# Invasion, alien control and restoration: legacy effects linked to folivorous insects and phylopathogenic fungi.

**Running title: Lasting ecological consequences of invasion**

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## Abstract

Invasive alien trees increase native tree stress and may increase attack by herbivores and pathogenic fungi. Alien tree removal should ameliorate such impacts. Here we compared levels of damage by phylopathogenic fungi and folivorous insects on *Brabejum stellatifolium* and *Metrosideros angustifolia* (native trees) and *Acacia mearnsii* (invasive tree species) among near pristine, invaded and restored sites. Generally, foliar damage levels were higher at invaded than at near pristine sites. Damage levels at restored sites were similar, or even higher than those at invaded sites. Decreased native tree species richness did not explain these patterns, as restored sites had similar native tree species richness levels to the near pristine sites. Increased host abundance and leaf nitrogen content did not significantly correlate to increased damage in most cases. Therefore, plant species richness recovers following restoration, but native trees still experience increased pressure from folivores and phylopathogenic fungi which may even exceed levels experienced at invaded sites, thus impacting recovery trajectories.

**Keywords:** *Acacia mearnsii*, Fynbos, leaf damage, leaf nutrients, pathogen, riparian system

## Introduction

Pest and pathogen loads in plant communities vary with plant stress levels (Gilbert & Hubbell 2002), native plant species richness (Scherber et al. 2010), host abundance (Mitchell et al. 2002; Bell et al. 2006) and plant nutritional status (Forkner & Hunter 2000; Moran & Scheidler 2002). Fragmentation of tropical forests, for example, leads to increased plant stress and, in turn, increased damage by pathogenic fungi and folivores (Benítez-Malvido et al. 1999). Invasive alien plants may also increase native plant stress by altering environmental conditions such as water availability, amount of sunlight and nutrient availability (Brooks et al. 2004). As these factors are known to affect host-plant/pest (and pathogen) dynamics (White et al. 2006), we hypothesise that invasive alien plant’s would increase the susceptibility of native plants to natural enemies (folivorous insects and pathogenic fungi) in invaded systems.

In South Africa, woody invasive alien trees have been targeted for removal in riparian areas in a 15 year old landscape level restoration initiative led by the extended public works programme, Working for Water. Although this initiative is credited with the creation of thousands of jobs, a body of evidence relating to the lack of effectiveness of the Working for Water initiative for curbing the spread of invasive alien trees is emerging (van Wilgen et al. 2012). In addition, clearing of invasive alien plants results in gap formation, alters the ecosystem microenvironment (including light, moisture, and temperature regimes) and increases patchiness and habitat heterogeneity (Torchin & Mitchell 2004). As folivore and plant pathogen populations may directly depend on these factors, their impact on host plants is also likely to change in response to these altered conditions (Folgarait et al. 1995).

The influence of plant species diversity on performance of natural enemies has been a focus of several experimental studies (Pfisterer et al. 2003; Agrawal et al. 2006; van Hezewijk et al. 2008; Scherber et al. 2010). Specifically, many studies found that altered native plant diversity can alter the stability, diversity and performance of their natural enemies (Mitchell et al. 2002; Johnson et al. 2008). Two hypotheses have been proposed to explain such effects (Root 1973). The ‘resource concentration’ hypothesis predicts that folivores, especially specialist folivores, increase in abundance with increasing concentration of preferred resources in more homogenous stands of host plants as compared heterogeneous stands (Grez & González 1995). The ‘enemy’ hypothesis states that the lower folivory levels in heterogeneous communities are a result of increased effectiveness of predators and parasitoids in controlling insect pest densities (Bach 1980; Riihimäki et al. 2005).

Apart from plant species diversity and leaf nutrient levels (Peeters 2002a, 2000b) host abundance can also influence the prevalence of folivorous arthropods and phylopathogenic fungi (Agrawal et al. 2006). Hypotheses relating to host abundance and folivore attack rates generally predict increased rates as host abundance increases (Agrawal et al. 2006; Cook & Holt 2006). Some studies have however found evidence that natural enemy attack is reduced on plants in dense stands compared to plants in sparse stands (Bach 1980; Garrett et al. 2000). Changes in host abundance might influence: (i) the quality and quantity of resources (host plant individuals) available for pathogenic fungi or folivores (Bach 1980; Cook and Holt 2006), (ii) the spatial arrangement of host plants (Folgarait et al. 1995) where increased host density reduces search time and increases occupation time for some folivore species (Cook & Holt 2006) and (iii) the microclimatic conditions under which the folivore/pathogen and host interact (Folgarait et al. 1995).

How native tree species richness, host plant abundance, and leaf nutrient levels affect plant exposure to attack by pathogenic fungi and folivorous insects in a restoration scenario has received little focussed attention (Covington et al. 1997). Here, we investigate whether differences exist in levels of leaf damage caused by folivorous insects and phylopathogenic fungi on an invasive alien plant (*Acacia mearnsii* DeWild (L.)) and two native plants

(*Brabejum stellatifolium* (L.) and *Metrosideros angustifolia* (L.)) in riparian sites that differ in invasion status. Three categories were chosen; a) near pristine (few *A. mearnsii* individuals), b) heavily invaded (*A. mearnsii* dominated communities); and c) restored sites that were cleared of invasive *A. mearnsii* more than seven years ago. The roles of variation in native tree species richness, host abundance and leaf nutrients in explaining observed differences in damage levels were evaluated. These data may provide tools for evaluating restoration success in the future.

## Methods

### Study sites and focal species

The study was conducted in mountain stream and foothill sections of three riparian systems within the south-western Cape, South Africa (Fig. 1). The area is characterised by a Mediterranean-type climate with cool rainy winters (June-August) and hot dry summers (December–February) (Deacon et al. 1992). The riparian ecosystems contain some Fynbos vegetation (dominated by Ericaceae, Proteaceae and Restionaceae) (Mucina & Rutherford, 2006) and include a variety of tree taxa that form forest pockets (Goldblatt & Manning 2000). Nine study sites in three different catchments were selected based on invasion status: three near pristine sites (reference sites), three heavily invaded sites (dominated by *A. mearnsii*), and three restored sites (formerly invaded sites that had been cleared of invasive plants more than 7 years prior to this study) (Fig. 1). Site categorisation was based on visual scoring of *Acacia mearnsii* cover within two transects measuring 50 m in length (parallel to the river) and 5 m in width (perpendicular to the river crossing both wet and dry bank zones). For heavily invaded sites *A. mearnsii* canopy cover was > 75% and for near pristine sites cover was < 5%. For restored sites, site categorisation was based on historical records and cover was < 5%. Reference sites are frequently used in restoration projects to provide direction for restoration goals (Blanchard & Holmes 2008). Potential sites were located using information

