

# Soil recovery after removal of the N<sub>2</sub>-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration

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**Abstract** Invasion by *Acacia longifolia* alters soil characteristics and processes. The present study was conducted to determine if the changes in soil C and N pools and processes induced by *A. longifolia* persist after its removal, at the São Jacinto Dunes Nature Reserve (Portugal). Some areas had been invaded for a long time (>20 years) and others more recently (<10 years). For each type of invasion, (i.e., long-invaded and recently invaded), three treatments were used: (1) *A. longifolia* left intact; (2) *A. longifolia* was removed; and (3) both *A. longifolia* and litter layer were removed. Soil samples were collected once a year for four and half years and analysed for chemical and microbial properties. In general, microbial parameters responded faster than C and N pools. In long-invaded areas, two and half years after removal of plants and litter, basal respiration and microbial biomass had already decreased >30%,  $\beta$ -glucosaminidase activity (N mineralization index) >60% and potential nitrification >95%. Removal of plants and litter resulted in a >35% decrease in C and

N content after four and half years. In recently invaded areas,  $\beta$ -glucosaminidase activity and potential nitrification showed a marked decrease (>54% and >95%, respectively) after removal of both *A. longifolia* and litter. Our results suggest that after removal of an N<sub>2</sub>-fixing invasive tree that changes ecosystem-level processes, it takes several years before soil nutrients and processes return to pre-invasion levels, but this legacy slowly diminish, suggesting that the susceptibility of native areas to (re)invasion is a function of the time elapsed since removal. Removal of the N-rich litter layer facilitates ecosystem recovery.

**Keywords** Ecosystem processes · Microbial activity and biomass · C and N-cycling · Potential nitrification · Soil ecology ·  $\beta$ -glucosaminidase activity

## Abbreviations

- LI Areas long-invaded by *A. longifolia* (>20 years)  
LIA Areas long-invaded where *A. longifolia* was left intact  
LIAR Areas long-invaded where only *A. longifolia* was removed  
LIALR Areas long-invaded where both *A. longifolia* and the litter layer were removed  
RI Areas recently invaded by *A. longifolia* (<10 years)

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RIA	Areas recently invaded where <i>A. longifolia</i> was left intact
RIAR	Areas recently invaded where only <i>A. longifolia</i> was removed
RIALR	Areas recently invaded where both <i>A. longifolia</i> and the litter layer were removed

## Introduction

It is oftentimes assumed that the ecological impacts of an invasive species will diminish immediately after its control or eradication (Wittenberg and Cock 2005), but this is not always the case. When an invasive plant has caused impacts at the ecosystem level, for example leaving a legacy of altered soil processes and pools, removal of the species may not be sufficient to allow the ecosystem to revert to its original state for many years. This “legacy effect” has been referred to in the literature (Corbin and D’Antonio 2004; D’Antonio and Meyerson 2002; Hawkes et al. 2005; Heneghan et al. 2006), and is considered an important factor for invasion success (Blumenthal 2005; Davis and Pelsor 2001) which may hamper restoration of native ecosystems (Gordon 1998; Hobbs and Humphries 1995; Prober et al. 2005). The duration of invasion may be important for the extent of invader impacts on ecosystems, and as such, a long-term perspective should be considered when studying impacts of invasive species (Strayer et al. 2006). However, long-term impacts of invaders are only rarely measured and studies experimentally demonstrating whether changes promoted at soil level prevail after removal of the invader are scarce. If this legacy does persist in soil, it can potentially create obstacles for restoration by: (1) facilitating re-invasion by the same or other alien species (Maron and Connors 1996; Pickart et al. 1998; Vinton and Burke 1995; Vinton and Goergen 2006); or (2) preventing recovery of native plants (Maron and Jefferies 1999).

Mediterranean regions, including Portugal, are particularly degraded by invasions of exotic plant species. Portuguese coastal areas have been extensively invaded by several species of *Acacia* (*Leguminosae*), namely *A. longifolia* (Andrews) Willd. and *A. saligna* (Labill) H.L.Wendl., which were initially introduced to stabilise dunes and curb

sand erosion (Alves et al. 1998; Marchante et al. 2003). Conservation and restoration of coastal ecosystems are fundamental since they are important barriers against the advance of the ocean. The dynamic of dune ecosystems relies on their high diversity of native plants, especially adapted to bind the sand without preventing its characteristic mobility, and minimizing the effects of erosion (van der Putten and Peters 1995). Invasion of Portuguese coastal dunes by *A. longifolia* has resulted in the development of woodlands dominated by this exotic species accompanied by a decrease in native plant diversity (Marchante 2001; Marchante et al. 2003), and consequently preventing the dune natural dynamic.

In Portuguese coastal systems, soil of areas invaded by *A. longifolia* for a long time (>20 years) has been more altered than soil of areas invaded for a shorter time (<10 years). The high amount of litter produced by *A. longifolia* led to changes of soil microbial processes in both invaded areas while C and N pools increased only after a long time of invasion (Marchante et al. 2007, 2008). Leaf litter is thought to be one of the main causes of altered inputs to soil by *A. longifolia*, because litter is a major source of soil organic matter, which strongly influences soil pools and microbial processes (Sayer 2006). The impacts at soil level suggest that *A. longifolia* may leave a legacy in the soil that persists after its removal and hamper restoration of native communities. In order to explore whether the changes induced by *A. longifolia* remain after its removal, we removed *A. longifolia* from invaded areas and examined the changes in soil properties during four and half years. Additionally, we wanted to determine the effect of the removal of the litter layer and to evaluate whether the legacy of *A. longifolia* on soil was influenced by the duration of the invasion. We hypothesized that high levels of soil C and N pools and microbial activity would remain several years after the removal of *A. longifolia* but that the removal of the litter layer would facilitate the decrease of these soil properties. Soil C and N pools are large relative to the active fractions and slow to change, whereas microbial activity levels may be more sensitive to the quality and quantity of inputs provided by the plant community. Thus, we expected that after removal of the invader soil C and N pools would remain unchanged for longer time than microbial activity. Because soil of areas invaded for shorter time was less altered than soil of long-invaded areas,

we predicted that the previous areas would recover faster.

