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Novel technique shows different hydrophobic chemical signatures of exotic and indigenous plant soils with similar effects of extracts on indigenous species seedling growth

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Abstract

Changes to ecosystem abiotic parameters are regarded as possible mechanisms facilitating plant invasion and community composition shifts. This study compared the hydrophobic chemical signatures of soil from exotic bitou bush (Chrysanthemoides monilifera spp. rotundata) invaded, indigenous acacia (Acacia longifolia var. sophorae) dominated and bare sand (unvegetated) habitats using a novel, rapid, capturing technique which utilised AmberliteA (R) XAD4 resin filled bags that were placed in situ. The hydrophobic chemical signature of the bitou bush soil extract was significantly different to the acacia soil and bare sand extracts. High concentrations of 18 sesquiterpenes dominated the hydrophobic signature of the bitou bush extract. Low concentrations of all three extracts did not significantly affect the seedling growth of three indigenous test species under laboratory conditions, however, at higher concentrations, the extracts from soil inhabited by plants, whether exotic or indigenous, similarly inhibited the seedling growth of two species, while seedling growth of the third species was inhibited by extracts from all three soil types. These results do not support the hypothesis that exotic invasive species are more likely to exhibit allelopathic effects than indigenous plant species.

Keywords

plant, indigenous, exotic, signatures, chemical, hydrophobic, different, seedling, similar, technique, extracts, effects, shows, soils, growth, novel, species, CMMB

Disciplines

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REGULAR ARTICLE

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Abstract Changes to ecosystem abiotic parameters are regarded as possible mechanisms facilitating plant invasion and community composition shifts. This study compared the hydrophobic chemical signatures of soil from exotic bitou bush (*Chrysanthemoides monilifera* spp. *rotundata*) invaded, indigenous acacia (*Acacia longifolia* var. *sophorae*) dominated and bare sand (unvegetated) habitats using a novel, rapid, capturing technique which utilised Amberlite® XAD4 resin filled bags that were placed in situ. The hydrophobic chemical signature of the bitou bush soil extract was significantly different to the acacia soil and bare sand extracts. High concentrations of 18 sesquiterpenes dominated the hydrophobic signature of the bitou bush extract. Low concentrations of all three extracts did not significantly affect the seedling growth of three indigenous test species under laboratory conditions, however, at higher concentrations, the extracts from soil inhabited by plants, whether exotic or indigenous, similarly inhibited the seedling growth of two species, while seedling growth of the third species was inhibited by extracts from all three soil types. These results do not support the hypothesis that exotic invasive species are more likely to exhibit allelopathic effects than indigenous plant species.

Keywords Allelopathy · Sesquiterpenes · Phenolic compounds · Fatty acids · Exotic plant invasion · Species co-existence

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Introduction

Modifications to abiotic ecosystem parameters can drive vegetation community composition change (Lawton 1994) and are demonstrated mechanisms of plant invasion (Levine et al. 2003). Shifts in ecosystem nutrient (Ehrenfeld et al. 2001; Yelenik et al. 2004; Lindsay and French 2005), fire (van Wilgen and Richardson 1985; D'Antonio and Vitousek 1992; Rossiter et al. 2003) and water (Dunbar and Facelli 1999) dynamics in response to plant invasion have been studied and hence, ecosystem process change has been accepted as a possible invasion mechanism

(Levine et al. 2003). Here we propose that plant invaders can also affect ecosystem processes by altering the complex soil chemical profile which includes compounds such as organic acids, phenols, terpenes and fatty acids. Complex soil chemistry changes by invasive plants have been suggested in the past, typically through allelopathy studies (Inderjit 2001, 2002). However, the difficulties inherent in the demonstration of unequivocal cause and effect of complex soil chemistry changes has prevented broad acceptance of allelopathy as a possible invasion mechanism (Inderjit 2001, 2002; Hierro and Callaway 2003; Schenk 2006). We aimed to address this deficiency, as suggested by Levine et al. (2003), and show that changes to the complex soil chemistry can affect indigenous plant growth, by comparing the effects of exotic plant invaded soil extracts to the effects of indigenous acacia and unvegetated soil extracts. To our knowledge, this is the first study to compare the complex soil chemical profiles of exotic invaded, indigenous plant and unvegetated soils.