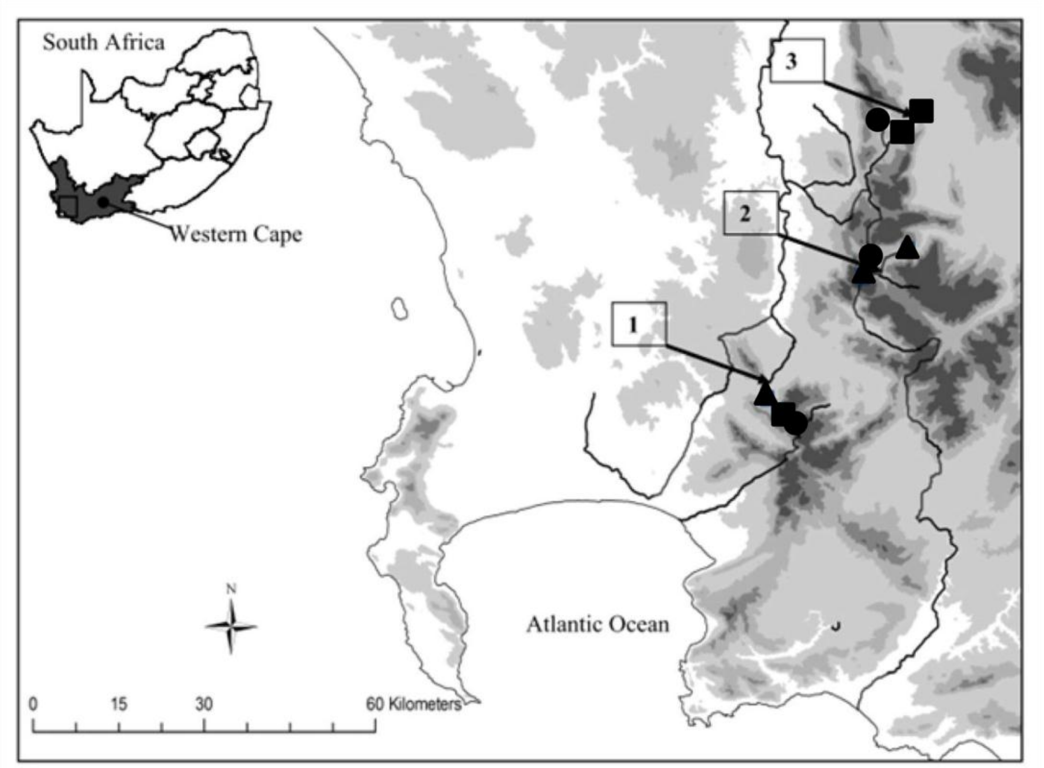

Figure 1. Location of the three Western Cape rivers: 1= Dwars River, 2 = Molenaars River, and 3 = Wit River and the nine sites (circles = near pristine sites, squares = heavily invaded sites and triangles = restored sites) identified in this study.

from previous studies (Blanchard & Holmes 2008) and through discussions with Western Cape nature conservation managers, members of Water for Water and private landowners.

Two species native to Fynbos riparian zones, *Brabejum stellatifolium* (Proteaceae) and *Metrosideros angustifolia* (Myrtaceae) (Crous et al. 2012), were selected. These evergreen trees are common components of these riparian habitats and considered key species in south-western Cape Mediterranean-type riparian systems (Galatowitsch & Richardson 2005). Within the riparian ecosystems studied, invasive taxa include numerous Australian acacias such as *A. cyclops* (A. Cunn. ex G. Don), *A. longifolia* (Andrew) Wild), *A. saligna* ((Labill.) H.L.Wendl.) and *A. melanoxylon* (R.Br). However, an evergreen *A. mearnsii* is by far the most abundant and was subsequently selected for comparisons with the selected native species. It is also considered the most problematic species in these riparian systems with individuals also commonly found scattered in near pristine areas (Dye & Jarmain 2004).

## Extent of folivorous insect and pathogen damage

At each of the nine sites, five individuals of each of the three focal tree taxa were randomly selected for comparisons of extent of damage caused by folivorous insects and phylopathogenic fungi. Selected individuals had similar heights and stem diameters for each species respectively across all sites (to minimize possible difference in susceptibility due to plant age) and all were within 5 m of the water's edge (to minimize possible differences in susceptibility due to water stress) (Crous et al. 2012).

During August and October 2011, five small branches (about 5 cm diameter and 30 cm long) were randomly collected from each individual tree at canopy level. All leaves were detached from branches and mature leaves were combined into a single bulk sample per individual tree in a brown paper bag. From these bulk samples, 100 leaves for each tree individual were randomly chosen (blindly drawing from brown paper bag) in the laboratory. Symptom assessments were conducted for each leaf in the laboratory using a dissecting microscope, allowing careful confirmation of damage type.

Leaves were assigned to one of the following damage types: (i) fungal damage alone, (ii) folivore damage alone and (iii) intact leaves (García-Guzmán & Dirzo 2004). Wounds created by folivorous insects are often entry points for pathogenic fungi (Agrios 2005). Therefore, leaves with both fungal and insect damage were not included in analyses as this would bias enumeration of leaves with fungal pathogen damage. Insect damage was categorised into two types – that caused by insects with chewing mouthparts and those with piercing and sucking mouthparts. Damage caused by leaf mining insects was negligible and not included in the analyses. Leaves colonised by phylopathogenic fungi were separated according to four predominant disease symptoms (blight, chlorotic areas, necrotic spots and black mould). Identifications of the phylopathogenic fungi that caused these main symptoms were based on a combination of characters including: the symptoms themselves, fungal micro-morphology (studied with the aid of a light- and dissecting microscope), culture characteristics (as grown for two weeks in the dark at 25ºC on Malt Extract agar, Biolab, South Africa) and published literature (Doidge & Bottomley 1931; Morris et al. 1988; Arzanlou et al. 2007).

One leaf of each of the five sampled individual trees per tree species per site from each the three catchments was used for DNA extraction and amplification. Therefore for each disease type on each tree species we sequenced nine separate fungal individuals. DNA extraction and PCR procedures followed Musvuugwa et al. (2015) without modification. Primers ITS1-f (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used for amplification and sequencing reactions. Preliminary identifications were confirmed by comparisons to those of taxa published on GenBank (http://www.ncbi.nlm.nih.gov/genbank/) using standard BLAST searches. Pathogenicity tests were not performed because repeated isolations always resulted in the isolation of the same fungus species from the same lesion type and we therefore assumed that the identified fungal species were the causal agents of the necrotic lesions.

