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Temporary wetland restoration after rice cultivation: is soil transfer required for aquatic plant colonization?

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ABSTRACT

Key-words: *former ricefield, restoration, spontaneous colonization, soil transfer, temporary wetland*

Mediterranean temporary wetlands have considerably declined in recent decades. Today, opportunities arise for the restoration of these wetlands due to land-use changes, such as the abandonment of cultivation. One critical question is whether communities, such as those observed in natural temporary wetlands, can develop alone or if active restoration should be implemented. In a series of experimental mesocosms, we transferred soil from several temporary wetlands chosen as a set of reference ecosystems. Four months after soil transfer, vegetation in transfer mesocosms was compared to that derived from spontaneous colonization (control mesocoms). Transfer mesocosms are colonized by all target hydrophyte species transferred with the soil and the resulting communities are similar to those of reference ecosystems. They also have fewer non-target species than the control mesocosms. Even though the study period was not sufficient to draw any definitive conclusion regarding the utility of forced dispersion by soil transfer, the preliminary results are promising for an application on a larger scale.

RÉSUMÉ

Restauration d'un milieu humide temporaire après culture du riz : le transfert de sol est-il nécessaire à la recolonisation des plantes aquatiques ?

Mots-clés : *anciennes rizières, restauration, colonisation spontanée, transfert de sol, zone humide*

Les marais temporaires méditerranéens ont vu leur superficie se réduire considérablement au cours des dernières décennies. Aujourd'hui, des opportunités apparaissent pour la restauration de ces zones humides, en particulier à partir d'espaces agricoles abandonnés. L'une des questions majeures est alors de savoir si les communautés telles que l'on peut les observer dans les marais temporaires naturels peuvent se développer seules ou si une restauration active doit être mise en place. Dans un ensemble de mésocosmes expérimentaux, nous avons transféré du sol provenant de plusieurs marais temporaires choisis comme un ensemble d'écosystèmes de référence. Quatre mois après le transfert de sol, la végétation des mésocosmes transférés a été comparée à celle issue uniquement de la colonisation spontanée (témoins). Les mésocosmes ayant subi un transfert de sol sont colonisés par la totalité des hydrophytes cibles transférées par le sol et présentent des communautés similaires à celles des écosystèmes de référence. Ils présentent d'autre part moins d'espèces jugées indésirables que les mésocosmes témoins. Même si la durée de l'étude ne nous permet pas de conclure définitivement sur la nécessité d'opérer un forçage de dispersion par transfert de sol, ces résultats préliminaires sont prometteurs pour une application à plus large échelle.

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INTRODUCTION

The remaining global extent of wetlands is estimated to be over 1.2 million square kilometers. During the twentieth century, in North America, Europe, and Australia more than 50% of certain types of wetlands were destroyed (Millennium Ecosystem Assessment, 2005). Due to this wetland loss, many countries have implemented specific regulations to protect wetlands (*e.g.* the US "clean water act" of 1972 (McMahon Jr, 1972) or the European Water Framework Directive of 2000 (European Commission, 2000)). These regulations include restoration and conservation of water integrity by limiting pollution and maintaining the overall integrity of wetlands. Indeed, wetlands provide multiple ecosystem services, such as water purification and waste water treatment, regulation of hydrological flow, climate and erosion, primary production, and biodiversity conservation (Millennium Ecosystem Assessment, 2005; Zedler and Kercher, 2005). Therefore, in recent decades, wetland restoration (Society for Ecological Restoration, 2004) has received increased attention (Zhang *et al.*, 2010).

In the Mediterranean Basin, wetlands have been greatly impacted because they are particularly productive systems which were converted for agriculture and tourism (Hollis, 1992). The remaining wetlands are of important ecological, social and economic values (Grillas *et al*., 2004). Natural temporary wetlands of the region are characterized by winter and spring flooding, with durations that greatly vary from year to year, and by a complete drying-out in summer (Grillas *et al.*, 2004). They represent one of the most remarkable Mediterranean habitats, comprising a high plant diversity of particularly annuals species (some of which are rare and endangered) adapted to the specific climate (*i.e.* necessity to be annual species to support the dry summer with short favorable periods for reproduction), such as *Zannichellia obtusifolia, Callitriche lenisulcaor Tolypella hispanica* (Grillas and Duncan, 1986; Grillas *et al.*, 2004). During the 20th century, temporary wetlands were subject to degradation and drastic area reduction in the Mediterranean region due to agriculture, industry, recreational activities, and hunting (Hollis, 1992; Grillas *et al.,* 2004). One of the main causes of the degradation of Mediterranean temporary wetlands is the water management for hunting activities that maintains the water level in spring and/or in summer and that has led to a decline of plant communities that are restricted to temporary wetlands along with an increase in perennial and cosmopolitan species (Aznar *et al.*, 2003; Tamisier and Grillas, 1994). Mediterranean temporary wetlands are thus considered a priority habitat (code 3170) according to the Natura 2000 Network of the European Union Habitats directive (European Commission, 1992). Wetland restoration (Society for Ecological Restoration, 2004) is urgently needed to stop the loss of this habitat type. In order to restore a wetland, two strategies can be adopted to establish plant communities: i) one based on spontaneous succession with recruitment from residual seed bank or from seed dispersal, or ii) active restoration which requires propagule introduction.

Spontaneous colonization may provide satisfying results in terms of plant composition and may also promote wetland "self-design" capacity as a response to hydrological conditions (Mitsch *et al.*, 1998; Prach *et al.*, 2001a). If the appropriate environmental conditions, mainly consisting of flooding regimes, water depth, and salinity, are restored (Grillas, 1990), vegetation can rapidly establish from the residual seed bank (Leck, 2003; De Steven *et al.*, 2006). Short distance dispersal (Reinartz and Warne, 1993), long distance endozoochorous (Brochet *et al.*, 2010; Figuerola *et al.*, 2002; Zedler and Black, 1992), and ectozoochorous (Figuerola and Green, 2002) dispersal may also contribute to spontaneous colonization Active revegetation methods may not be needed if sources populations of desired seeds are available nearby and if physical barriers do not hamper dispersal (Moreno-Mateos and Comin, 2010).