Plants directly influence the local soil chemistry by passively diffusing or actively transporting complex compounds from the roots, or via leachates from decaying plant material (Waller and Feng 1996; Kuzyakov and Domanski 2000; Bertin et al. 2003). Plants may also indirectly alter the soil chemistry by influencing the microbial (Hattenschwiler and Vitousek 2000; Kourtev et al. 2003; Reinhart and Callaway 2006) or faunal community (Kourtev et al. 1999; Dudareva et al. 2006). Additionally, the plant derived soil compounds may be biotically (Huang et al. 1999; Inderjit 2001; de Boer et al. 2006) or abiotically (Weidenhamer and Romeo 2004; Inderjit 2005) modified. Moreover, fluctuations in plant compound release can occur as a function of season, microclimate and developmental stage of the plants (Kuhn et al. 2004) and in response to neighbour identity (Ormeno et al. 2007). For example Lin et al. (2007) have shown that the release of monoterpenes is higher from well watered roots than drought-stressed roots. Hence the localized soil chemistry within a community has high spatio-temporal variability (Russell 1988).

The complex interactions within and between the soil, plants, microbes and fauna through space and time complicate studies aiming to determine allelopathy, to the point where it may be impossible to unequivocally demonstrate such a phenomenon (Inderjit 2002). Past allelopathy studies have typically isolated plant extracts and tested them for plant growth inhibition (Bousquet-Melou et al. 2005; Dorning and Cipollini 2006) or utilised competition experiments to test the relative contribution of resource and chemical interference competition to species dominance (Nilsson 1994; Weidenhamer 1996). However, these studies predominantly failed to investigate the soil chemistry. Soil chemistry studies are crucial to allelopathy research, although they have been under-utilised (Inderjit 2001; Inderjit and Weiner 2001) as they can elucidate the presence of a chemical continuum, or pathway, between the allelochemical donor and target plant (Cheng 1995). For example, Kelsey et al. (1978) showed that a chemical continuum existed between *Artemisia tridentata* spp. *vaseyana* litter and the soil and that these chemicals inhibited the germination of sagebrush. Cheng (1995) proposed a three step procedure for preliminary allelopathy studies: (a) detection of potential allelochemicals in the root, (b) transport of the compounds from donor to target plant, or detection in soil, and (c) inhibition of target plant by the compounds. Allelopathy studies often base conclusions on evidence from parts (a) and (c) as detection of compounds in the soil (part b) has been problematic (Cheng 1995; Inderjit 2001), or simply overlooked. However, some studies have since incorporated assessment of soil allelochemical dynamics (Nishimura and Mizutani 1995; Wallstedt et al. 2000; Weidenhamer and Romeo 2004) which has become more tractable with the advancement of analytical techniques (Dudareva et al. 2006).

We surmised that soil chemical interference might be more likely to occur between plants that have not co-evolved (Callaway and Aschehoug 2000; Rausher 2001) as a result of different historical selection pressures. Thus invasive exotic plants would show greater levels of interference competition than indigenous dominant species. However, interspecific competition between indigenous species is also suspected to be ubiquitous in nature (Amarasekare 2002) and may influence species composition. We have investigated coastal dune systems in south eastern Australia where coastal vegetation dominated by indigenous coastal *Acacia longifolia* var. *sophorae* Labill. (Mimosaceae) has been invaded by South African *Chrysanthemoides monilifera* spp. *rotundata* (L.) T. Norl. (Asteraceae) and indigenous plant recruitment is limited (Ens and French 2008). We have found that the *C. monilifera* spp. *rotundata* root and soil have similar complex

chemical profiles with high concentrations of hydrophobic terpenes and that these compounds were present in *A. longifolia* var. *sophorae* roots but not in *A. longifolia* var. *sophorae* soil in winter (Ens et al. 2009). The hydrophobic, solvent-derived extracts of *C. monilifera* spp. *rotundata* roots and soil inhibited a range of indigenous species and were more inhibitory than hydrophilic extracts (Ens et al. 2009).

This study aimed to contribute to our understanding of soil chemical interference between plants, using a novel resin-bag compound adsorption technique to trap soil compounds in situ. Similar resin-based methods are used in the water purification industry to adsorb pollutants (Xu et al. 2003; Kujawski et al. 2004) and Weidenhamer (2005) has reported the use of the polydimethylsiloxane (PDMS) materials to adsorb and measure levels of a photosynthesis inhibitor, sorgoleone, in the rhizosphere of sorghum plants. Here we present a simplified and modified version of the Tang and Young (1982) continuous trapping method for allelochemicals. As the compounds of interest were hydrophobic, we used resin specifically designed to adsorb hydrophobic compounds, however different types of resin could be used to adsorb and assess compounds with different chemical functional groups such as polar compounds, if warranted. The hydrophobic chemical signatures of soil invaded with *C. monilifera* spp. *rotundata*; soil inhabited by the dominant shrub, *A. longifolia* var. *sophorae*; and unvegetated soil were compared. The activity of these extracts was tested using bioassays with indigenous species to facilitate ecological relevance of the study.