## Severity of pest and pathogen damage

Damage severity was defined as the percentage of leaf area visibly infected by a pathogen or by feeding activities of arthropods. The percentage of area lost to disease or folivory was determined using a subjective estimate of lamina damage (1 = intact; 2 = 1–11%; 3 = 12–49% and 4 = 50–100% of leaf area damaged) based on previous studies (Benítez-Malvido 1999; García-Guzmán & Dirzo 2004; Eichhorn et al. 2010). Leaf damage for all tree individuals was scored by the same person. The estimates of leaf area lost were based on comparisons with a standard leaf of the same size as that being scored. Using these methods we could therefore not account for leaves that were completely removed from the plants and our estimations may therefore be lower than actual damage levels.

To test the effects of invasion status on numbers of leaves damaged (extent of damage) and categories of damage severity, for each host tree, Generalised Linear Models were performed. These variables were fitted to a Poisson distribution model with a log-link function using generalised estimating equations using R software (R Development Core Team 2014). The Poisson distribution model was selected to minimize the deviance statistic. For significant terms, the individual levels were compared using a Tukey's HSD test (García-Guzmán & Dirzo 2004). Significant differences are reported where $p < 0.05$.

## Leaf nutrient analyses

In the present study, the term 'leaf' denotes the primary photosynthetic organ of the plant. Leaf nutrients were measured for *B. stellatifolium, M. angustifolia,* and *A. mearnsii*. Only mature leaf samples were taken, and leaves were considered mature when fully expanded. These were collected from five replicate trees of each tree species from each of the nine sites selected. Samples from each tree individual were removed separately with scissors and placed in plastic re-sealable bags with moistened paper towelling. Hands were gloved to avoid contamination of leaf material. Samples were placed in insulated containers with ice, and were transferred to the Institute for Plant Production, Western Cape Department of Agriculture, Stellenbosch, South Africa for processing.

Leaves were dried, ground in a Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA) and analysed for foliar phosphorus (P) (expressed as percentage of dry mass) using a CE Instruments NC2100 CN Analyser (CE Elantech, Incorporated, Lakewood, New Jersey, USA). Total nitrogen (expressed as percentage of dry mass) was determined by the semimicro Kjeldahl method (Ofosu-Budu et al. 1992) using a Tecator Kjeltec Auto 1030 Analyser (Tecator, Höganäs, Sweden). Mean leaf nutrient levels (as percentage) were compared for each plant species across sites within three different invasion treatments using analysis of variance (ANOVA) followed by Tukey's multiple comparison tests in STATISTICA.

### Effect of native tree species richness, host abundance and leaf nutrients on host damage

At each of the nine sites, two transects were established measuring 50 m in length (parallel to the river) and 5 m in width (perpendicular to the river crossing both wet and dry bank zones; for description of wet and dry bank zones see Reinecke et al. (2015)). In these, native tree species richness and host abundance were determined. Data from the two transects were combined per site, and compared among sites with different invasion status similarly to leaf nutrient content.

To investigate possible interactions between host species abundance, native plant species richness and leaf nutrients on extent of folivorous insect and phylopathogen damage, regression analyses were performed with percentage of leaves damaged by the respective organism groups as the response variables and native tree species richness, host abundance and nutrient levels as predictors. Analyses were performed using STATISTICA. Unadjusted $P < 0.05$ values are reported.

## Results

### Differences in folivorous insect and fungal damage levels

All three tree species exhibited significant differences in levels of folivory in relation to invasion status of the site. Levels of folivory on *Brabejum stellatifolium* were significantly higher at invaded sites than at near pristine sites and increased significantly from invaded sites at restored sites ($Z_{[2,42]} = 78.53$, $p < 0.001$) (Fig. 2a). For *Metrosideros angustifolia* folivore damage showed a similar pattern, but the difference was only significant for comparisons between restored and near pristine sites ($Z_{[2, 42]} = 72.53$, $p < 0.001$) (Fig. 2a). *Acacia mearnsii* showed a different pattern, with significantly more leaves damaged by folivorous insects at heavily invaded sites compared to near pristine sites and restored sites ($Z_{[2, 42]} = 49.42$, $p < 0.001$), which were statistically similar (Fig. 2a).

In addition to damage caused by folivorous insects, the only other organisms causing significant leaf damage to the study plants were fungi. Each host species had at least one common phylopathogenic species. *Brabejum stellatifolium* was infected by the biotrophic pathogen *Periconiella velutina* (G.Winter) which causes black mould and the necrotrophic pathogen *Phyllosticta owaniana* (G.Winter), which causes leaf spot. *Metrosideros angustifolia* was infected by an *Alternaria* sp. that caused necrotic spots. *Acacia mearnsii* was infected by the biotrophic heteroecious pathogen *Uromycladium alpinum* (McAlpine) that caused chlorotic spots.

For *B. stellatifolium* the extent of fungal disease was significantly higher at restored sites and heavily invaded sites ($Z_{[2, 42]} = 17.59$, $p < 0.001$) compared to near pristine sites (Fig. 2b). For *M. angustifolia*, the extent of fungal disease was significantly higher in restored riparian