Vegetation recovery is often limited by a low density and a high distance of seed sources (Bischoff, 2002). Several studies have demonstrated dispersal limitation despite the proximity of natural temporary wetlands (Collinge and Ray, 2009; Galatowitsch and Valk, 1996). Moreover, the site to be restored may be isolated from the network of wetlands (McKinstry and Anderson, 2005; Reinartz and Warne, 1993) or may have been submitted to a long cultivation period (Prach *et al.*, 2001), which often limits re-colonization. In such cases, active restoration, including reestablishment of dispersal vectors, is needed to restore plant communities (Bischoff, 2002). Community translocation involves the removal of the full species assemblage of a site and the establishment of a functioning community at a new receptor site (Bullock, 1998). Transfer of bulk soil is such a community translocation method. It is often used in wetland restoration and has already shown promising results: the imported soils contribute considerably to species richness and native wetland species establishment, indicating that soil transfer may enhance the success of wetland restoration projects compared to natural colonization (*e.g.* Balcombe *et al.*, 2005; Nishihiro *et al.* 2006; Reinartz and Warne, 1993). Moreover, this technique could be the most efficient method for transferring a large number of temporary wetland plant species that have a short life cycle but can produce large quantities of seeds and rapidly form a large seed bank (Mouronval and Baudoin, 2010).

In the present study we investigate the benefits of soil transfer when compared to spontaneous succession in temporary wetland restoration of former ricefields. Before testing spontaneous colonization *vs.* active revegetation, physical manipulations need to be included, *e.g.* hydrological and topography restoration, in particular if environmental conditions are not appropriate. This is often the case if the wetland has been destroyed for cultivation, resulting in strong modifications of the former topography and of the inundation periods. Only after restoration of abiotic conditions, the potential of spontaneous colonization can be evaluated. We thus used experimental on-site wetland mesocosms to address the following questions: (1) is spontaneous succession sufficient to restore typical Mediterranean wetlands on former ricefields? (*i.e.* what can we expect from the seed bank of former ricefields considered as wetland?); (2) does soil transfer accelerate the colonization of target species and does it increase species richness and the abundance of wetland plants?; (3) is soil transfer required if the site to be restored is close to natural wetlands?

MATERIALS AND METHODS

> *STUDY SITE*

The experiments were conducted at the Cassaïre site (c. 43°31′N, 4°44′E, 3 meter maximum elevation) located east of the Camargue area (Rhône delta, Southern France, Figure 1) with na average substrate salinity of 0.22 g·L^{-1}. The climate is typically Mediterranean, characterized by an annual average temperature of 15 °C, an annual rainfall of 550 mm mainly concentrated in autumn, and a summer drought. The site has been submitted to recurrent leveling for rice cultivation since the 1940s, eliminating the natural topography. Cultivation definitively stopped in 2004. On the old Cassini map dating from the eighteenth century, the site was marked as a wetland, and the final aim of the restoration project is to create 35 hectares of Mediterranean temporary wetlands with native aquatic flora including the rare and endangered species found nearby in the Camargue area. The objective of the present study was to identify methods that may also used in large scale restoration projects. The experiments were set up in 2011.

> *DONOR SITES*

In order to maximize the number of locally adapted aquatic plant species at the Cassaïre site, we selected five temporary wetlands as donor sites in the surroundings (between 1 and 6 km, Figure 1) resulting in an inventory of plant communities and abiotic conditions of all the temporary wetlands of the Camargue area. The environmental conditions of these five donor wetlands corresponded to the range of expected environmental conditions at the Cassaïre site after the creation of the temporary wetland: *i.e.* having a flooding period from September to June only, a maximum water depth of 40 cm, and a salinity below 6 g·L^{-1}. Not all the target hydrophyte species were found in a single donor wetland but the five donor sites together represent the regional target hydrophyte species pool quite well. We attempted to select the most appropriate species to the different abiotic filters in the Cassaïre site driven by the environmental conditions of our recreated wetland. Indeed, plant communities of temporary Mediterranean wetlands vary with salinity, hydroperiod, depth, so we maximized the pool

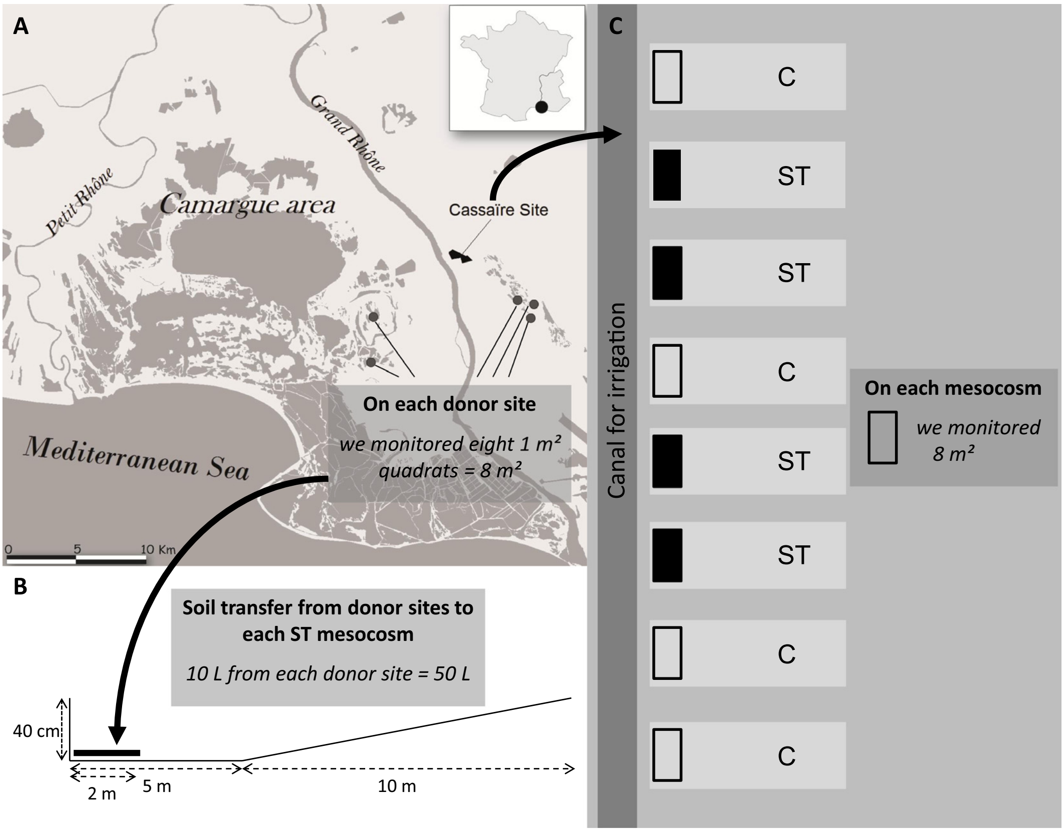

Figure 1
Location of the Cassaïre site (in black) and of the five donor sites (grey circle). The light grey shading indicates the wetlands of the Camargue area (A). Side view of one soil transfer mesocosm (B). Experimental design of restoration treatments at Cassaïre site (C). C = control mesocosms and ST = soil transfer mesocosms (black rectangle indicates the soil transfer).

of species, allowing the most suitable species to grow at the environmental conditions of the Cassaïre site.