## Materials and methods

### Site description

The experimental area is located in the São Jacinto Dunes Nature Reserve, at the central-northern coast of Portugal (40° 39' N, 8° 44' W). The area of the Reserve is approx. 660 ha. The climate is Mediterranean with an Atlantic influence. Mean annual precipitation is 920 mm and mean monthly temperatures range from 10.2°C in January to 20.2°C in June. In the experimental area, native vegetation is characterized by small shrubs, herbs and few trees, including some non-dominating N<sub>2</sub>-fixing species: *Cytisus striatus* (Hill) Rothm.D.Don, *Stauracanthus genistoides* (Brot.) Samp. subsp. *genistoides*, *Ulex europaeus* L. subsp. *europaeus* and *Myrica faya* Aiton. Approximately 2/3 of the reserve has been invaded by the N<sub>2</sub>-fixing *A. longifolia* and, to a lesser extent, by *Carpobrotus edulis* (L.) N.E.Br. (iceplant) and *Cortaderia selloana* (Schultes) Asch. & Graebner (pampas grass).

### Experimental design

*Acacia longifolia* was introduced into some parts of the reserve at the beginning of the 20th century. Although control has been attempted, reinvasion occurred and some areas have been densely vegetated by *A. longifolia* for more than 20 years. We classified these areas as long-invaded (LI). Following a severe fire in the Reserve in 1995, *A. longifolia* appeared and formed dense thickets in an area of the reserve that previously had very few, widely scattered plants of this species. We classified these areas as recently invaded (RI); at the time we began our study (2002), this area had been invaded for less than 10 years. A non-invaded (NI) area was selected as a reference for native systems, but no treatments (see next paragraph) were applied. Apart from the fire in 1995, there is no recent register of fire. Long-invaded and non-invaded areas have not burned at least in the last 50 years. The sites were comparable because they were located in areas with similar altitudes and distances from the ocean, and were originally covered

by the same dune vegetation. The sites were at least 100 m inland of the primary dune system in a zone where sediments are stable and sand mobility is low. Soil is sandy with 95% of the particles retained by a 0.18 mm mesh.

In December 2002, in each of the two invaded areas (LI and RI), a complete randomized block design was used to define five blocks, each consisting of three 10 × 10 m plots. One of three treatments was randomly assigned to one plot in each block. The treatments were: *A. longifolia* not removed (A); *A. longifolia* felled (with chainsaws at ground level) and removed but leaving the litter layer in place (AR); and *A. longifolia* felled and removed along with the litter layer (ALR). Felled trees were hand removed and litter layer was removed with rakes, trying to minimize soil disturbance as far as possible. A treatment leaving *A. longifolia* standing intact and only removing the litter layer was not included because this study is part of a larger project where treatments were planned with the perspective of control of the invasive and as such removal of litter layer alone was not considered.

### Soil samples

In invaded areas, three soil samples were collected in each of the plots (15 samples per treatment), in January 2003, April 2004, April 2005, May 2006 and May 2007. In non-invaded areas, samples were collected in April 2004 and April 2005, from 3 replicate plots ( $n = 9$ ). Each sample consisted of two sub-samples (collected 1–2 m apart) taken to a depth of 10 cm with a coring device of 8 cm diameter. The litter layer was excluded. Samples were passed through a 4 mm sieve to remove coarse roots and organic debris and soil was kept at 4°C until microbial analyses. Mineral N and water content were analysed on fresh soil while all the other analyses were made on air-dried soil. All samples were analysed for chemical composition. Microbial processes were analysed in 2004 and 2005 only.

### Soil analysis

#### *Chemical analysis*

Soils were analysed for organic C (Tinsley method, adapted by Silva 1977), Kjeldahl N (Bremner 1965),

nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) (extracted with 0.1 M  $\text{CaCl}_2$  and analyzed with autoanalyzer), and soil gravimetric water content (oven dried at 105°C for 48 h).

#### Basal respiration and microbial biomass C

Soil basal respiration (BR) was used as a measure of overall soil microbial activity. Soil microbial biomass C (Cmic) via substrate-induced respiration (SIR) was determined to provide a measure of the total, physiologically active part of the microflora (Anderson and Domsch 1978). For SIR, 1 g field moist soil samples were placed in 20 ml serum bottles to which 50  $\mu\text{l}$  of water was added. The samples were kept overnight for acclimation. The following day, 2 mg glucose  $\text{g}^{-1}$  field moist soil (50  $\mu\text{l}$ ) was added to the samples. Water and glucose solution were added to achieve 60% of water holding capacity. Bottles were capped airtight and incubated at room temperature. After 4 h, 0.5 ml gas from the headspace was sampled with a syringe and  $\text{CO}_2$  was measured in a gas chromatograph equipped with TC detector (TCD 180°C, carrier gas He, column GS-CPL0T, oven 90°C, average velocity: 100  $\text{cm s}^{-1}$ ). BR was measured in the same way as SIR but water was added instead of glucose solution and  $\text{CO}_2$  measured after 24 h incubation.

The microbial  $\text{qCO}_2$  was determined dividing BR ( $\mu\text{g CO}_2\text{-C g}^{-1}$  dry soil  $\text{h}^{-1}$ ) by Cmic ( $\mu\text{g Cmic g}^{-1}$  dry soil)  $\times 10^3$ . Cmic:Corg ratio was also calculated, where Cmic is microbial biomass C and Corg is organic C in the soil.

#### $\beta$ -glucosaminidase activity

4-MUF *N*-acetyl- $\beta$ -D-glucosaminide (Sigma Chemical Co.) was used as substrate to quantify *N*-acetyl- $\beta$ -D-glucosaminidase (EC 3.2.1.30, hereafter  $\beta$ -glucosaminidase).  $\beta$ -glucosaminidase is a chitinase involved in chitin degradation, which has been used as an index of N mineralization (Ekenler and Tabatabai 2004). Ekenler and Tabatabai (2004) studied N mineralization (using different biological and chemical methods) and  $\beta$ -glucosaminidase activity, in a range of soils with distinct properties, and found significant correlations between the two activities. The protocol used to quantify  $\beta$ -glucosaminidase was described by Miller et al. (1998) and later modified by Andersson et al. (2004).