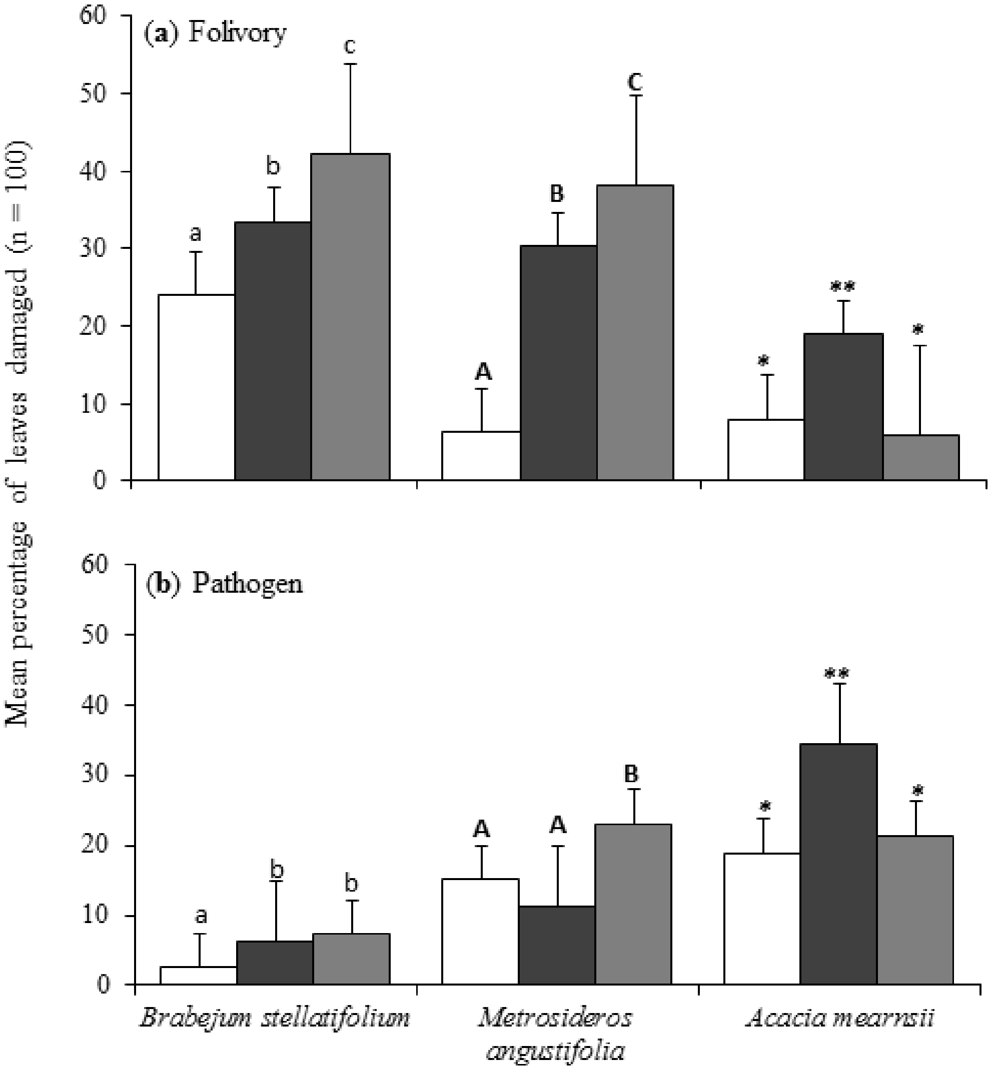

**Figure 2.** Mean percentage (+SE) of leaves damaged by either folivorous insects (**a**) or pathogens (**b**) for *B. stellatifolium*, *M. angustifolia*, *A. mearnsii* from riparian sites with different invasion status: near pristine (*clear*), heavily invaded (*dark grey*) and restored (*light grey*). Significant differences are indicated above error bars by different lower case letters for *B. stellatifolium*, different uppercase letters for *M. angustifolia* and differences in the number of asterisks for *A. mearnsii*. (n = 15 for each tree species in each invasion status).

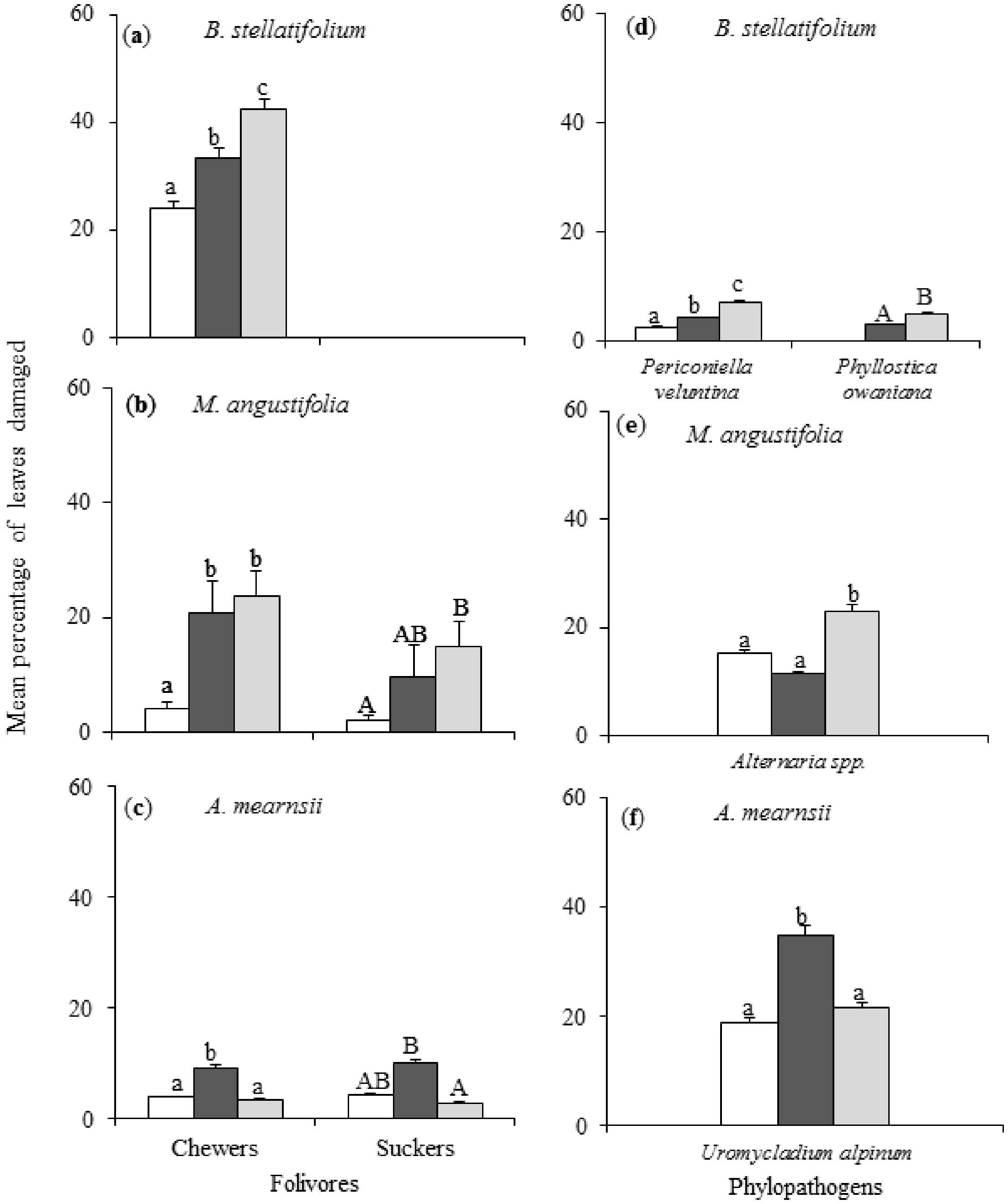

Figure 3. Frequency of leaves damaged by folivorous insects with chewing and sucking mouthparts (**a, b** and **c**) and diseases caused by four phylopathogenic fungi (**d, e** and **f**) for *B. stellatifolium*, *M. angustifolia* and *A. mearnsii* at sites that differed in their invasion status: near pristine (*clear*); heavily invaded (*dark grey*); and restored riparian zones (*light-grey*). Different letters indicate a significant difference between groups within each damage type.

vegetation ($Z_{[2, 42]} = 31.82$ $p < 0.001$) than in near pristine and heavily invaded sites which did not differ significantly (Fig. 2b). For *A. mearnsii*, fungal disease was higher in heavily invaded sites ($Z_{[2, 42]} = 80.37$, $p < 0.001$) than in restored and near pristine sites, which were statistically similar (Fig. 2b).