Method

Resin bags

Seventy five small cotton fabric bags (15 cm×5 cm) were each filled with 10 g of Amberlite® XAD4 industrial grade, polymeric, resin (Rohm and Haas Co., Philadelphia, U. S. A). The filled bags were thoroughly washed with distilled water, then washed twice with dichloromethane (DCM; HPLC grade) before being dried in a fume cupboard at room temperature. The clean resin bags were then stored in an air-tight glass jar prior to use. Use of plastic utensils was avoided to prevent plasticizer contamination.

Study site

We studied the soil chemistry of the fore dune at Corrimal Beach, NSW, Australia, (34°23′09″, 150°24′55″) where the extant indigenous vegetation was dominated by coastal *Acacia longifolia* var. *sophorae* and *Spinifex sericea* towards the strandline. South African *Chrysanthemoides monilifera* spp. *rotundata* had invaded patches of the site. The soil substrate was characterised by Holocene parallel sand dunes with very little organic matter below the leaf litter layer.

During November (late Spring), five bags were buried under each of five *C. monilifera* spp. *rotundata* plants, five *A. longifolia* var. *sophorae* plants and in five patches of bare sand at 10 cm below the ground surface. Each plant/ bare sand patch was at least 10 m apart. For the *C. monilifera* spp. *rotundata* and *A. longifolia* var. *sophorae* conditions, the resin bags were also buried laterally within 10 cm of visible plant roots. *C. monilifera* spp. *rotundata* and *A. longifolia* var. *sophorae* produce both lateral roots, which aids in nutrient and water interception, and deeper vertical roots for anchorage (Ens personal observation). Bags were left in-situ for 10 days. This procedure was performed twice in order to obtain enough extract for use in bioassays of three different species. Chemical signatures of duplicate extracts were similar.

Compound extraction and identification

Five resin bags from each plant or bare patch were pooled to produce five replicates from each condition (*C. monilifera* spp. *rotundata*, *A. longifolia* var. *sophorae* and bare sand). To obtain a soil extract, the five resin bags for each replicate were placed in a conical flask, DCM (250 mL) was added, and the flask sealed for 24 h with intermittent agitation. After soaking, the solution was removed by filtration and the DCM evaporated under reduced pressure (Büchi rotary evaporator) from a water bath (38°C). A resin bag control extract was also derived from five clean resin bags that had been kept in a sealed glass jar and were not placed in the field.

Equal concentrations (4.13 mg/ mL; *w/v* of DCM) of each extract (*C. monilifera* spp. *rotundata*, *A. longifolia* var. *sophorae*, bare sand and resin bag control) were prepared and 0.5 μL was injected into a Varian 3,700 gas chromatograph (GC) coupled to a VG Autospec mass spectrometer system (GC-MS).

The GC-MS was fitted with a fused silica BP5 capillary column (30 m×0.25 mm) (SGE Australia) in the split mode with helium as the carrier gas. The oven temperature program began at 80°C, was increased by 4°C/ min until 100°C, then increased by 10°C/min to 280°C and held at 280°C for 10 min. The compounds were subsequently identified by comparison with mass spectra and Kovats retention indices published in the electronic NIST (2002) and SciFinder Scholar libraries (2006) and in Adams (2001). Relative amounts of each compound in each extract were quantified by calculating the peak areas in the chromatograms. Peaks detected in the resin bag control extract and in the column blank were omitted from further analysis. These peaks were comprised of small branched and some longer chain hydrocarbons.