#### Potential nitrification

Ten grams of field moist soil was added to 100 ml nutrient solution (5 mM NaCl, 1 mM  $\text{KH}_2\text{PO}_4$ , 1 mM  $\text{MgSO}_4 \cdot 7$  and 1  $\text{g l}^{-1}$   $\text{CaCO}_3$ , pH 7.2), supplied with 5 mM  $(\text{NH}_4)_2\text{SO}_4$  and incubated at 25°C (adapted from Aaronson 1970), with continuous shaking, at 150 rpm. After 14 days, sub-samples were collected and extracted for  $\text{NO}_3^-$  with 1 M KCl, centrifuged for 15 min at 3,500  $\text{rev min}^{-1}$  and the supernatant filtered through N-free filter.  $\text{NO}_3^-$  was measured on Aquatec equipment (measured nitrite ( $\text{NO}_2^-$ ) and  $\text{NO}_3^- + \text{NO}_2^-$ , from which  $\text{NO}_3^-$  was calculated). Potential nitrification was calculated as  $\mu\text{g NO}_3\text{-N g}^{-1}$  dry soil  $\text{day}^{-1}$ .

Both microbial and chemical results were expressed on a dry soil basis.

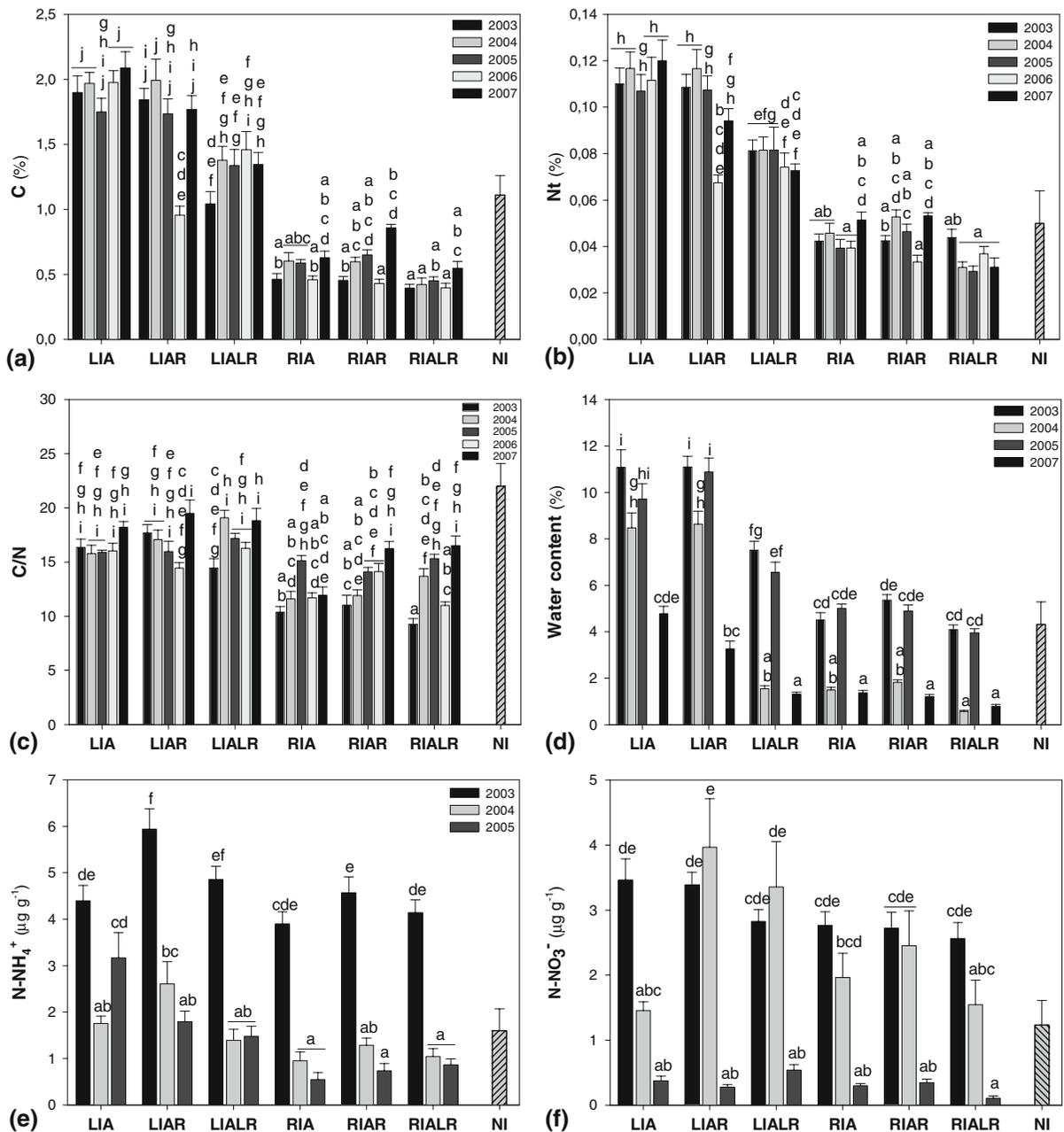
#### Statistical analysis

The results from the two areas—recent invasion and long invasion by *A. longifolia*—were analysed together. The non-invaded areas were used only for reference, not included in the statistical analysis. To analyse the recovery of the different parameters over time, and the interaction between duration of invasion and recovery, a repeated measure ANOVA, with within-subjects factors, was performed. The factors considered were “duration of invasion”, “treatment” and “sampling year”. Mean differences were compared with Tukey’s test at 5% level of significance. STATISTICA 6.0 (StatSoft, Inc., 2001, <http://www.statsoft.com>) was used for the statistical analysis.