*Brabejum stellatifolium* mostly incurred damage caused by folivorous insects with chewing mouthparts ($Z_{[2,42]} = 77.79$, $p < 0.001$) (Fig. 3a), while *M. angustifolia* and *A. mearnsii* were almost equally damaged by insects with chewing- and those with piercing-sucking mouthparts (*M. angustifolia*: $Z_{[2,42]} = 53.75$, $p < 0.001$ and *A. mearnsii*: $Z_{[2,42]} = 25.71$, $p < 0.001$) (Fig. 3b & c). Damage levels caused by both chewing and sucking insects followed a similar pattern to those observed for the combined data presented in figure 2a. Similarly, for *B. stellatifolium*, both fungal taxa showed the same pattern (Fig. 3d) as was found for their combined effect shown in Figure 2b.

### Severity of pest and pathogen damage

Severity of leaf damage is presented in Figure 4. For all three species, the proportion of damaged leaves in each severity category varied significantly among study sites as denoted by different letters. For *B. stellatifolium* and *M. angustifolia*, damage severity caused by both folivorous insects and phylopathogenic fungi was higher at heavily invaded and restored sites than at near pristine sites, but for both taxa, damage severity tended to be highest at the restored sites. *Acacia mearnsii* tended to be more severely damaged by both folivorous insects and phylopathogenic fungi at heavily invaded sites (Figs. 4c and f).

### Effect of native tree species richness, host abundance and leaf nutrients on host damage

Mean native tree species richness was significantly lower at heavily invaded sites than at near pristine sites ($F_{[2, 6]} = 6.86$, $p = 0.03$), but returned to pre-invasion levels after alien removal. Abundance of *B. stellatifolium* was highest at the near pristine sites ($F_{[2, 6]} = 16.89$; $p = 0.003$)

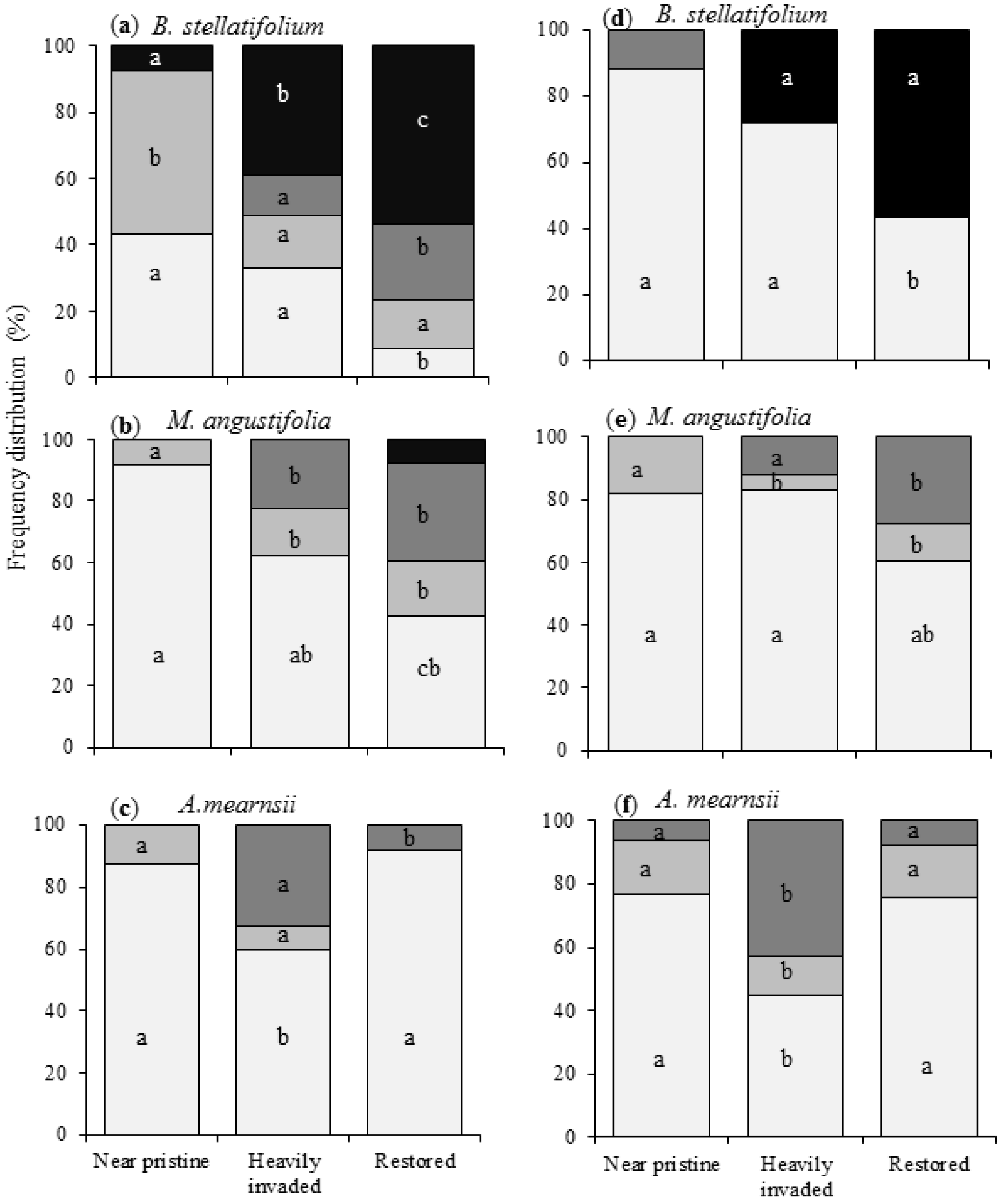

**Figure 4.** Frequency distributions of percentage of leaf area damaged by folivorous insects (**a, b** and **c**) and phylopathogens (**d, e** and **f**). The darker the bars, the more severe the damage caused by the various organisms were. Categories of leaf area damaged were as follows: 0% (*light grey*); 1-11% (*mid grey*); 12-49% (*dark grey*) and 50-100% (*black*). Significant differences across sites for each category are denoted by different letters.