In March and May of 2011 (representing vernal and late season vegetation; Grillas *et al.*, 2004), we analyzed the vegetation in each of the five donor sites. In each site, we placed eight 1 m^2 quadrats covering the full humidity gradient. In each quadrat, we recorded the total percent cover of aquatic vegetation and the cover of each species using a modified Braun-Blanquet scale (Braun-Blanquet *et al.*, 1952): 0.5 for species covering less than 1%, 1 for species covering between 1% and 5% of the quadrat, 2 between 5% and 25%, 3 between 25% and 50%, 4 between 50% and 75% and the coefficient 5 for species covering more than 75% of the quadrat. In August 2011, during the dry period when all plants are dormant as seeds in the seed bank, we collected per donor site 45 × 45 cm to a depth of 3 cm soil samples in each quadrat (eight soil samples in total per donor site), resulting approximately in a total of 40 L of soil per donor site. Our assumption was that these collected soils would contain the seeds of the species recorded in the quadrats a few months earlier. We pooled the 8 samples of each donor site to one bulk sample using a cement mixer and we stored them under dry until the transfer to the Cassaïre site.

> MESOCOSM EXPERIMENTS AND SOIL TRANSFER

To simulate the suitable environmental conditions of a Mediterranean temporary wetland, eight mesocosms with a gentle slope were dug out (15 m long × 5 m large × 40 cm deep; Figure 1B) along a canal that was used for irrigation. Four mesocosms were used to test soil transfer. The four other mesocosms were used as control to monitor spontaneous vegetation establishment. The position of treatments was randomized (Figure 1C). We pooled 10 L samples from each of the five sites and we spread this 50 L of soil on a 4×2 m plot at the bottom of each transfer mesocosm (Figure 1B). A pump maintained a constant 20 cm water level from the day after the transfer in January 2012 (inundation beginning four months after classical temporary wetland conditions but allowing even so the vernal species germination in March) to the end of May 2012. Mid-May to mid-June corresponds roughly to the local dry out of temporary wetlands.

> *VEGETATION MONITORING*

Our aim was to compare soil transfer with control plots in term of similarity to the donor sites. Vegetation monitoring was carried out in May 2012 (four months after the transfer), when most species show their biomass peak. In each of the mesocosm 4 m $\times$ 2 m plot (8 m^2; Figure 1C), we estimated the total cover of aquatic vegetation (%) and the cover of each species using the same method as for the donor sites of the previous year. Area of the donor site vegetation analyses was adjusted to that of the mesocosm plots (8 m^2) by pooling the eight 1 m^2 quadrats of each donor site.

Among all plant species occurring in the experiment (Appendix), we assigned aquatic species to one of the following categories:

1. Target hydrophyte species: Present at donor sites according to the classification of temporary wetlands (Grillas and Duncan, 1986), corresponding to the native or typical flora of the temporary wetlands of the Camargue area, adapted to the Mediterranean climate, with protection status and threatened by some types of water management, such as fresh water production in the summer (*Callitriche* sp., *Callitriche truncata, Chara aspera, Chara canescens, Chara globularis, Ranunculus peltatus, Ranunculus trichophyllus, Tolypella glomerata, Tolypella hispanica, Zannichellia obtusifolia and Zannichellia pedicellata*).
2. Ricefield hydrophyte weeds: Present with rice cultivation (Marnotte *et al.*, 2006). Ricefield weeds are often exotic species introduced by rice cultivation (*Lindernia dubia*) but can also correspond to banal algae enhanced by rice water management (C*hara vulgaris*), and therefore associated with eutrophic and flooded summer wetlands. These widespread algae may occur in the reference ecosystems, but cannot be considered as target hydrophyte species.
3. Green filamentous algae: Occurring in temporary wetlands at high temperatures and high nutrient levels (*Cladophora vagabunda* and *Spirogyra* sp.).

All the target hydrophyte species and ricefield hydrophyte weeds are annual species, produce large quantities of seeds that survive several years and are very resistant to drought (Marnotte *et al.*, 2006; Mouronval and Baudoin, 2010). However, for some Characeae (*Chara globularis* and *Chara vulgaris*) as well as the two ranunculus (*Ranunculus peltatus* and *Ranunculus trichophyllus*), plants can be annual or perennial. The Mediterranean climate with temporary wetlands selects the annual nature of these species, perennials do not tolerate the summer dry season.

> *SOIL NUTRIENTS*

To compare soil conditions between the Cassaïre and the donor sites, the following soil properties were measured by the soil analysis laboratory of INRA (The French National Institute for Agricultural Research, Aras, France): organic matter, total C, total N, P_2O_5, pH and conductivity. Before setting up the mesocosm experiment, five soil samples were taken from the surface (0–10 cm) and from 40–60 cm depth (corresponding to the digging depth of the mesocosm *i.e.* the new soil surface). In each sample, three sub-samples of 1 L were taken at random and subsequently pooled for analysis. The same method was applied to the five donor sites (one sample from the surface (0–10 cm) comprising three sub-samples per donor site) to obtain a reference for the soil nutrient status in the target communities. Samples were dried and sieved (to 200 μm).

> *DATA ANALYSIS*

We used different factorial and multivariate analyses to compare transfer and control mesoscosms with donor sites. Contrary to the position of soil transfer and control within the mesocosm experiment, the position of donor site and Cassaïre site is not randomized. We still include donor sites and mesocosm treatments in the same models because we are convinced that environmental conditions are very similar and that we can use donor sites to characterize the target community of restoration.

In order to compare soil data between the donor sites and the Cassaïre site, and to assess the habitat suitability of the Cassaïre site, we performed a Principal Component Analysis on soil nutrient contents (15 samples × 6 variables).

To analyze differences between control and soil transfer, we used nonparametric multivariate analysis of variance (nonparametric MANOVA) (Anderson, 2001). We used Bray-Curtis similarity index (Raup and Crick, 1979) with 999 permutations based on species coefficient cover to compare the species composition We also performed a Correspondence Analysis (CA) on the vegetation data of the donor sites, of the transfer mesocosms, and of the control mesocosms (13 quadrats × 28 species). In order to characterize the success of the soil transfer, we calculated the Bray-Curtis similarity index. For each plot surveyed in a mesocosm, the mean Bray-Curtis index between this plot and the 5 plot surveyed in donor sites (one per donor site corresponding to an average of the eight 1 m^2 quadrats per donor site) was calculated. In order to assess donor site variability, we compared the plot of each donor site to that of the other donor sites, obtaining also a mean Bray-Curtis similarity index for donor sites.

