	x	x	x	x	x	x	x	
Hypericum	*perfoliatum*						x		x			
Hypochaeris	*glabra*					x			x	x	x	x
Lactuca	*serriola*										x	
Lagurus	*ovatus*										x	
Lathyrus	*cicera*									x		
Leontodon	*tuberosus*							x				
Limonium	*echioides*							x				
Linaria	*arvensis*											x

Linum	*strictum*								x		x	x
Linum	*trigynum*											x
Lobularia	*maritima*					x	x		x	x	x	x
Logfia	*gallica*									x		x
Lolium	*rigidum*	x	x	x	x	x	x	x	x	x	x	
Lysimachia	*arvensis*	x			x		x	x	x	x	x	
Lysimachia	*foemina*		x		x		x		x			
Lysimachia	*linum-stellatum*											x
Malva	*sylvestris*	x			x	x	x		x	x	x	
Marrubium	*vulgare*					x	x		x	x	x	
Medicago	*minima*	x		x		x		x		x	x	x
Medicago	*monspeliaca*	x	x	x	x	x	x	x	x	x	x	x
Medicago	*orbicularis*	x		x		x		x				
Medicago	*polymorpha*	x	x	x	x	x	x	x	x	x	x	
Medicago	*rigidula*	x			x	x	x	x			x	x
Medicago	*truncatula*	x		x	x	x	x	x	x	x	x	x
Melilotus	*indicus*	x		x	x	x		x	x	x		
Misopates	*orontium*			x						x		
Neatostema	*apulum*		x									x
Oloptum	*miliaceum*	x	x		x		x		x		x	
Pallenis	*spinosa*			x	x	x		x	x			
Papaver	*rhoeas*						x				x	
Parapholis	*incurva*	x	x	x	x	x	x	x	x		x	
Petrorhagia	*prolifera*							x				x
Plantago	*afra*											x
Plantago	*bellardii*					x						x
Plantago	*coronopus*	x	x		x	x	x	x	x	x	x	
Plantago	*lagopus*	x			x			x		x		x
Plantago	*lanceolata*	x	x			x					x	

Poa	*bulbosa*											x
Podospermum	*laciniatum*				x	x	x	x	x	x	x	
Polygonum	*aviculare*							x				
Polypogon	*maritimus*	x										
Polycarpon	*tetraphyllum*											x
Poterium	*sanguisorba*		x	x		x	x					x
Prospero	*autumnale*											x
Psilurus	*incurvus*											x
Reichardia	*picroides*											x
Rostraria	*cristata*	x	x	x	x	x	x	x	x	x	x	x
Rumex	*pulcher*								x			x
Sagina	*apetala*		x		x		x		x	x	x	x
Scabiosa	*atropurpurea*	x	x	x	x			x			x	x
Scolymus	*hispanicus*									x	x	
Scorpiurus	*muricatus*		x							x		
Senecio	*vulgaris*											x
Sherardia	*arvensis*							x	x	x	x	x
Sideritis	*romana*		x						x			x
Silene	*gallica*											x
Silene	*nocturna*			x			x	x	x	x		x
Silybum	*marianum*	x	x	x	x	x	x	x	x	x	x	
Sonchus	*oleraceus*				x				x			x
Sonchus	*asper*	x	x	x	x	x	x	x	x	x	x	x
Sonchus	*tenerrimus*					x		x		x	x	
Stipa	*capillata*											x
Symphyotrichum	*squamatum*	x	x	x	x	x	x	x	x	x	x	
Taeniatherum	*caput-medusae*											x
Thymus	*vulgaris*											x
Tolpis	*umbellata*											x

Torilis	*nodosa*								x			
Tragopogon	*porrifolius*		x			x		x	x			
Trifolium	*angustifolium*	x	x	x	x	x	x	x	x	x	x	
Trifolium	*campestre*	x	x	x	x	x	x	x	x	x	x	x
Trifolium	*cherleri*							x				x
Trifolium	*glomeratum*			x	x	x	x	x	x	x	x	x
Trifolium	*scabrum*	x	x	x	x	x	x	x	x	x	x	x
Trifolium	*stellatum*					x		x	x			x
Trifolium	*suffocatum*						x				x	
Trifolium	*tomentosum*						x			x		
Urospermum	*dalechampii*		x									x
Urospermum	*picroides*		x		x	x	x	x	x	x	x	x
Valantia	*muralis*								x			
Verbascum	*sinuatum*		x	x	x		x		x	x	x	x
Veronica	*arvensis*							x		x	x	
Veronica	*persica*				x		x	x	x		x	
Veronica	*polita*										x	
Vicia	*lathyroides*									x		
Vicia	*sativa*						x	x		x	x	
Vulpia	*ciliata*	x	x	x	x	x	x	x	x	x	x	x
Vulpia	*myuros*	x	x	x	x	x	x		x	x	x	x