**Table 1**. Differences in native tree species richness among studied sites that vary in their invasion status and size classes (diameter (cm) measured at breast height) and host abundance of the three tree species considered in this study. Significant differences in native tree species richness, host abundance and size-classes are indicated by different lower case letters for *B. stellatifolium*, different uppercase letters for *M. angustifolia* and differences in the number of asterisks for *A. mearnsii*.

| Invasion status | Mean native tree species richness ± standard error | Plant species | Host abundance | Mean stem diameter (cm) ± standard error |
|---|---|---|---|---|
| Near pristine | $20 \pm 0.9^{\mathbf{a}}$ | *B. stellatifolium* | $25 \pm 1.7^{a}$ | $36 \pm 1.1^{a}$ |
| | | *M. angustifolia* | $26 \pm 1.0^{A}$ | $31 \pm 2.4^{B}$ |
| | | *A. mearnsii* | $6 \pm 0.3^{*}$ | $30 \pm 1.3^{*}$ |
| Heavily invaded | $7 \pm 2.3^{\mathbf{b}}$ | *B. stellatifolium* | $11 \pm 2.1^{b}$ | $29 \pm 0.95^{a}$ |
| | | *M. angustifolia* | $8 \pm 2.0^{B}$ | $19 \pm 1.1^{A}$ |
| | | *A. mearnsii* | $63 \pm 30.9^{**}$ | $36 \pm 2.4^{*}$ |
| Restored | $17 \pm 0.7^{\mathbf{a}}$ | *B. stellatifolium* | $15 \pm 1.3^{b}$ | $32 \pm 1.3^{a}$ |
| | | *M. angustifolia* | $17 \pm 3.3^{AC}$ | $22 \pm 2.2^{A}$ |
| | | *A. mearnsii* | $6 \pm 0.3^{*}$ | $13 \pm 0.9^{**}$ |

with no difference detected between the restored and invaded sites (Table 1). Abundance of *M. angustifolia* was highest at the near pristine sites and restored sites while at heavily invaded sites, abundance was significantly lower ($F_{[2,\ 6]} = 6.31$; $p = 0.03$) (Table 1). *Acacia mearnsii* and *Metrosideros angustifolia* showed differences in size of individuals from sites that differ in invasion status, with individuals at restored sites significantly smaller than those at near pristine or invaded sites (Table 1). The size of *B. stellatifolium* did not vary across sites that differ in invasion status (Table 1).

Regressions between native plant species richness and percentage of leaves damaged by phylopathogenic fungi and insects were not significant in all cases except for damage caused by phylopathogenic fungi on *A. mearnsii* (Fig 5); in this case, damage caused by phylopathogenic fungi was negatively correlated to native plant species richness. Native host tree densities were always negatively correlated to the number of leaves damaged by phylopathogenic fungi or folivorous insects, although only significantly so in the case of damage by folivorous insects (Fig. 5). The invasive *A. mearnsii* showed the opposite, with both damage types significantly and positively correlated to host abundance (Fig. 5).

Leaf P content did not vary among sites for any plant species tested (Fig. 6). Leaf N varied significantly among sites only for *B. stellatifolium* and *A. mearnsii* (Fig. 6). For *B. stellatifolium*, levels of leaf N were significantly higher in restored sites compared to heavily invaded and near pristine sites ($F_{[2,42]} = 68.12$, $p < 0.00001$) that did not significantly differ from each other (Fig. 6). Significantly higher levels of N for *A. mearnsii* were found in heavily invaded sites as compared to near pristine sites ($F_{[2,42]} = 8.39$, $p = 0.0009$). Leaves of *A. mearnsii* at restored sites had intermediate N levels (Fig. 6). No significant correlations were found between leaf N content and number of leaves damaged by phylopathogenic fungi for any tree species (Table 2). However, leaf N content was significantly correlated to number of leaves damaged by folivorous insects for *B. stellatifolium* and *A. mearnsii*. This was in

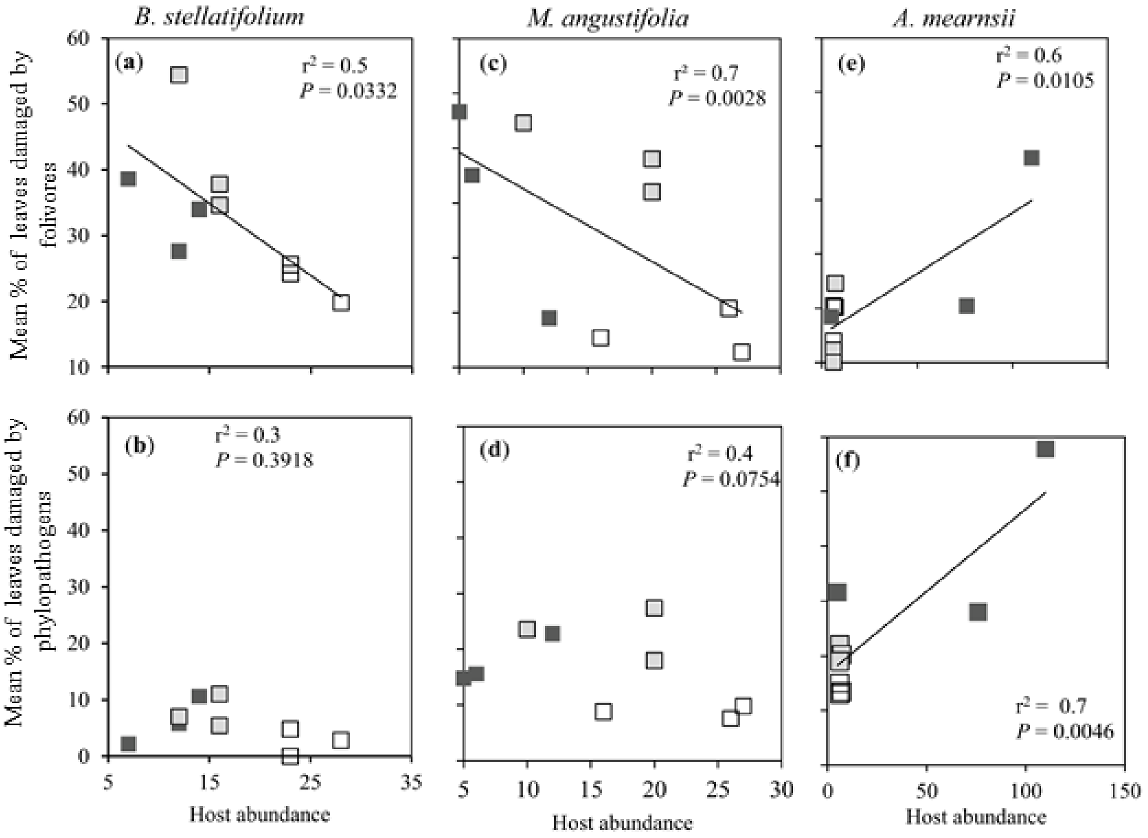

Figure 5. The effects of host abundance on the percentage of leaves damaged by folivorous insects (**a, c** and **e**) or phylopathogens (**b, d** and **f**) for three tree species (*B. stellatifolium*, *M. angustifolia* and *A. mearnsii*) at sites that differ in their invasion status: near pristine (*clear square*); heavily invaded (*dark grey square*); and restored (*light-grey square*) riparian zones. Reported P-values are for partial regression coefficients; reported $r^2$ is for each overall regression. All trends were linear.