We compared the means of the soil nutrient variables, the species richness, the total cover of aquatic vegetation, the means of the Bray-Curtis index and the plant species categories (*i.e.* target hydrophyte species, ricefield hydrophyte weeds and green filamentous algae) between soil transfer, control and donor sites using a one-way analysis of variance (ANOVA), followed by Tukey post-hoc tests (Sokal and Rohlf, 1995) if the data met the assumptions of ANOVA. If data did not comply with assumptions, we performed Kruskal-Wallis and pairwise Wilcoxon tests with a p-value adjustment according to the simple Bonferroni method, in which the p-values are multiplied by the number of comparisons.

All tests were performed using R 2.12.0 (R Development Core Team, 2010) with a $p = 0.05$ threshold using "ade4" package (Dray *et al.*, 2007) and "vegan" package (Oksanen *et al.*, 2008).

RESULTS

> *SOIL NUTRIENTS*

The soil found at a depth of 40–60 cm on the Cassaïre site became surface soil in the redesigned wetland topography (future soil surface), and it differed from the existing surface soil in Cassaïre and from that of the donor sites (Figure 2). P_2O_5 content was significantly higher in the Cassaïre current surface soil, while pH was significantly higher in Cassaïre deep soil layer. The other nutrients, organic matter, total C, total N, and conductivity were significantly higher in the donor site soils. Organic matter, total C and total N clearly distinguished the soils from the donor sites from those of the Cassaïre along the first axis (73.2%; with an eigenvalue of 5.13 for a total represented of 7) of the PCA (Figure 3). The second axis (18.0%; with an eigenvalue of 1.26 for a total represented of 7) discriminates the deeper Cassaïre soil from surface Cassaïre soil, and clearly delineates their respective differences in phosphorus and pH (Figures 2 and 3).

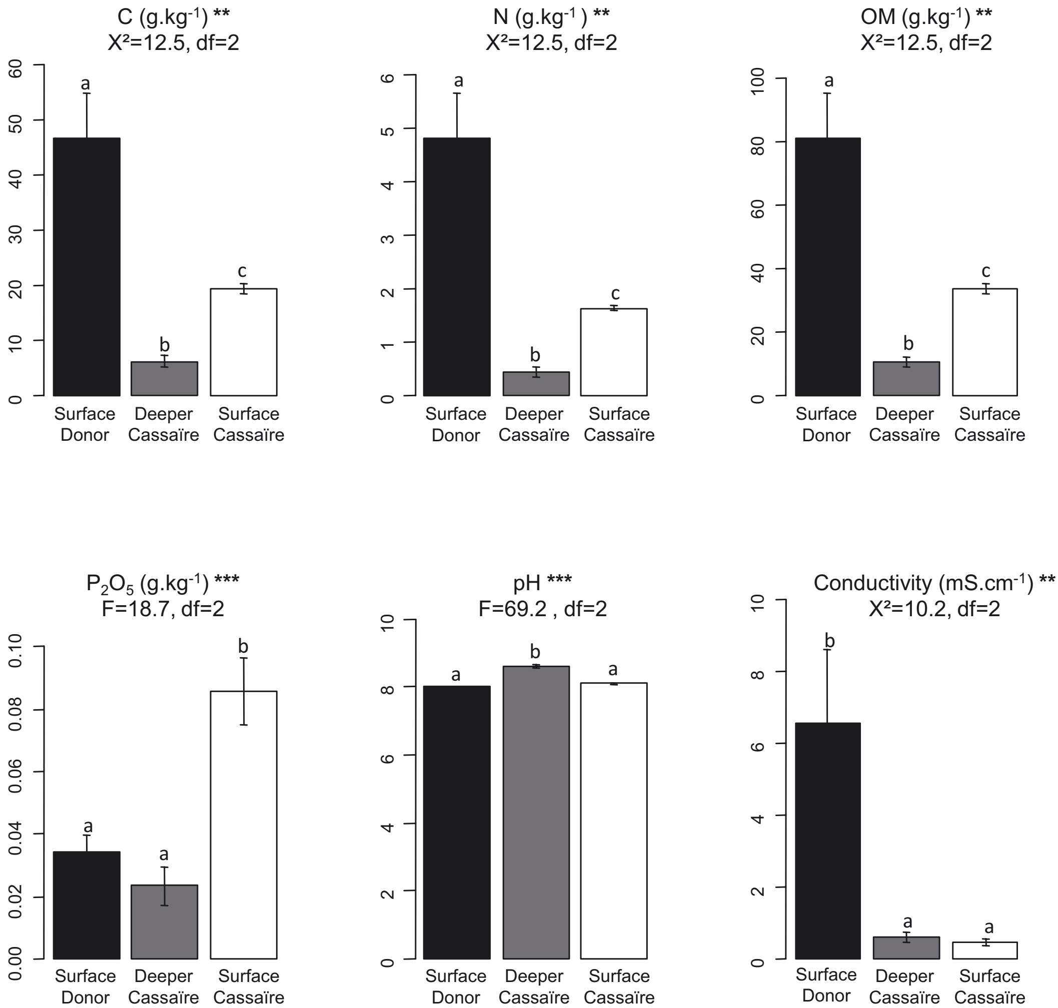

Figure 2
*Mean values ± standard errors of total Carbon (C), total Nitrogen (N), Organic Matter (OM), Phosphorus (P_2O_5), pH and Conductivity in the surface soil of the donor sites (black bars, n = 5), in the surface soil of the Cassaïre site (current soil surface; white bars, n = 5) and in the deeper soil (40–60 cm deep) of the Cassaïre site (future soil surface; grey bars, n = 5). Df are the degrees of freedom. The F of ANOVA or the X^2 of Kruskal-Wallis tests are shown above the bars (***: $p < 0.001$, **: $p < 0.01$), different letters above bars indicate significant differences according to Tukey post-hoc tests or pairwise multiple comparisons with Bonferroni p adjustment.*

> EFFECT OF SOIL TRANSFER ON AQUATIC VEGETATION

Plant species richness significantly increased with soil transfer relative to the control and ended up being comparable to that of the donor sites (Figure 4A). The aquatic vegetation cover was significantly lower in the control mesocosms than in the soil transfer mesocosms (Figure 4B). The aquatic vegetation cover in the latter and at the donor sites was not significantly different (Figure 4B). The Bray-Curtis similarity index was significantly higher in the soil transfer mesocosms than in the control and approached the values of the donor sites (Figure 4C). The first axis of the CA (41.2%; with an eigenvalue of 0.61 for a total represented of 1.48) discriminated the control mesocosms from the two other communities: donor sites and soil transfer mesocosms (Figure 5); control mesocosms were composed of *Rumex crispus, Poa trivialis* and the exotic species *Lindernia dubia*. The species composition of the transfer mesocosms was very similar to that of the donor sites that were characterized by target hydrophyte species, such as *Chara aspera*, *Ranunculus peltatus* and *Tolypella glomerata*. These results were confirmed by the nonparametric MANOVA, showing that the soil transfer