## Results

#### Chemical parameters

Total N (Kjeldahl) and organic C pools were lower in recently invaded areas (RI) than in long-invaded ones (LI) (Fig. 1a and b). In long-invaded areas, removal of *A. longifolia* alone (LIAR) resulted in a significant decrease in organic C and N after three and a half years, but these pools increased again the following year. Where both *A. longifolia* and litter were removed (LIALR), organic C and N were generally lower, approx. 65% of the content in soils with



**Fig. 1** (a) Organic C, (b) total N, (c) C/N ratio, (d) water content, (e) ammonium, and (f) nitrate, in areas invaded by *A. longifolia*, in the upper 10 cm of soil, in the São Jacinto Dunes Nature Reserve. Bars are means + SE ( $n = 15$ ). Bars

with the same letter are not significantly different (Tukey test,  $P < 0.05$ ). LI, stands for areas invaded for a >20 years; RI, for areas invaded <10 years; A, with *A. longifolia*; AR, *A. longifolia* removed; ALR, *A. longifolia* and litter removed

*A. longifolia* by the end of the study period. In recently invaded areas, organic C and N showed some tendency to be lower in areas where both *A. longifolia* and litter were removed (RIARL), but

this trend was rarely significant. The C/N ratio was generally lower in recently than in long-invaded areas (Fig. 1c). In both invaded areas, this ratio did not change significantly with the treatments.

Soil water content was lower in recently invaded (RI) than in long-invaded areas (LI) (Fig. 1d) and higher in 2003 and 2005 than in 2004 and 2007. In long-invaded areas, soil water content was lowest when both *A. longifolia* and litter were removed. In recently invaded areas, there was a similar tendency, but the difference was not significant.

In general, the  $\text{NH}_4^+$  pool showed no significant differences in both invaded areas (Fig. 1e). Ammonium was higher in 2003 than in 2004 and 2005 in all treatments. In long-invaded areas, in 2005,  $\text{NH}_4^+$  pool was higher in areas with *A. longifolia* than in the other two treatments. Generally, there were no significant differences between the  $\text{NO}_3^-$  pool in recently and in long-invaded areas (Fig. 1f). In both invaded areas,  $\text{NO}_3^-$  concentrations were lower in 2005 than in 2003 and 2004. In long-invaded areas, in 2004, the  $\text{NO}_3^-$  pool was lower in areas with *A. longifolia* than in the plots where plants had been removed.

#### Microbial parameters

Generally, basal respiration and microbial biomass C were lower in recently invaded than in long-invaded areas (Fig. 2a and b). In long-invaded areas, basal respiration decreased by approximately 25% after removal of *A. longifolia* alone (LIAR) and after removal of both *A. longifolia* and litter (LIALR). In recently invaded areas, there were no differences in basal respiration between treatments, except for RIAR being higher than RIA in 2005. Microbial biomass C was not affected by the removal of *A. longifolia* alone, but in long-invaded areas there was a decrease both years when litter was also removed (LIALR). In areas where both *A. longifolia* and litter were removed (LIALR and RIALR), microbial biomass C was lower in 2004 than in 2005. Cmic:Corg ratio was higher in recently invaded areas than in long-invaded (Fig. 2c). This ratio was not affected by any treatment in long-invaded areas. On the other hand, in areas invaded for shorter time, Cmic:Corg ratio was higher where litter was removed (RIALR) than in the treatments where litter remained (RIA and RIAR). When *A. longifolia* alone was removed, the  $\text{qCO}_2$  was higher in recently than in long-invaded areas (Fig. 1d). In 2004,  $\text{qCO}_2$  was lower in LIAR than in LIA and LIALR while in 2005 there was no difference between the treatments. In

recently invaded areas, on the contrary, 2004 showed similar  $\text{qCO}_2$  values in the three treatments, while in 2005 RIAR was higher than RIA and RIALR.

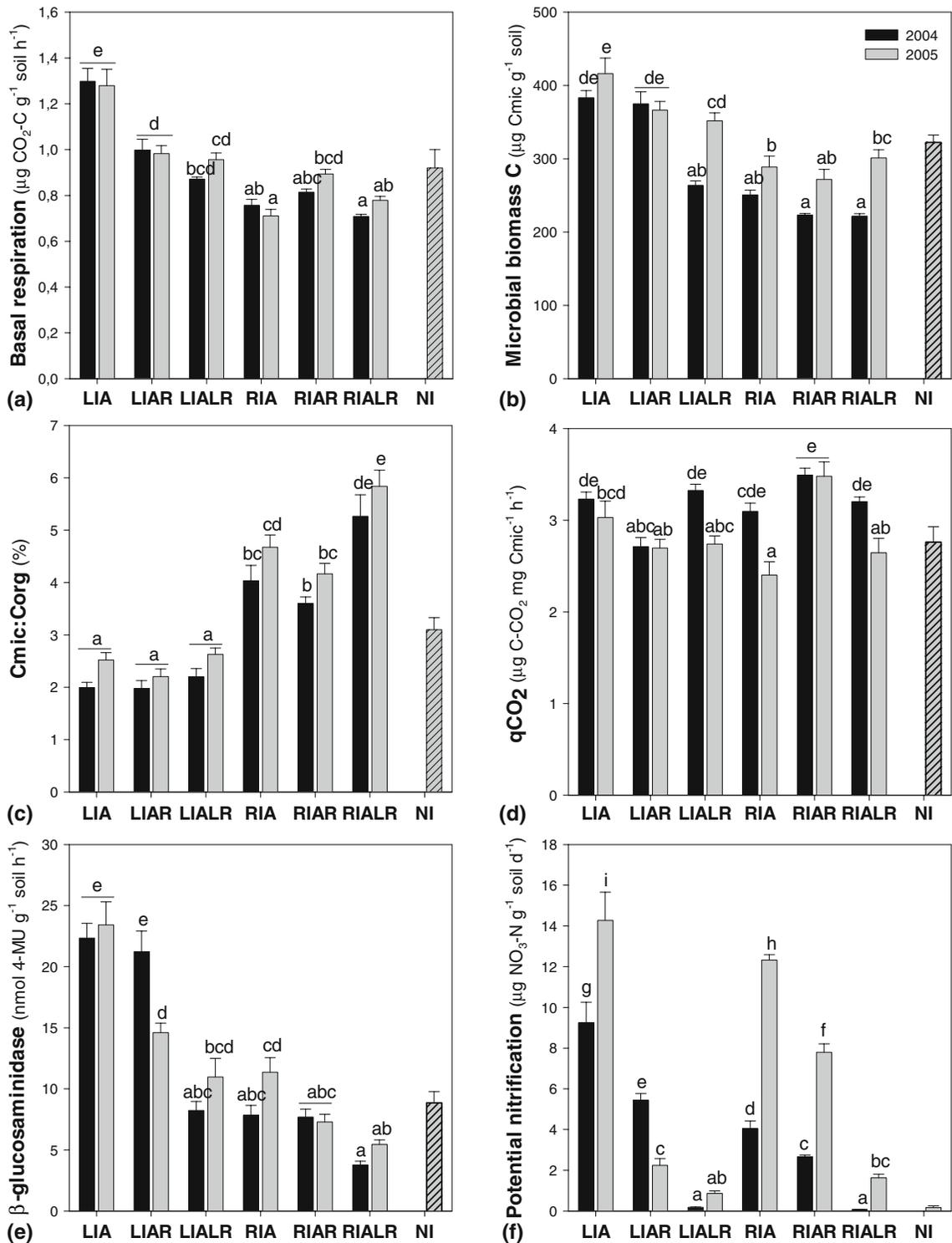
$\beta$ -glucosaminidase activity was 2–3 times higher in long-invaded than in recently invaded soil (Fig. 2e). In long-invaded areas, removal of *A. longifolia* alone promoted a decrease in  $\beta$ -glucosaminidase activity after two and half years (2005 sampling) while after removal of both *A. longifolia* and litter the activity of this enzyme was lower both years. In recently invaded areas, the trend was similar to the one observed in long-invaded areas, but the differences were significant only between RIA and RIALR in 2005.