Seedling growth bioassay

To emulate field concentrations of each extract, we prepared concentrations of samples using the range of weights adsorbed by one resin bag in 1 day, which was between 1–5 mg (Ens 2007). Amounts of 1 mg, 3 mg and 5 mg/ Petri dish were therefore used in the *Isolepis nodosa* (Rott.) R. Br (Australian indigenous Cyperaceae) bioassay, and 1 mg and 5 mg/ Petri dish were used in the *Acacia longifolia* var. *sophorae* (Labill.) F. (Muell.) and *Banksia integrifolia* (L) bioassays. Each sample was dissolved in DCM (1 mL) and added to a glass Petri dish (9 cm diameter) fitted with Whatman No. 1 filter paper. The DCM was allowed to evaporate in a fume cupboard for 15 min; distilled water (2 mL) was added (producing concentrations of 0.5 mg, 1.5 mg and 2.5 mg extract/mL water), followed by 20 equidistant seeds. Seeds of indigenous species: *I. nodosa* and *A. longifolia* var. *sophorae* seeds were collected from five sites within the Wollongong region and indigenous *B. integrifolia* seeds were purchased from the Australian Seed Company. In total, for each test species we prepared three replicate Petri dishes for each concentration of extract from each replicate plant/ bare soil condition. Three controls were included: one with distilled water (2 mL) (tested using *I. nodosa*); one where DCM had evaporated from the filter paper and distilled water (2 mL) added (tested using *I. nodosa*, *A. longifolia* var. *sophorae* and *B. integrifolia*); and the third tested the effect of an extract derived from clean resin bags (using *A. longifolia* var. *sophorae* and *B. integrifolia* assays). Petri dishes were sealed with Parafilm® and incubated in a diurnal (12 h/12 h) temperature (15/25°C) and light (Six 8 Watt cool white fluorescence tubes) regime. Percentage germination and seedling root and shoot length were measured after 23 days.

Statistical analyses

Comparison of the chemical composition of each extract

Differences between the total weights of each extract and the amounts of each compound in each extract ($n=5$) in each condition ($n=3$) were compared using one-way ANOVAs with habitat (*C. monilifera* spp. *rotundata*, *A. longifolia* var. *sophorae* and bare sand) as a fixed factor (SPSS 2003). The Student-Neumann-Keuls (SNK) test was conducted to test differences between habitats.

An ANOSIM (PRIMER 2001) was used to determine whether the chemical signatures of each habitat significantly differed from each other. A similarity percentage analysis (SIMPER; PRIMER 2001) quantified the level of similarity between the hydrophobic chemical signatures of the habitats with five replicates (plants or bare sand patch) per habitat.

Seedling growth bioassay

Comparison of the effect of the water and DCM controls on *I. nodosa* root and shoot length was assessed using ANOVA (SPSS 2003). The effects of the resin bag extract (5 ppm) and the DCM control on the germination and seedling growth of *B. integrifolia* and *A. longifolia* var. *sophorae* were compared using a t-test (SPSS 2003).

The effects of increasing concentrations of the *A. longifolia* var. *sophorae* soil, *C. monilifera* spp. *rotundata* soil and bare sand extracts on the germination percentages of *I. nodosa*, *B. integrifolia* and *A. longifolia* var. *sophorae* were assessed using probit analysis (SPSS 2003). Pearson's goodness of fit test was used to ascertain whether the regression models adequately fit the data. A Z score was used to investigate whether the slopes differed from zero and a parallelism test was conducted to determine whether the slopes of the relationship between germination and concentration of each extract were similar. If the

two slopes were not parallel, then the relationship between germination and concentration was analysed to determine significance for each extract separately.

The effects of each extract on the root and shoot lengths of *I. nodosa*, *B. integrifolia* and *A. longifolia* var. *sophorae* were compared using ANOVA with habitat (*C. monilifera* spp. *rotundata*, *A. longifolia* var. *sophorae* and bare sand) as a fixed factor and concentration (zero from DCM control; 0.5 mg/ml; 1.5 mg/ml for *I. nodosa*; 2.5 mg/ml) as a covariate in the model (SPSS 2003). Post hoc SNK multiple comparisons tests between habitats and concentrations were conducted when significant differences were detected by the ANOVA.

Results

Comparison of the chemical composition of each extract

The resin bags extracted similar weights of material from soil below *A. longifolia* var. *sophorae* and *C. monilifera* spp. *rotundata* canopies, and significantly less from the bare sand condition ($F_{2,12}=16.26$, $P<0.001$; Fig. 1).