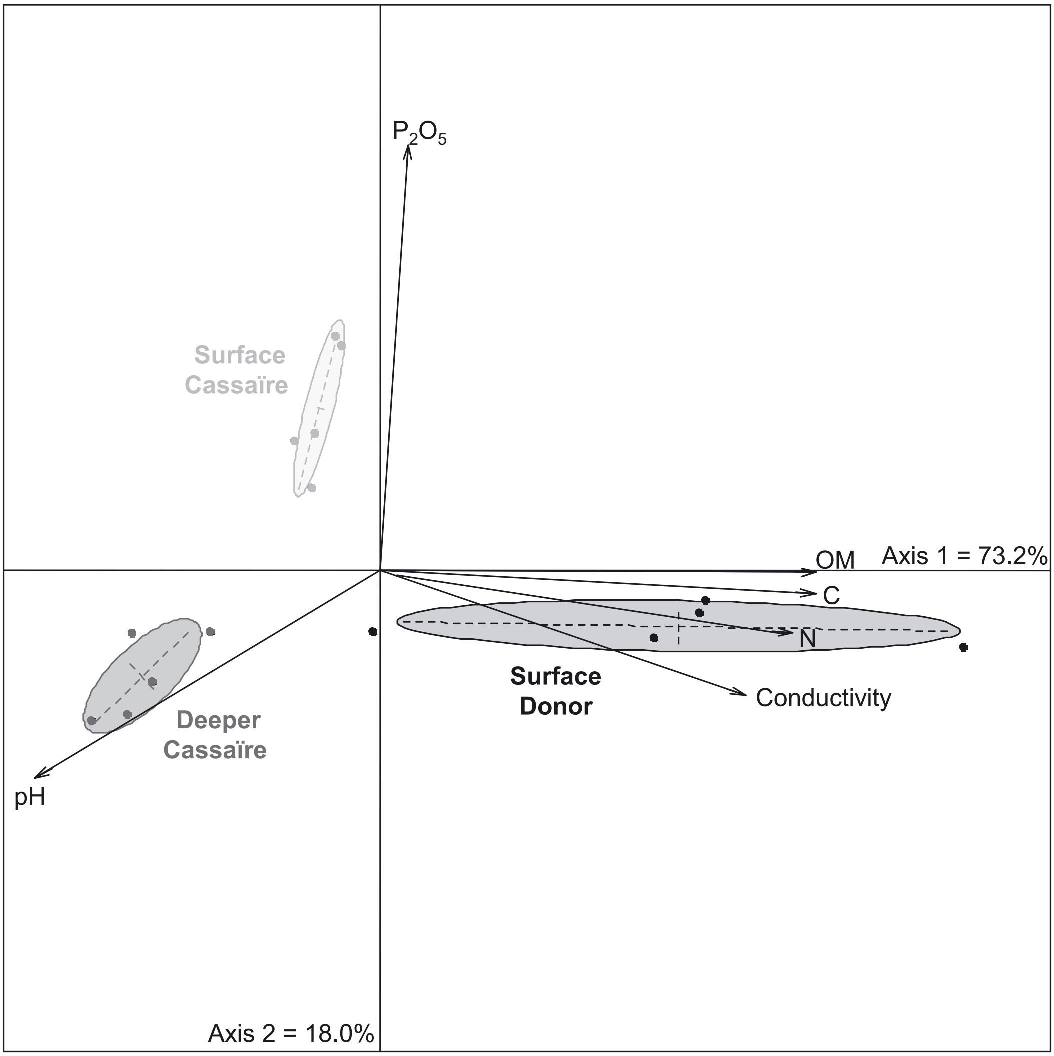

Figure 3
Ordination plot of the Principal Component Analysis based on soil nutrient contents (15 samples × 6 variables) of surface soil of the donor sites (black, 5 plots), of surface soil of the Cassaïre site (current soil surface; light grey, 5 plots), and of deeper soil (40–60 cm deep) of the Cassaïre site (future soil surface; dark grey, 5 plots). Ellipses are centred on the barycentre and their forms are weighted by the distribution of all points corresponding to the same treatment (surface donor, surface soil, deeper soil).

treatment did significantly affect plant community composition between control and transfer mesocosms ($dF = 1$, $F = 11.98$, $p = 0.024$).

> EFFECT OF SOIL TRANSFER ON THE DIFFERENT SPECIES CATEGORIES

Active restoration significantly increased the number of target species recorded in the first months after the flooding: compared with the donor sites, the transfer mesocosms had a significantly higher number of the target species, which were totally absent from the control mesocosms (Figure 6A). Ricefield hydrophyte weeds were present in all control mesocosms but absent from the transfer mesocosms and from the donor sites (Figure 6B). We found significantly more filamentous algae in the soil transfer mesocosms than in the control mesocosms and at the donor sites (Figure 6C).

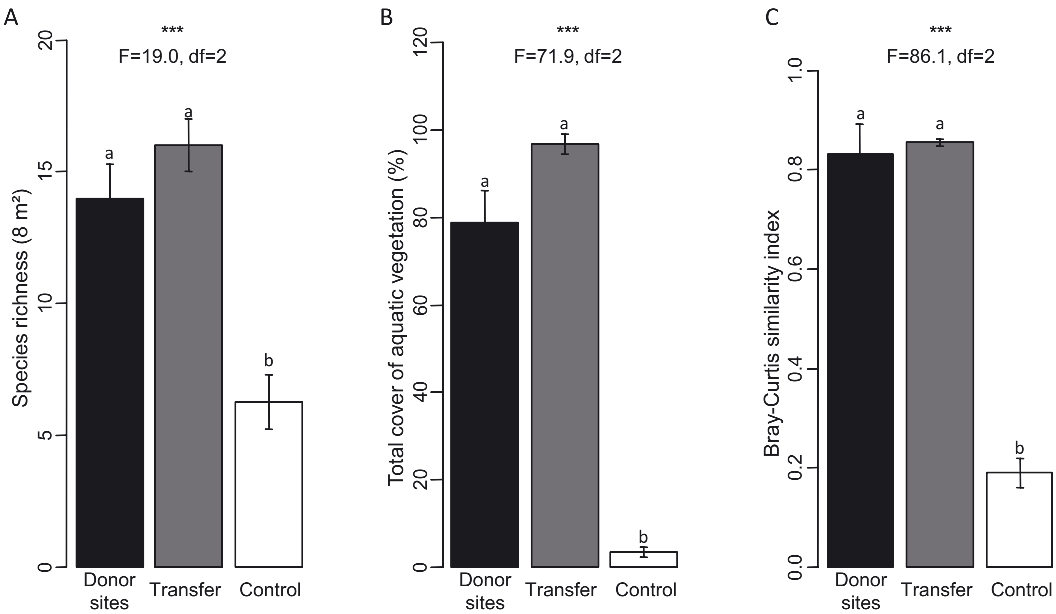

Figure 4
*Mean and standard errors of species richness (8 m^2) (A), total cover of aquatic vegetation (%) (B) and Bray-Curtis similarity index on aquatic vegetation (C) between donor sites and i) donor sites (black bars, $n = 5$ plots), ii) soil transfer mesocosms (grey bars, $n = 4$ plots) and iii) control mesocosms (white bars, $n = 4$ plots). Df are the degrees of freedom. The F of ANOVA are shown above the bars (***: $p < 0.001$), different letters above bars indicate significant differences according to Tukey post hoc tests.*