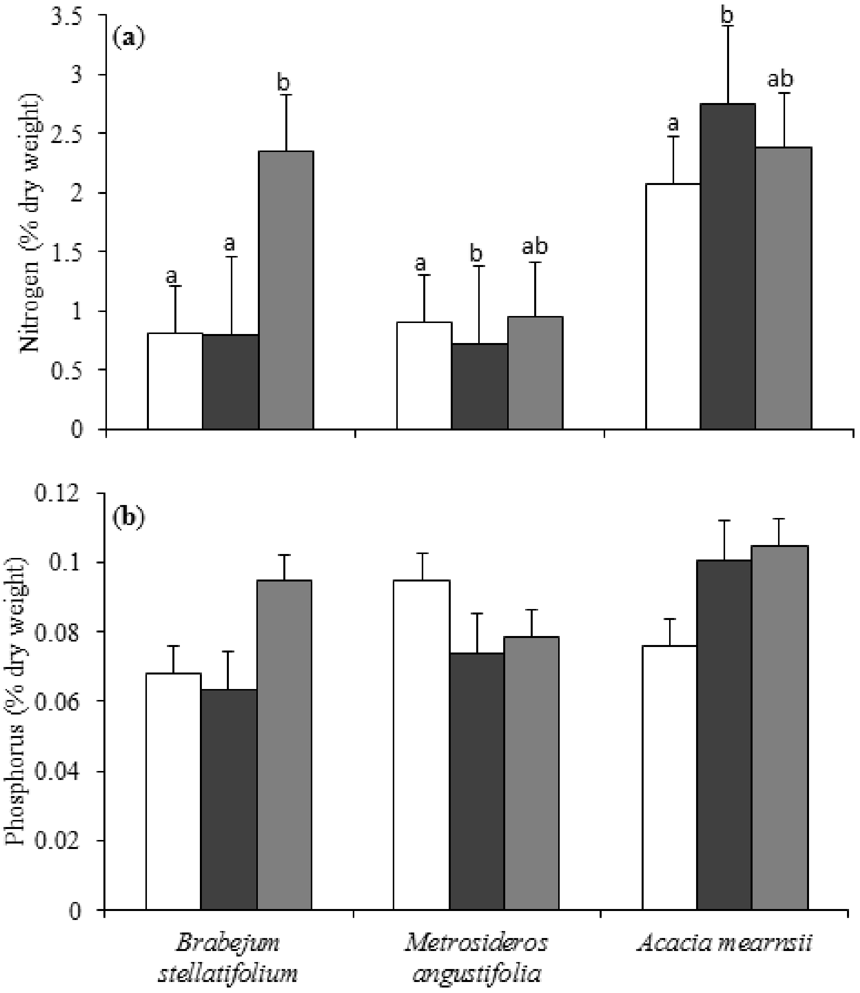

**Figure 6.** Mean + SE of (**a**) foliar Nitrogen content and (**b**) Phosphorus content measured as percentage of dry mass of *B. stellatifolium*, *M. angustifolia*, *A. mearnsii* from riparian sites with different invasion status: near pristine (*clear*), heavily invaded (*dark grey*) and restored (*light grey*). Significant differences are indicated above bars by different lower case letters for each tree species.

**Table 2.** Correlations for foliar damage caused by phylopathogenic fungi and folivorous insects as response variables versus native tree species richness and leaf nitrogen as the predictors (*n* = 9).

| Plants species | Number of leaves damaged by phylopathogenic fungi | | Number of leaves damaged by folivorous insects | |
|---|---|---|---|---|
| | Tree species richness | Leaf nitrogen | Tree species richness | Leaf nitrogen |
| *B. stellatifolium* | 0.21n.s | 0.21n.s | 0.02n.s | 0.51*** |
| *M. angustifolia* | 0.00n.s | 0.03n.s | 0.13n.s | -0.11n.s |
| *A. mearnsii* | 0.59*** | 0.21n.s | 0.25n.s | 0.52*** |

*Note:* Data presented are for the fitted intercept, the coefficient of determination ($r^2$)

Significance: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s $p > 0.05$

contrast to *M. angustifolia* that showed no significant correlation between folivore damage and nitrogen content of leaves (Table 2).

## Discussion

In this study, both native and alien trees in riparian zones varied in their susceptibility towards pathogenic fungal colonisation and folivorous insect (natural enemies) damage across sites, in accordance with differences in invasive alien plant invasion status. For the native species *B. stellatifolium*, both folivore and pathogen damage was significantly higher at invaded sites than at near pristine sites and these levels did not return to their original levels after restoration. In fact, folivore damage levels were even higher at restored sites than at heavily invaded sites. Similarly, damage caused by leaf phylopathogenic fungi and folivorous insects was also higher for the other native species *M. angustifolia* at restored sites than it was at heavily invaded and near pristine sites. The pattern was different for the invasive *A. mearnsii* where damage caused by both folivorous insects and phylopathogenic fungi increased at heavily invaded sites, but returned to near pristine site levels at restored sites. The high levels of folivory and fungal diseases on *A. mearnsii* could not be credited to biological control agents since the two known biocontrol agents (*Melanterius maculatus* and *Dasineura rubiformis*) do not damage the vegetative parts of *A. mearnsii*, but rather cause reductions in seed availability (Impson et al. 2011). The lower levels of folivory and fungal disease in near pristine sites concurs with literature which shows that higher heterogeneity in plant species composition and structure of near pristine sites possibly explains observed lower levels of damage by natural enemies (Bach 1980; Agrawal et al. 2006). The higher levels of damage by folivorous insects and phylopathogenic fungi on native plants in restored areas is intriguing, particularly because this study was undertaken seven years after restoration efforts.