An ANOSIM, using the weight of the different hydrophobic compounds in the soil determined from the gas chromatograms (Fig. 2), identified differences in the chemical signatures of the different habitats (Global $R=0.133$, $P=0.03$). Only an alkane series trace was detected in the resin bag control extract. Pairwise comparisons and SIMPER analysis detected that the chemical signatures of soil from *A. longifolia* var. *sophorae* and bare areas were not significantly different (Global $R=-0.028$, $P=0.563$, 26.9% dissimilarity), however the soil in the *C. monilifera* spp. *rotundata* habitat had a significantly different signature to the *A. longifolia* var. *sophorae* (Global $R=0.204$, $P=0.04$, 30.9% dissimilarity) and bare sand conditions (Global $R=0.276$, $P=0.04$, 28.1% dissimilarity). Compounds common to all conditions included: alkanes, alkanols, fatty acids and some phytosterols. One-way ANOVAs indicated that significantly higher concentrations of 18 terpenes were found in *C. monilifera* spp. *rotundata* invaded soil, while higher concentrations of a phenolic compound were found in *A. longifolia* var. *sophorae* dominated soil and more fatty acids were found in the bare sand compared to the other habitats (Table 1).

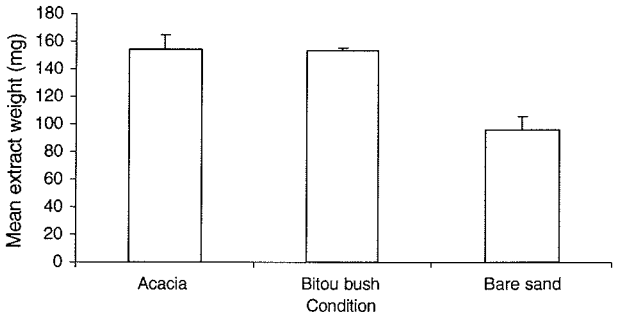

Fig. 1 Mean weights of each extract (five resin bags pooled) from the acacia, bitou bush and bare sand conditions. Error bars represent one standard error. Difference letters denote significant differences

Seedling growth bioassay

There was no difference between the effect of the water and DCM controls on the shoot length ($F_{1,6}=0.78$; $P=0.410$) and root length ($F_{1,6}=0.16$; $P=0.707$) of *I. nodosa*. The effect of the resin bag extract (2.5 mg/ml) did not differ from the effects of the DCM control on the germination and seedling growth of *A. longifolia* var. *sophorae* (germination: $t_{1,1}=3.67$, $P=0.17$; shoot length: $t_{1,1}=9.40$, $P=0.07$ root length: $t_{1,1}=2.57$, $P=0.24$) and *B. integrifolia* (germination: $t_{1,1}=3.13$, $P=0.20$; shoot length: $t_{1,1}=4.33$, $P=0.14$; root length: $t_{1,1}=8.04$, $P=0.08$).

I. nodosa seed germination was not significantly affected by the *A. longifolia* var. *sophorae*, *C. monilifera* spp. *rotundata* and bare sand extracts ($Z=0.25$; $P=0.803$; Fig. 3). Overall, *A. longifolia* var. *sophorae* germination was promoted by increasing concentrations of the extracts ($Z=2.23$; $P=0.026$), although not above the control level (Fig. 3). *B. integrifolia* germination was inhibited by all extracts ($Z=-4.20$; $P<0.01$), most notably, to approximately 60% of the control by the *C. monilifera* spp. *rotundata* and *A. longifolia* var. *sophorae* soil extracts at 0.5 mg/ml (Fig. 3). High unexplained variability in germination resulted in significant deviations in the Goodness of fit tests, indicating that the models did not tightly fit the data (analyses not presented). Despite this high variability, regression coefficients and tests of differences in slopes between extract species yielded significant differences indicating that while only a small proportion