In areas where *A. longifolia* is still present (LIA and RIA), potential nitrification was lower in recently invaded soil than in long-invaded (Fig. 2f). In general, this parameter was higher in 2005 than in 2004. In both invaded areas, potential nitrification was significantly lower in areas where *A. longifolia* was removed (LIAR and RIAR), and even lower where also litter was removed (LIALR and RIALR), than in untreated areas (LIA and RIA). After removal of both *A. longifolia* and litter, the decrease in potential nitrification activity was as high as 68 and 45 times in long and recently invaded areas, respectively, in 2004.

#### Discussion

##### Recovery of soil properties in areas invaded by *A. longifolia* after short and long time

Previous work in the Portuguese coastal dunes showed that areas invaded for a long time (>20 years) by *A. longifolia* accumulated more N-rich litter and had higher soil C and N pools and microbial activity than non-invaded areas while after recent invasion (<10 years) only soil N fluxes increased significantly (Marchante et al. 2007, 2008). Our results in the present study show that four and half years after removal of *A. longifolia* the C and N pools were still much higher in areas invaded >20 years than in native areas. The high C and N pools slowly decreased, and recovery was accelerated by removal of litter. Microbial parameters, particularly the ones more related to N-cycling (nitrification and  $\beta$ -glucosaminidase activity), decreased faster than the chemical pools. In areas invaded <10 years,



**Fig. 2** (a) Basal respiration, (b) microbial biomass C, (c) Cmic:Corg ratio, (d)  $q\text{CO}_2$ , (e)  $\beta$ -glucosaminidase activity, and (f) potential nitrification, in areas invaded by *A. longifolia*, in the upper 10 cm of soil, in the São Jacinto Dunes Nature

Reserve. Bars are means + SE ( $n = 15$ ). Bars with the same letter are not significantly different (Tukey test,  $P < 0.05$ ). Abbreviations as for Fig. 1

potential nitrification and Cmic:Corg were still higher than in native areas two and half years after removal of litter and/or *A. longifolia*, showing that despite the impacts of invasion are less pronounced in these areas the legacy of altered soil also remains for several years. This happens even though in native areas several N<sub>2</sub>-fixing shrubs or trees are present (see site description), stressing the effects of *A. longifolia* on N-cycling.

Persistence of soil changes caused by invasive species has previously been observed in areas cleared of N<sub>2</sub>-fixing invaders: N mineralization rate in fynbos invaded by *A. saligna* (Yelenik et al. 2004) or in coastal prairies invaded by *Lupinus arboreus* (Maron and Jefferies 1999, 2001) were not different from stands where the invaders had been removed. Effects of such species, which alter both soil N pools and fluxes, are expected to remain long after removal as a result of the slow release of available N from the large soil organic N pool (Yelenik et al. 2007), and because increasingly recalcitrant N fractions dominate turnover dynamics (Maron and Jefferies 1999). Since C and N pools increased after *A. longifolia* invasion for >20 years (Marchante et al. 2008), these pools and the high microbial activities were expected to remain long after removal of the trees. When the litter layer was removed, the input of organic matter to the soil diminished and nutrients may leach, further explaining the faster reduction of C and N pools. Based on the rate of turnover of the highly labile fraction of N, Maron and Jefferies (1999) predicted that it would take at least 25 years for the N pool to decrease by 50% after *Lupinus arboreus* was removed from a coastal prairie. When *A. longifolia* and litter were removed, N pool decreased >35% after four and half years, suggesting a faster decrease than in *L. arboreus* invaded areas. However, the remaining pool is probably more recalcitrant and consequently decrease slower (Maron and Jefferies 1999), indicating that the high soil C and N pool in areas invaded for >20 years will last for many years. This may be confirmed by the faster decrease in microbial activities, showing that the availability of labile substrates, easily used by the microbial communities, is decreasing.

Corbin and D'Antonio (2004) hypothesized that soils changed by invasive species that influence only soil N fluxes, might be more easily reverted to background levels. In this study, N related processes

(potential nitrification and  $\beta$ -glucosaminidase activity, as a measure of N mineralization) decreased after trees and litter have been removed, suggesting that N fluxes were approaching the background levels, even in long-invaded areas where also N pools had increased. Nevertheless, after removal of *A. longifolia* alone these activities only decreased after two and half years. In areas invaded after 1995, basal respiration and microbial biomass C remained similar after removal of the invader, probably because these parameters had not been changed by the invasion. However, litter removal represents a decline in fresh organic matter inputs, and this could be expected to affect microbial communities and decrease soil respiration after some years (Sayer 2006 and reference therein). In both invaded areas, where trees and litter were removed, a higher microbial biomass C was observed in 2005 compared to 2004 possibly because new vegetation covers the plots and therefore increases inputs to the soil. The corresponding decrease in qCO<sub>2</sub>, however, indicated that the efficiency of microbial biomass was lower. Additionally, results were shown on a dry soil basis and consequently increases in microbial biomass C (like basal respiration and  $\beta$ -glucosaminidase activity) may be related to lower organic C in areas cleared of trees and litter.