After removal of invasive alien plants, host abundance of *B. stellatioflium* and *M. angustifolia* (two native trees) was similar to near pristine sites. However, abundance of these species was low at heavily invaded sites. Abundance of *A. mearnsii* was greater in heavily invaded sites than in restored and near pristine sites. Apart from decreased host abundance of the two native trees in heavily invaded sites, native plant species richness was also lower than that in near pristine sites. Results therefore support previous observations that alien plant species have a negative impact on native species richness and abundance by replacing native plant species in the communities they invade (French et al. 2008; Vilà et al. 2011). Most invasive Australian *Acacia* spp. in south Africa are transformers, posing serious threat to structure and function of pristine Fynbos areas by elevating soil nitrogen levels and converting them into species-poor ecosystems (Richardson et al. 2000). Despite these severe impacts, native plant species richness can return to its original level once the invasive alien plants are removed (Holmes & Cowling 1997; Blanchard & Holmes, 2008; Marchante et al. 2011 and this study). The recovery of native plant species in riparian areas referred to in these studies suggests that, in each case, no “threshold of irreversibility’’ (as defined by Aronson et al. 1993) had been reached (see Holmes & Cowling 1997), thereby allowing regeneration after clearing.

There is a debate over whether or not the levels of leaf damage by folivorous insects and phylopathogenic fungi depends on native plant species richness, with homogenous tree stands being more damage-prone than heterogeneous stands (Mitchell et al. 2002; Pfisterer et al. 2003; Haas et al. 2011). Consequently, one would expect to see decreasing levels of folivory and fungal disease with increasing native plant species richness. However, results from this study suggest that activities of folivores and phylopathogenic fungi in diverse riparian habitats (near pristine and restored) and simple habitats (heavily invaded) are independent of plant species richness, a pattern observed in other ecosystems (e.g. Scherber et al. 2010). Bach (1980) suggested that folivore activity is linked to host abundance/density rather than plant species richness. An area which has a high abundance/density of a preferred host favours folivore survival more so than a diverse area where access to the preferred host is more limited; a phenomenon known as the ‘resource concentration’ hypothesis (Grez & González 1995).

Apart from host abundance, Mitchell et al. (2002) highlighted the importance of understorey richness in maintaining and controlling natural enemies of plants. We have not included understory plant species in our analyses which may have influenced results obtained for restored and near pristine sites. Therefore, even though tree species richness was similar, understory plant richness could have been less diverse in restored sites leading to a decrease in natural enemies and increased levels of folivory. Future studies should therefore include understory plant richness as an explanatory variable for altered herbivore pressure.

Several studies have tested relationships between levels of folivory, fungal diseases and the abundance of hosts, with some studies showing positive (Mabry & Wayne 1997), some negative (Underwood and Halpern 2012; Halpern et al. 2014) and some neutral (Rhainds & English-Loeb 2003) effects. In this study, increased host abundance was negatively correlated to native tree folivory and positively to the levels of both folivory and fungal diseases for *A. mearnsii*. The negative relationship between folivory and host abundance for the two native trees could be explained by dilution of folivore effects with increased abundance (e.g. see Halpern et al. 2014). Alternatively, at low abundance, when host plants are thinly dispersed, folivores may show fidelity to a host, as finding another host both increases energy use and exposure to predaceous arthropods (Schuldt et al. 2010).

Levels of damage by folivores and pathogens on *A. mearnsii* seemed to follow a more classic pattern as is well known in agricultural monotypic stands. *Acacia mearnsii* forms thick near-monocultures that make it easy for insects to find hosts (if pre adapted) and for spores of pathogens to land on susceptible hosts (e.g. Bell et al. 2006). It is also possible that predator communities may have been disrupted. There is some evidence that in their introduced range, alien plants may assemble communities of folivores as abundant and rich as those of native hosts (Frenzel & Brandl 2003) especially if the invasive alien plants is closely related to several native trees (Procheş et al. 2008). The Fabaceae are well represented in South Africa (18 % of the 198 listed trees species in South Africa belong to the Fabaceae (Coates-Palgrave 2002)). Hence, it is possible that a rich regional pool of pre-adapted folivores is available to counteract chemical defences of *A. mearnsii* (e.g. Litt et al. 2014).

Apart from the influences of host abundance and native plant species richness, the observed higher levels of folivory and fungal diseases may be related to changes in leaf chemical components due to high rates of nutrient inputs to the ecosystems through the presence of nitrogen-fixing invasive alien plants (e.g. *A. mearnsii* is a putative nitrogen fixing legume (Tye & Drake 2012), that are known to elevate soil nutrient levels (van der Waal, 2009). A study by Naudé (2012), at some of the same riparian sites used in this study, showed that a legacy effect remained after clearing invasive alien plants in that levels of available nitrogen in soil remained high. This implies that plants in restored sites may be more vigorous than in near pristine sites due to abundant available resources (such as was seen for the native trees in this study). This, in turn, may make these plants more attractive to folivorous insects and/or phylopathogenic fungi (Moran & Scheidler 2002), thereby increasing their abundance (and causing an increase in damage) in riparian systems after alien clearing.

Recorded levels of foliar nitrogen of the host trees fell within the range of values reported for other Mediterranean trees (Fife et al. 2008) and for other evergreen trees such as Australian *Acacia* spp. (Peeters 2002b). Likewise, leaf phosphorus levels were within the lower and upper ranges of reported values for these hosts (Fife et al. 2008). Foliar nutrient content data confirmed that *B. stellatifolium* had greater levels of foliar nitrogen at restored sites, as did *Acacia mearnsii*. Phosphorus levels did not vary significantly for any plant species tested across all invasion statuses. Of the covariates tested, leaf nitrogen was the only factor that was positively correlated with folivores and leaf chewers for *B. stellatifolium* and with folivores for *A. mearnsii*. This result suggests that nitrogen content is an important driver of arthropod population dynamics for *B. stellatifolium* and *A. mearnsii*.

In conclusion, the results of this study suggest that, although plant communities often recover after invasive alien removal, native trees continue to be damaged by folivorous insects and pathogenic fungi resulting in damage levels in excess of those seen at invaded sites. This implies that restoration efforts of invaded riparian zones do not remove the legacy effect of the invasion with many unexpected and lasting ecological consequences. Of the explanatory factors assessed, host abundance (resource concentration) and nitrogen availability (resource quality) seemed to be the most influential on damage caused by arthropods. Neither of these two factors explained the similar observed patterns for damage caused by phylopathogenic fungi. Although these correlative analyses cannot ascertain the exact mechanism by which host plant abundance affected folivory, it is highly possible that the interplay between folivore densities, predaceous arthropod densities and/or plant nutritional status may be important (see Forkner & Hunter 2000; Moran & Scheidler 2002). Investigating the effect of these factors on pest and pathogen populations under controlled conditions is therefore a necessary next step for future research.

## Acknowledgments

The authors are grateful to Marno Fourie and Oliver Kambaj for field assistance, and two reviewers for helpful comments and suggestions to improve this manuscript. We thank private landowners and CapeNature for access to field sites. This study was funded by the DST/NRF Centre of Excellence in Tree Health Biotechnology. We are also thankful to Dr. Kenneth Oberlander for assistance with molecular techniques for identification of phylopathogenic fungi isolates in this study.

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