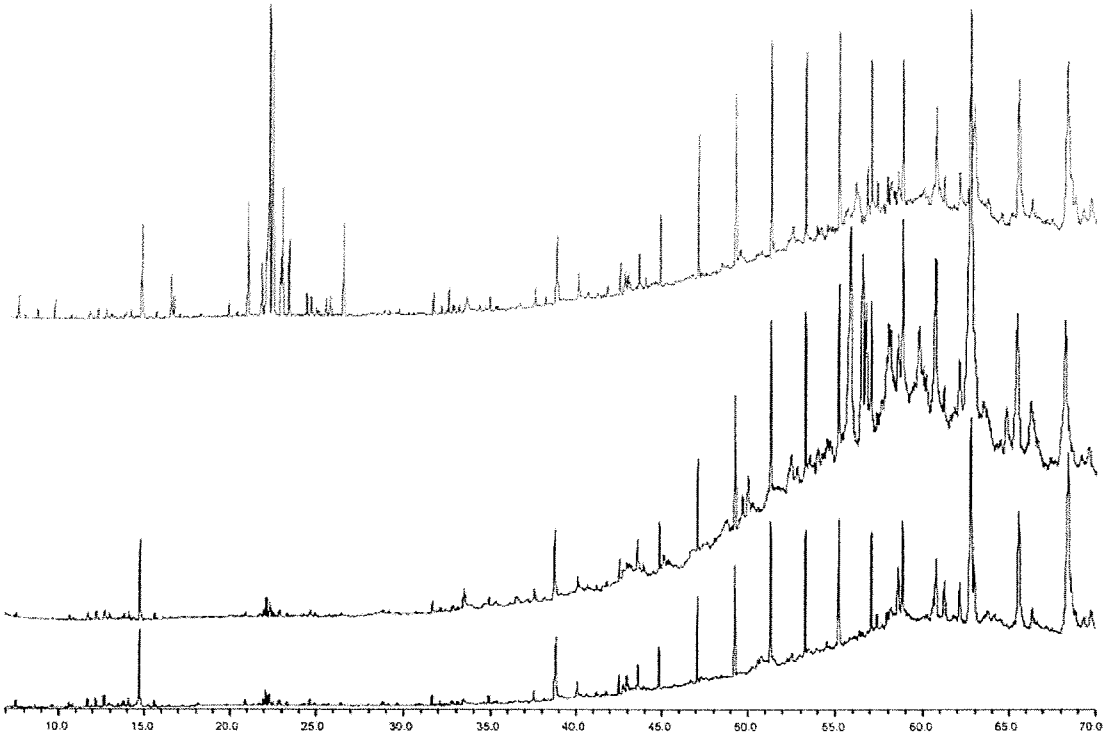

Fig. 2 Representative overlaid gas chromatograms of the *C. monilifera* spp. *rotundata* (*top*), *A. longifolia* var. *sophorae* (*middle*) and bare sand (*lower*) extracts

of the variability is explained by the treatments, it is nevertheless a predictable component.

Extract concentration overall had a significant inhibitory effect on the root length ($F_{2,85}=11.34$; $P<0.001$) and shoot length ($F_{2,85}=5.61$; $P=0.005$) of *A. longifolia* var. *sophorae* (Fig. 3). SNK tests showed that the shoot length of *A. longifolia* var. *sophorae* was significantly higher in the DCM control compared to those exposed to the 2.5 mg/ml concentrations of all extracts, whereas the *A. longifolia* var. *sophorae* root length was highest in the 1.5 mg/ml concentrations of all extracts. There were no differences between the effects of the different extracts on *A. longifolia* var. *sophorae* seedling growth (root: $F_{2,85}=0.10$; $P=0.904$; shoot: $F_{2,85}=0.41$; $P=0.664$). In contrast, the shoot length of *B. integrifolia* was significantly lower when exposed to the bitou bush and *A. longifolia* var. *sophorae* soil extracts compared to the bare sand extract ($F_{2,86}=6.42$; $P=0.003$), although lengths were above those of the control (Fig. 3). However, the mean *B. integrifolia* root length did fall to approximately 50% of the control length at 2.5 mg/ml of the *A. longifolia* var. *sophorae* soil extract, while there was no significant change in root length with increasing concentration of the bare sand extract (Fig. 3). The root ($F_{2,163}=18.20$; $P<0.001$) and shoot ($F_{2,184}=3.92$; $P=0.022$) length of *I. nodosa* were significantly affected by extract type: the *A. longifolia* var. *sophorae* soil extract reduced the shoot length more than the *C. monilifera* spp. *rotundata* soil and bare sand extracts which elicited similar effects, while *I. nodosa* root length was significantly greater in the bare sand extract that both the *A. longifolia* var. *sophorae* and bitou bush soil extracts (Fig. 3). Additionally, increasing concentrations of extract inhibited the root length of *I. nodosa* ($F_{2,163}=4.82$; $P=0.009$), so that the mean root length was reduced to less than 60% of the control by the *C. monilifera* spp. *rotundata* and 50% by the *A. longifolia* var. *sophorae* soil extracts (Fig. 3).