We must consider that although we have carefully applied the treatments, soil disturbance during felling and litter removal may have stimulated microbial activity, especially the N processes. If so, this was a temporary effect since one and half years later, both nitrification and  $\beta$ -glucosaminidase activity were either similar or lower than in areas where *A. longifolia* persisted.

The legacy of higher C and N pools and microbial activities that remains after removal of *A. longifolia* may have consequences for the restoration of native communities and therefore should be taken into account when planning a management strategy.

#### Implications for restoration of native plant communities

The consequences of altered N cycling may be especially important after invader death or removal, when shade or allelopathy do not influence the native species anymore (Levine et al. 2003). Since increased C and N pools persist for more than four and half years

after removal of *A. longifolia*, the restoration of native communities may be hampered for a long period. First, *A. longifolia* may ameliorate the conditions for its own growth. Although not well confirmed, this kind of feedback is probably important in facilitating invasive species (Corbin and D'Antonio 2004; Ehrenfeld 2003; Vinton and Goergen 2006). *Acacia longifolia* was grown in soils collected under different plant species and grew better in its own soil (higher C and N pools) than in soils collected under other N<sub>2</sub>-fixing species (Echeverría et al. 2008). N<sub>2</sub>-fixing species are able to colonize N poor environments, but they may be favoured by higher N availability. When planted with increasing soil N, *A. longifolia* growth was enhanced by higher concentrations of N (Peperkorn et al. 2005). The prevalence of larger N pools may also favour other fast growing exotic species. Particularly in nutrient-poor ecosystems, nutrient enrichment has been shown to favour invasion by exotic plants, (Maron and Connors 1996; Pickart et al. 1998; Vinton and Burke 1995; Vinton and Goergen 2006; Yelenik et al. 2004), although higher nutrient availability not always favour exotics (Gross et al. 2005). In our study site, the vegetation recovering after removal of *A. longifolia* included several generalist, nitrophilic and exotic/invasive plants (Marchante et al. 2004). Finally, nutrient enrichment in N-poor soils may prevent establishment of native species (Maron and Jefferies 1999). However, the reduction in soil N and organic matter is not a prerequisite for the restoration of dunes invaded by N<sub>2</sub>-fixing species (Pickart et al. 1998). In fact, although N is a limiting resource for dune plants, it is the increase of N that foster dune succession (Olf et al. 1993) and some dune plants react positively to N addition (van den Berg et al. 2005).

Despite all these implications, soil properties slowly recover to values closer to the native areas, suggesting that with time the vulnerability to (re)invasion will decrease, and recovery of native plant communities' increase.

#### Contributions for management

Recently invaded areas should be controlled prior to long-invaded, before further ecosystem level changes take place. When *A. longifolia* invasion has increased C and N pools, these pools remain for a long time, and as discussed above this may complicate

restoration. Therefore, invaded areas should be controlled before C and N pools increase. In recently invaded areas, potential nitrification increased after invasion (Marchante et al. 2008) but recovered to levels closer to the native areas after one and half years (when both *A. longifolia* and litter were removed), suggesting that native communities are easier to restore. Our results confirmed that removal of litter, although time-consuming, should be considered as a suitable approach to diminish C and N pools. Even though it may eliminate seeds (Allison and Ausden 2006) of both *A. longifolia* and native species, this practice has been shown to reduce non-native weeds, favour native plants and restore soil nutrient pools (Coleman and Levine 2007; Mitchell et al. 1999; Pickart et al. 1998). Furthermore, deep litter layers (sometimes >10 cm thick), serve as a physical barrier that diminish light availability and may prevent seed germination and seedling growth.

Since higher C and N pools prevail long after *A. longifolia* removal, restoration of the site may depend on restoring soil nutrient balance. The reduction in soil N may prevent fast growing exotic species and ultimately favour re-establishment of native species (Hulme 2006; Kulmatiski et al. 2006; Maron and Jefferies 2001; Perry et al. 2004). Controlled burns are sometimes used to reduce C and N pools (Haubensak et al. 2004), but it may not be efficient in decreasing available N in the short term (Prober et al. 2005). In our study site, the fire that occurred in 1995 may have reduced the C pool and possibly the N pool to a lower extent (Marchante et al. 2008), but as the germination of *A. longifolia* seeds is fire stimulated, the fire also led to the rapid invasion of the burnt areas. The addition of a C source can also be used to reduce N availability because it reduces plant-available N by stimulating microbial uptake of N. This may result in the inhibition of fast growing nitrofilic exotic weeds and facilitation of slower-growing natives (Blumenthal et al. 2003; Prober et al. 2005); but may also inhibit specific native species (Alpert and Maron 2000; Haubensak et al. 2004).

#### Conclusions

The higher soil C and N pools lasted at least four and a half years after removal of *A. longifolia*, and may persist in soil as a long lasting legacy, while the

microbial activities persisted for a shorter time. Soil chemical and microbial properties are slowly recovering to background levels, pools slower than activities, and this recovery process is faster in areas where litter had also been removed. In long-invaded areas, the high nutrient content of the soil may hamper the recovery of dune communities for several years and promote establishment of other exotic species. The susceptibility of native areas to (re)invasion seems to be a function of the time elapsed since removal. Management practices that reduce N availability may be necessary for the restoration of invaded areas. Further studies on the recovery of vegetation are being conducted, and may lead to a better understanding of system restoration processes.