DISCUSSION

> *SOIL PROPERTIES*

Abiotic conditions can adversely affect the success of plant community transfer, particularly when they are very different from those of the donor ecosystem (Bullock, 1998; Dawe *et al.*, 2000). In coastal wetlands, such as the Camargue area, plant communities are mainly driven by the hydroperiod, water depth, and salinity (Grillas, 1990) but soil nutrient conditions also play an important role by directly affecting plant growth (Zedler, 2000). Obtaining soil characteristics close to those of the reference ecosystem is a major objective in restoration projects (Zedler, 2000), in order to establish suitable conditions for target species recolonization (Marrs, 2002). In our study, the nutrient concentrations considered were higher at donor sites except for phosphorus, which was higher at the site to be restored. The fertilizer use during cultivation may explain the results because the restoration period following abandonment was quite short for depletion of fertilizer residues. Indeed, organic matter and nutrient concentrations are often higher in the reference wetlands (Galatowitsch and Valk, 1996; Zedler, 2000), except for phosphorus, which is strongly related to previous fertilizer use. On one hand soil transfer appears to be an appropriate method to increase organic matter and nutrients concentrations, on the other hand it may also favor the establishment of filamentous algae (Burkholder, 2009), that we found more abundantly in the transfer mesocosms than in the control. The higher pH in the deeper soil that became the surface soil of the mesocosms did not seem to affect the plant germination probably because it still remained within the pH range appropriate for basophilous plants (*i.e.* 7.5 to 9; Wilde, 1954).

Upper soil layers of former agricultural lands contain high levels of nutrient favoring most of the ruderal species of the seed bank, and increasing competition (Marrs, 2002). The topsoil removal to construct mesocosms (which will also be removed at the scale of the site to restore the wetland) led reduced significantly the content of phosphorus, organic matter, total C and total N, and also to the reduction of unwanted plant species by reducing the seed bank (Muller *et al.*, 2013). However, the role of nutrients is probably time limited. If for terrestrial

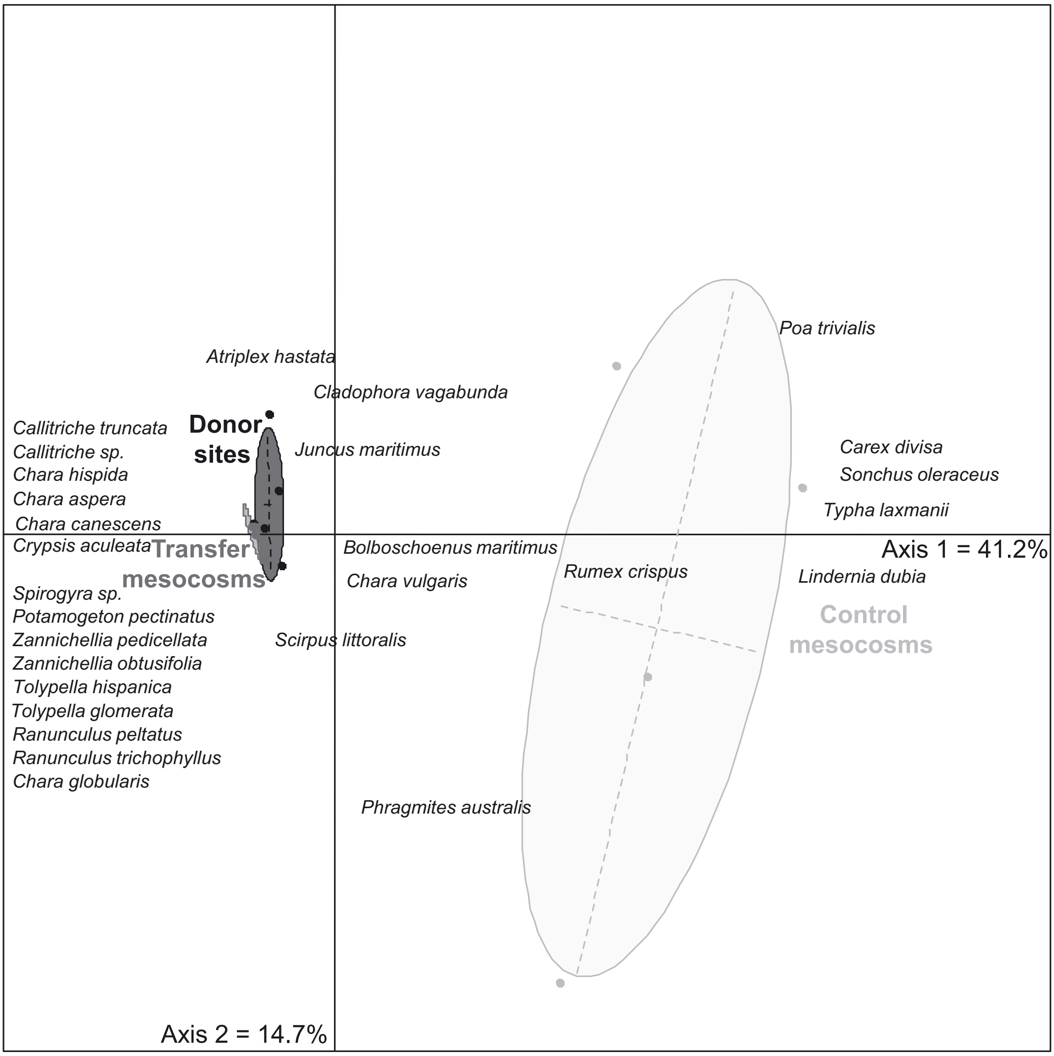

Figure 5
Ordination plot of the Correspondence Analysis of species abundances (13 plots × 28 species) on donor sites (dark, 5 plots), transfer mesocosms (dark grey, 4 plots) and control mesocosms (light grey, 4 plots). Ellipses are centred on the barycentre and their forms are weighted by the distribution of all points corresponding to the same treatment (donor sites, transfer mesocosms, control mesocosms).

oligotrophic community restoration, soil conditions play an important role for success (Muller *et al.*, 2013), requiring nutrient poor site conditions, in wetland ecosystem, hydrology seems to play a determining role on plant communities which can buffer the effects of soil conditions, by eliminating species that are not adapted to summer drought or to winter flooding. However high nutrient levels may lead to a spread of filamentous algae, that may prevent the installation of temporary wetland communities (Hosper, 1998).