Discussion

The present study demonstrated that different plants are likely to induce unique soil chemical signatures which can function as mediators of plant-plant interference competition. Despite varying levels of susceptibility, *I. nodosa* and *B. integrifolia* seedlings showed a trend for increased seedling growth inhibition with

Table 1 Mean percentage of, and ANOVA results comparing the proportional composition of each compound found to significantly differ between conditions

Compound	Retention time (mins)	Mean percentage of each compound in each condition			*F* ratio (*df* =2,13)	*P* value	Post hoc tests
		Acacia (A)	Bare sand (Ba)	Bitou bush (B)			
α-pinene	7.465	0.08	0.14	0.38	26.54	**<0.001**	A = Ba < B
camphene	7.833	0.01	0.01	0.05	16.26	**<0.001**	A = Ba < B
β pinene	8.571	0.02	0.02	0.10	15.08	**0.001**	A = Ba < B
3-carene	9.587	0.03	0.03	0.30	46.41	**<0.001**	A = Ba < B
Branched alkane	15.539	0.13	0.05	0.18	4.71	**0.031**	Ba ≤ A≤ B
3-methoxy-*p*-cymene	16.393	0.05	0.08	0.38	12.99	**0.001**	A = Ba < B
2-methoxy-*p*-cymol	16.583	0.04	0.04	0.16	8.10	**0.006**	A = Ba < B
carvacrol ethyl ether	19.820	0.03	0.03	0.14	15.95	**<0.001**	A = Ba < B
7-epi-silphiperfol-5-ene	20.898	0.13	0.14	0.83	7.92	**0.006**	A = Ba < B
(+)-cycloisosativene	21.715	0.06	0.10	0.54	12.69	**0.001**	A = Ba < B
copaene	21.947	0.15	0.21	0.76	7.06	**0.009**	A = Ba < B
maaliene	22.094	0.35	0.47	3.08	9.06	**0.004**	A = Ba < B
α isocomene	22.169	0.25	0.32	2.35	8.47	**0.005**	A = Ba < B
humulene	22.994	0.13	0.19	1.12	11.22	**0.002**	A = Ba < B
cymene	23.171	0.07	0.05	0.48	4.79	**0.030**	A = Ba < B
allo-aromadendrene	24.660	0.18	0.16	0.33	4.35	**0.038**	A = Ba < B
pentadecene	25.739	0.04	0.02	0.18	6.25	**0.014**	A = Ba < B
5-methoxycalamenene	32.483	0.10	0.08	0.11	4.29	**0.039**	A = Ba < B
5-hydroxycalamenene	36.199	0.04	0.03	0.05	4.15	**0.043**	Ba ≤ A≤ B
Palmitic acid	38.798	1.01	2.49	1.94	6.652	**0.011**	A ≤ B≤ Ba
Margaric acid	41.132	0.05	0.08	0.13	3.41	0.067	Ba < A<B
manool	42.717	0.19	0.36	0.50	7.47	**0.008**	A ≤ Ba ≤ B
9-hexadecenoic acid	42.909	0.18	0.50	0.43	4.81	**0.029**	A ≤ B≤ Ba
a phenol	58.080	2.89	1.71	1.50	12.36	**0.001**	Ba = B<A

increasing concentration of the *A. longifolia* var. *sophorae* and *C. monilifera* spp. *rotundata* soil hydrophobic extracts, demonstrating the potential for soil chemical interference, or allelopathy. This finding suggests that chemical interference competition may influence both species dominance within indigenous plant communities and facilitate exotic plant invasion in sand dune vegetation communities of low resource environments such as the coastal sand dune system of this study.

Previous studies have shown that both indigenous *A. longifolia* var. *sophorae* (Costello et al. 2000) and South African *C. monilifera* spp. *rotundata* (Mason and French 2008) have the potential to form monospecific stands and inhibit indigenous species recruitment (Ens and French 2008). To elucidate the mechanisms facilitating this inhibition, we have previously found that in winter, hydrophobic soil extracts from *C. monilifera* spp. *rotundata* inhibited the growth of indigenous species more than comparable extracts from *A. longifolia* var. *sophorae* soil (Ens et al. 2009). In contrast, the present study found that during spring, hydrophobic compounds in the soil surrounding *A. longifolia* var. *sophorae* and *C. monilifera* spp. *rotundata* roots similarly inhibited the growth of indigenous species. Hence we propose that chemical interference between plants in this system may follow seasonal or climatic dynamics and that *C. monilifera* spp. *rotundata* induces a longer period of soil chemical interference than *A. longifolia* var. *sophorae*. The present extracts were obtained from *C. monilifera* spp. *rotundata* soil in late spring (November) when