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## References

- Aaronson S (1970) Experimental microbial ecology. Academic Press, New York, p 236
- Allison M, Ausden M (2006) Effects of removing the litter and humic layers on heathland establishment following plantation removal. *Biol Conserv* 127:177–182. doi:[10.1016/j.biocon.2005.08.008](https://doi.org/10.1016/j.biocon.2005.08.008)
- Alpert P, Maron JL (2000) Carbon addition as a countermeasure against biological invasion by plants. *Biol Invasions* 2:33–40. doi:[10.1023/A:1010063611473](https://doi.org/10.1023/A:1010063611473)
- Alves JMS, Santo MDE, Costa JC, Gonçalves JHC, Lousã MF (1998) Habitats Naturais e Seminaturais de Portugal Continental. Instituto da Conservação da Natureza, Lisbon Portugal, 55 pp
- Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol Biochem* 10:215–221. doi:[10.1016/0038-0717\(78\)90099-8](https://doi.org/10.1016/0038-0717(78)90099-8)
- Andersson M, Kjølner A, Struwe S (2004) Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. *Soil Biol Biochem* 36:1527–1537. doi:[10.1016/j.soilbio.2004.07.018](https://doi.org/10.1016/j.soilbio.2004.07.018)
- Blumenthal D (2005) Interrelated causes of plant invasion. *Science* 310:243–244. doi:[10.1126/science.1114851](https://doi.org/10.1126/science.1114851)
- Blumenthal DM, Jordan NR, Russelle MP (2003) Soil carbon addition controls weeds and facilitates prairie restoration. *Ecol Appl* 13:605–615. doi:[10.1890/1051-0761\(2003\)013\[0605:SCACWA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2003)013[0605:SCACWA]2.0.CO;2)
- Bremner JM (1965) Total nitrogen. In: Black CA (ed) Methods of soil analysis. Part 2: chemical and microbiological properties. American Society of Agronomy, Inc. Publisher, Madison USA, pp 1149–1176
- Coleman HM, Levine JM (2007) Mechanisms underlying the impacts of exotic annual grasses in a coastal California meadow. *Biol Invasions* 9:65–71. doi:[10.1007/s10530-006-9008-6](https://doi.org/10.1007/s10530-006-9008-6)
- Corbin JD, D'Antonio CM (2004) Effects of exotic species on soil nitrogen cycling: implications for restoration. *Weed Technol* 18:1464–1467
- D'Antonio C, Meyerson LA (2002) Exotic plant species as problems and solutions in ecological restoration: a synthesis. *Restor Ecol* 10:703–713. doi:[10.1046/j.1526-100X.2002.01051.x](https://doi.org/10.1046/j.1526-100X.2002.01051.x)
- Davis MA, Pelsor M (2001) Experimental support for a resource-based mechanistic model of invasibility. *Ecol Lett* 4:421–428. doi:[10.1046/j.1461-0248.2001.00246.x](https://doi.org/10.1046/j.1461-0248.2001.00246.x)
- Echeverría SR, Crisóstomo JA, Nabais C, Freitas H (2008) Belowground mutualists and the invasive ability of *Acacia longifolia* in coastal dunes of Portugal. *Biol Invasions*. doi:[10.1007/s10530-008-9280-8](https://doi.org/10.1007/s10530-008-9280-8)
- Ehrenfeld JG (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems* (N Y, Print) 6:503–523. doi:[10.1007/s10021-002-0151-3](https://doi.org/10.1007/s10021-002-0151-3)
- Ekenler M, Tabatabai MA (2004)  $\beta$ -glucosaminidase activity as an index of nitrogen mineralization in soils. *Commun Soil Sci Plann* 35:1081–1094. doi:[10.1081/CSS-120030588](https://doi.org/10.1081/CSS-120030588)
- Gordon DR (1998) Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. *Ecol Appl* 8:975–989. doi:[10.1890/1051-0761\(1998\)008\[0975:EOINIP\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1998)008[0975:EOINIP]2.0.CO;2)
- Gross KL, Mittelbach GG, Reynolds HL (2005) Grassland invasibility and diversity: responses to nutrients, seed input, and disturbance. *Ecology* 86:476–486. doi:[10.1890/04-0122](https://doi.org/10.1890/04-0122)
- Haubensak KA, D'Antonio CM, Alexander J (2004) Effects of nitrogen-fixing shrubs in Washington and coastal California. *Weed Technol* 18:1475–1479
- Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecol Lett* 8:976–985. doi:[10.1111/j.1461-0248.2005.00802.x](https://doi.org/10.1111/j.1461-0248.2005.00802.x)
- Heneghan L, Fatemi F, Umek L, Grady K, Fagen K, Workman M (2006) The invasive shrub European buckthorn (*Rhamnus cathartica* L.) alters soil properties in Mid-western US woodlands. *Appl Soil Ecol* 32:142–148. doi:[10.1016/j.apsoil.2005.03.009](https://doi.org/10.1016/j.apsoil.2005.03.009)
- Hobbs RJ, Humphries SE (1995) An integrated approach to the ecology and management of plant invasions. *Conserv Biol* 9:761–770. doi:[10.1046/j.1523-1739.1995.09040761.x](https://doi.org/10.1046/j.1523-1739.1995.09040761.x)
- Hulme PE (2006) Beyond control: wider implications for the management of biological invasions. *J Appl Ecol* 43:835–847. doi:[10.1111/j.1365-2664.2006.01227.x](https://doi.org/10.1111/j.1365-2664.2006.01227.x)
- Kulmatiski A, Beard KH, Stark JM (2006) Soil history as a primary control on plant invasion in abandoned agricultural fields. *J Appl Ecol* 43:868–876. doi:[10.1111/j.1365-2664.2006.01192.x](https://doi.org/10.1111/j.1365-2664.2006.01192.x)