> *NATURAL COLONIZATION*

Many of the naturally colonizing species which established in the control mesocosms in the five first months were abundant in the seed bank of the area (seed bank sampling using the seedling emergence with sample concentration method, unpublished data). *Rumex crispus, Poa trivialis,* and *Sonchus oleraceus*, as well as the aquatic species *Lindernia dubia* and *Chara vulgaris*, were the main species found in the control mesocosms. *Lindernia dubia,* a common exotic species of ricefields and *Chara vulgaris*, a cosmopolitan algae had been favored by

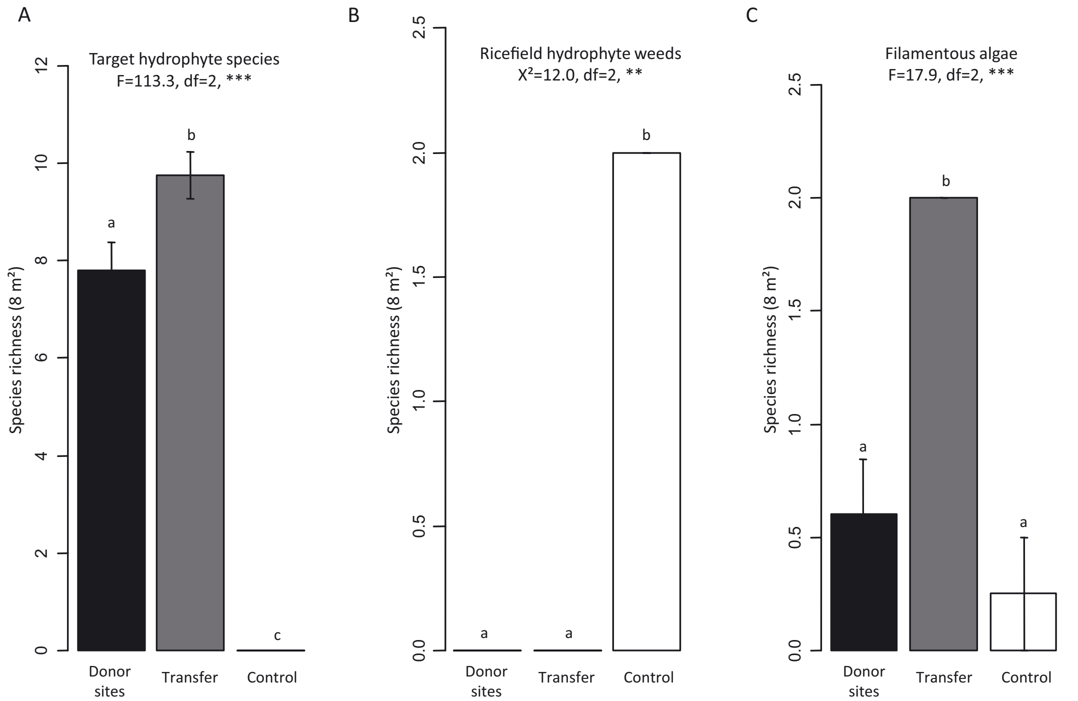

Figure 6
*Mean and standard errors in species number of the different species categories in 8 m^2 plots: the target hydrophyte species (A), the ricefield hydrophyte weeds (B) and the filamentous algae (C) for donor sites (black bars, $n = 5$ plots), soil transfer mesocosms (grey bars, $n = 4$ plots) and control mesocosms (white bars, $n = 4$ plots). Df are the degrees of freedom. The F of ANOVA or the X^2 of Kruskal-Wallis test are shown above the bars (***: $p < 0.001$, **: $p < 0.01$), different letters above bars indicate significant differences according to Tukey post-hoc tests or pairwise multiple comparisons with Bonferroni p adjustment.*

water management during rice cultivation (*i.e.* summer inundation), are typical ricefield weeds occurring in seed bank (Marnotte *et al.*, 2006) and reflecting land use history of the area. Nevertheless, their abundance will decrease as summer drought does often not allow to finish their life cycle (they flower in early summer). Ruderal and meadow species should also rapidly disappear after several flooding periods. The Mediterranean hydrology should play a major role as a filter to eliminate species that are not adapted to summer drought (ricefield weeds) or to winter flooding (ruderals and terrestrials species).

Five months after the creation of the mesocosms, most plant species established from the seed bank of the control mesocosms. Aquatic plants may establish from the seed bank (Leck, 2003; De Steven *et al.*, 2006) but also by seed deposition through water dispersal (Mitsch *et al.*, 1998), waterbirds (Brochet *et al.*, 2010; Figuerola and Green, 2002; Figuerola *et al.*, 2002), vertebrates (Zedler and Black, 1992), or wind dispersal (Reinartz and Warne, 1993). However, these mechanisms of colonization are not efficient, because dispersal is slow and sometimes unlikely (Moreno-Mateos and Comin, 2010). In our study, no target species were found in the control mesocosms. The time span is too short to evaluate the potential of natural colonization from the external seed pool. Although rice cultivation dominates the surrounding landscape, natural temporary wetlands still occur within distance of less than 1 km and dispersal by waterbirds, a major dispersal vector (Brochet *et al.*, 2010), seems to be possible. However, the size of our mesocosms may have been too small to be attractive for waterbirds. This weak attractiveness will be overcome when the 35 hectare wetland will be restored, as it will be more attractive for waterbirds (Pirot *et al.,* 1984); zoochorous processes will then be playing their role in the dispersion of aquatic species.

Spontaneous succession alone has been shown to allow the restoration of plant communities and active restoration is not necessary if target species occur at the site or if sites are connected to propagule sources (Dawe *et al.*, 2000). However, sites situated in agricultural landscapes or with a long history of cultivation, such as the Cassaïre area, often show a weak ability to restore passively due to the lack of target species in the seed banks and/or few target seed sources (Collinge and Ray, 2009; Galatowitsch and Valk, 1996; De Steven *et al.*, 2006). Moreover, on abandoned croplands, ruderal species may predominate, slowing down succession and preventing the establishment of target species (Prach *et al.*, 2001a). In such cases, soil transfer may be an efficient method to accelerate succession.