- Levine JM, Vila M, D'Antonio CM, Dukes JS, Grigulis K, Lavelle S (2003) Mechanisms underlying the impacts of exotic plant invasions. *P R Soc Lond B Bio* 270:775–781. doi:[10.1098/rspb.2003.2327](https://doi.org/10.1098/rspb.2003.2327)
- Marchante H (2001) Invasão dos ecossistemas dunares portugueses por *Acacia*: uma ameaça para a biodiversidade nativa. Master thesis, Faculty of Sciences and Technology, University of Coimbra, Coimbra
- Marchante H, Marchante E, Freitas H (2003) Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. In: Child LE, Brock JH, Brundu G, Prach K, Pyšek P, Wade PM, Williamson M (eds) *Plant invasion: ecological threats and management solutions*. Backhuys Publishers, Leiden, The Netherlands, pp 75–85
- Marchante HS, Marchante EM, Buscardo E, Maia J, Freitas H (2004) Recovery potential of dune ecosystems invaded by an exotic *Acacia* species (*Acacia longifolia*). *Weed Technol* 18:1427–1433
- Marchante E, Kjølner A, Struwe S, Freitas H (2007) Soil microbial activity in dune ecosystems in Portugal invaded by *Acacia longifolia*. In: Tokarska-Guzik B, Brock JH, Brundu G, Child L, Daehler CC, Pyšek P (eds) *Plant invasions: human perception, ecological impacts and management*. Backhuys Publishers, Leiden, The Netherlands, pp 247–257
- Marchante E, Kjølner A, Struwe S, Freitas H (2008) Short and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune ecosystem. *Appl Soil Ecol*. doi:[10.1016/j.apsoil.2008.04.004](https://doi.org/10.1016/j.apsoil.2008.04.004)
- Maron JL, Connors PG (1996) A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia* 105:302–312. doi:[10.1007/BF00328732](https://doi.org/10.1007/BF00328732)
- Maron JL, Jefferies RL (1999) Bush lupine mortality, altered resource availability, and alternative vegetation states. *Ecology* 80:443–454
- Maron JL, Jefferies RL (2001) Restoring enriched grasslands: Effects of mowing on species richness, productivity, and nitrogen retention. *Ecol Appl* 11:1088–1100. doi:[10.1890/1051-0761\(2001\)011\[1088:REGEOM\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2001)011[1088:REGEOM]2.0.CO;2)
- Miller M, Palojarvi A, Rangger A, Reeslev M, Kjølner A (1998) The use of fluorogenic substrates to measure fungal presence and activity in soil. *Appl Environ Microbiol* 64:613–617
- Mitchell RJ, Marrs RH, Le Duc MG, Auld MHD (1999) A study of the restoration of heathland on successional sites: changes in vegetation and soil chemical properties. *J Appl Ecol* 36:770–783. doi:[10.1046/j.1365-2664.1999.00443.x](https://doi.org/10.1046/j.1365-2664.1999.00443.x)
- Olf H, Huisman J, Van Tooren BF (1993) Species dynamics and nutrient accumulation during early primary succession in coastal sand dunes. *J Ecol* 81:693–706. doi:[10.2307/2261667](https://doi.org/10.2307/2261667)
- Peperkorn R, Werner C, Beyschlag W (2005) Phenotypic plasticity of an invasive acacia versus two native Mediterranean species. *Funct Plant Biol* 32:933–944. doi:[10.1071/FP04197](https://doi.org/10.1071/FP04197)
- Perry LG, Galatowitsch SM, Rosen CJ (2004) Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. *J Appl Ecol* 41:151–162. doi:[10.1111/j.1365-2664.2004.00871.x](https://doi.org/10.1111/j.1365-2664.2004.00871.x)
- Pickart AJ, Miller LM, Duebendorfer TE (1998) Yellow bush lupine invasion in Northern California coastal dunes I. Ecological impacts and manual restoration techniques. *Restor Ecol* 6:59–68. doi:[10.1046/j.1526-100x.1998.00618.x](https://doi.org/10.1046/j.1526-100x.1998.00618.x)
- Prober SM, Thiele KR, Lunt ID, Koen TB (2005) Restoring ecological function in temperate grassy woodlands: manipulating soil nutrients, exotic annuals and native perennial grasses through carbon supplements and spring burns. *J Appl Ecol* 42:1073–1085. doi:[10.1111/j.1365-2664.2005.01095.x](https://doi.org/10.1111/j.1365-2664.2005.01095.x)
- Sayer EJ (2006) Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Biol Rev Camb Philos Soc* 81:1–31. doi:[10.1017/S1464793105006846](https://doi.org/10.1017/S1464793105006846)
- Silva R (1977) Sector Fertilidade do Solo. Laboratório Químico-Agrícola. Ministério da Agricultura e Pescas, Lisbon
- Strayer DL, Eviner VT, Jeschke JM, Pace ML (2006) Understanding the long-term effects of species invasions. *Trends Ecol Evol* 21:645–651. doi:[10.1016/j.tree.2006.07.007](https://doi.org/10.1016/j.tree.2006.07.007)
- van den Berg LJJ, Tomassen HBM, Roelofs JGM, Bobbink R (2005) Effects of nitrogen enrichment on coastal dune grassland: a mesocosm study. *Environ Pollut* 138:77–85. doi:[10.1016/j.envpol.2005.02.024](https://doi.org/10.1016/j.envpol.2005.02.024)
- van der Putten WH, Peters BAM (1995) Possibilities for management of coastal foredunes with deteriorated stands of *Ammophila arenaria* (marram grass). *J Coast Conserv* 1:29–39
- Vinton MA, Burke IC (1995) Interactions between individual plant species and soil nutrient status in shortgrass steppe. *Ecology* 76:1116–1133. doi:[10.2307/1940920](https://doi.org/10.2307/1940920)
- Vinton MA, Goergen EM (2006) Plant-soil feedbacks contribute to the persistence of *Bromus inermis* in tallgrass prairie. *Ecosystems* (N Y, Print) 9:967–976. doi:[10.1007/s10021-005-0107-5](https://doi.org/10.1007/s10021-005-0107-5)
- Wittenberg R, Cock MJW (2005) Best practices for the prevention and management of invasive alien species. In: Mooney HA, Mack RN, McNeely JA, Neville LE, Schei PJ, Waage JK (eds) *Invasive alien species. A new synthesis*. Island Press, Washington, p 368
- Yelenik SG, Stock WD, Richardson DM (2004) Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. *Restor Ecol* 12:44–51. doi:[10.1111/j.1061-2971.2004.00289.x](https://doi.org/10.1111/j.1061-2971.2004.00289.x)
- Yelenik SG, Stock WD, Richardson DM (2007) Functional group identity does not predict invader impacts: differential effects of nitrogen-fixing exotic plants on ecosystem function. *Biol Invasions* 9:117–125. doi:[10.1007/s10530-006-0008-3](https://doi.org/10.1007/s10530-006-0008-3)