> *EFFECT OF SOIL TRANSFER ON THE AQUATIC PLANT COMMUNITY*

Soil transfer increased the total species richness and allowed the establishment of all the aquatic target species. The plant composition of the transfer mesocosms was close to that of the donor sites. The differences in soil nutrients between the donor sites and the transfer mesocosms did not prevent germination of the aquatic species that were transferred with the soil in the mesocosms.

In grassland ecosystems, Jaunatre *et al.* (2012) showed an increase in the non-target species after soil transfer compared with the reference grassland, resulting from soil disturbance induced by the transfer itself. Bullock (1998), also working in grassland ecosystems restoration, showed that the transfer led to communities with species that are very different from those of target communities. Highly selective stress conditions leading to very predictable successional trajectories (Mesléard *et al.*, 1999) may explain the observed general success of soil transfer in wetlands as well as the promising results of the present study in contrast to the observed response of terrestrial communities in which selection is less strong. In aquatic ecosystems, the selection of plant communities by the water filter is important, only aquatic species can survive in the environment. This filter has an important selective effect, especially in our case, where the water regime is temporary, increasing selection pressure (the species should be aquatic and tolerated stages of drought, *i.e.* be annual).

> *OTHER BENEFITS OF SOIL TRANSFER*

In addition to target species introduction, soil transfer seems to significantly reduce the establishment of undesired species emerging from the seed bank and from the surroundings, such as ricefield weeds. The increase in the cover of aquatic vegetation seems to prevent the germination and growth of these weeds. The initial species composition of the restored vegetation potentially affects the vegetation for a long time (Vécrin *et al.*, 2002) and non-desirable species installed at the beginning can persist, hampering succession and/or changing the vegetation trajectory (Prach and Pysek, 2001; Prach *et al.*, 2001a, 2001; De Steven *et al.*, 2006). Soil transfer may also reduce stochasticity, by immediately installing a stable community (Weiher and Keddy, 1995). Collinge and Ray (2009) and Reinartz and Warne (1993) have shown that wetlands that initially received more native seeds were less prone to colonization by exotic species, and that the early introduction of native wetland species may increase the long-term diversity of communities in created wetlands. Indeed, in our case, the ricefield weeds can compete with our target hydrophyte species. Although they are less adapted to the temporary wetland and tend to disappear over time, a certain plasticity of their phenology allowed their presence in the mesocosms and can thus compromise succession and prevent the natural reestablishment of target species. Soil transfer appears to be an appropriate method to accelerate succession towards the desired community and to attempt to bypass some of blocked stages of succession (Collinge and Ray, 2009; McKinstry and Anderson, 2005; Reinartz and Warne, 1993).

Soil transfer provides an advantage for rare species showing dispersal limitation. Indeed, some studies have shown that passive methods may not allow the full restoration of the reference species composition (Collinge and Ray, 2009; De Steven *et al.*, 2006).

In using soil transferred from several donor sites, we increase the number of target hydrophyte species at one site compared with a single donor site. We further increase the pool of available species, allowing the selection of the most appropriate species to the specific abiotic conditions of the Cassaïre site, and thereby increasing the probability of success (Zedler, 2000). Soil transfer also provides a soil seed bank ensuring survival in fluctuating environments. This dormant reservoir is a powerful mechanism for maintaining species diversity by promoting the coexistence of a greater number of species.

In addition, soil transfer allows i) the preservation of biotic interactions by transferring soil microorganisms (Bullock, 1998) which play an important role in structuring plant community (Moora and Zobel, 2009) and in improving substrate conditions (McKinstry and Anderson, 2005) and ii) the potential transfer of zooplankton and macroinvertebrate egg bank. Brady and coauthors (2002) demonstrated that soil transfer leads to a more natural invertebrate community structure, and can be a significant benefit for non-aerial invertebrates, which are not able to disperse alone, such as crustaceans (*Cladocera* and *Triops*).

> *RESTORATION PERSPECTIVES AND THE IMPORTANCE OF TIME AND MONITORING*

The positive results obtained in the mesocosms after only few months and the low technical effort demonstrate that soil transfer is a promising restoration method that may also be applied at larger scales (*i.e.* creating 35 hectares of Mediterranean temporary wetlands in our site). Indeed, unlike terrestrial ecosystems where soil transfer involves the destruction of the donor ecosystem (Jaunatre *et al.*, 2012; Vécrin and Muller, 2003) and cannot be a substitute for *in situ* conservation (McLean, 2003), the soil transfer technique used in our study appears a non-destructive method at the scale of the donor wetlands. Because the seed bank of the first few centimeters of the soil in temporary wetlands is rich in seed number and species diversity (Bonis and Grillas, 2002), only 8 m^2 of soil were collected in our case at the donor sites (corresponding approximately to less 0.001% of the total area of the donor sites) and were spread for half a day over 32 m^2 (corresponding to 200 L of soil) in the mesocosms. The low quantities of soil required allow an application of the method at a scale of several hectares. Moreover, instead of spreading the soil over the whole area, soil may be transferred in small patches, functioning as species-rich sources for spontaneous colonization of nearby areas not transferred.

Short-term observations are not sufficient in predicting community dynamics (Collinge and Ray, 2009; Mesléard *et al.*, 1991; Weiher *et al.*, 1996). An initial success may be compromised by long-term mortality, undesired successional trajectories, and does not reflect long-term success (Dawe *et al.*, 2000; Fahselt, 2007), although long-term studies confirm a beneficial role of soil transfer in wetland restoration (Balcombe *et al.*, 2005; Nishihiro *et al.*, 2006; Reinartz and Warne, 1993). Long-term monitoring of changes in plant communities of restored wetlands is required to evaluate the potential of this technique for restoring or creating Mediterranean temporary wetlands.

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APPENDIX

Table I
Species occurring in mesocosms, in donor sites and target hydrophyte species.

Species recorded in mesocosms	Species present in donor sites	Aquatic target species
Atriplex hastata	×	
Bolboschoenus maritimus	×	
Callitriche sp.	×	×
Callitriche truncata	×	×
Carex divisa		
Chara aspera	×	×
Chara canescens	×	×
Chara globularis	×	×
Chara hispida	×	
Chara vulgaris	×	
Cladophora vagabunda	×	
Crypsis aculeata	×	
Juncus maritimus	×	
Lindernia dubia		
Phragmites australis	×	
Poa trivialis		
Potamogeton pectinatus	×	
Ranunculus peltatus	×	×
Ranunculus trichophyllus	×	×
Rumex crispus		
Scirpus littoralis	×	
Sonchus oleraceus		
Spirogyra sp.		
Tolypella glomerata	×	×
Tolypella hispanica	×	×
Typha laxmanii		
Zannichellia obtusifolia	×	×
Zannichellia pedicellata	×	×