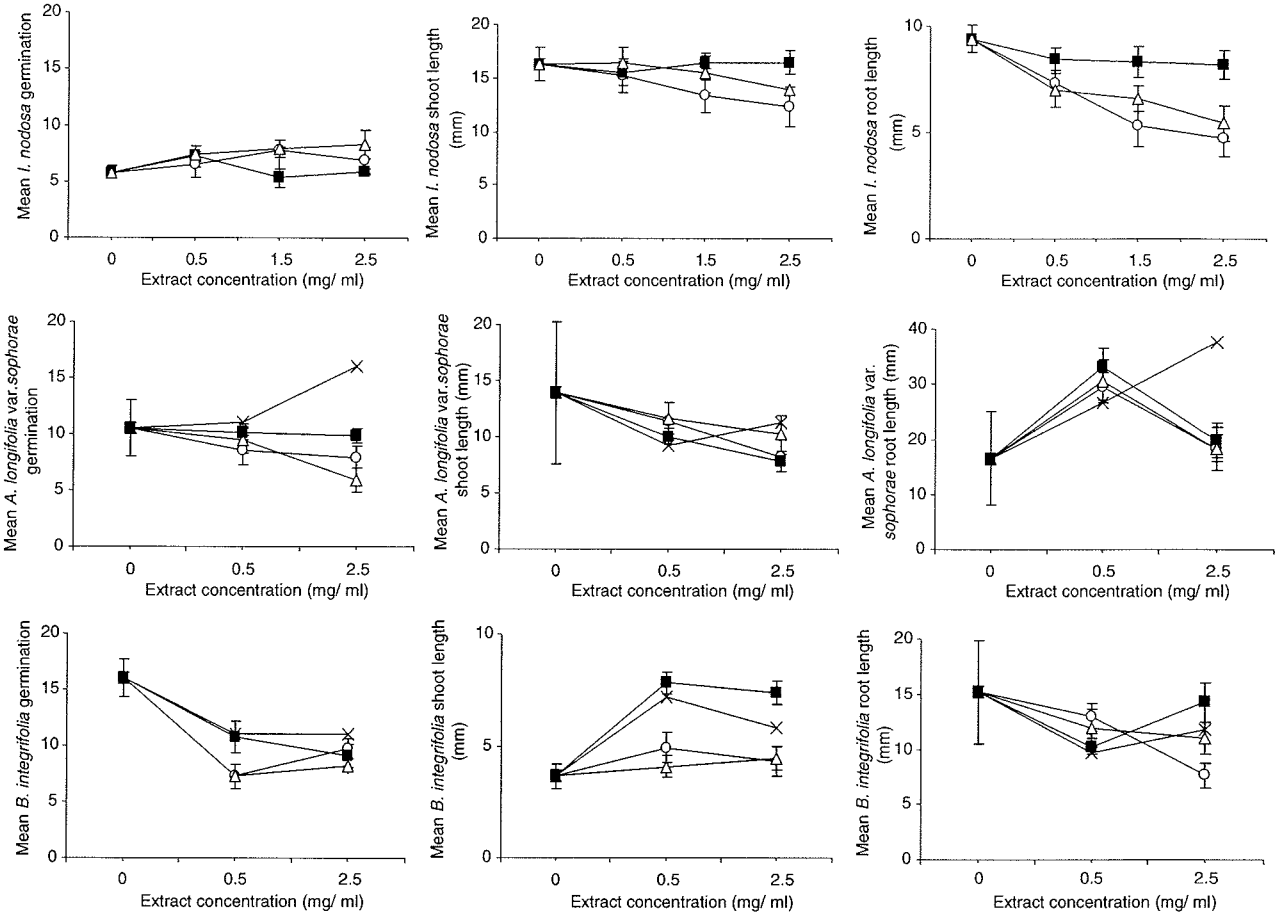

Fig. 3 The mean germination, shoot lengths and root lengths of *I. nodosa*, *A. longifolia* var. *sophorae* and *B. integrifolia* with increasing concentrations of the bare sand (■), acacia soil (○), bitou bush soil (Δ) and resin bag control (x; for *A. longifolia* var. *sophorae* and *B. integrifolia* only) extracts. The zero values represent the DCM control values. *Error bars* represent one standard error

the hydrophobic chemical profile was much the same as that detected in winter (July) (Ens et al. 2009). This is logical considering the persistent growth, reproduction and hence metabolism of *C. monilifera* spp. *rotundata* throughout the year (Weiss 1984). However the *A. longifolia* var. *sophorae* soil chemistry differed markedly between the winter and spring sample times, which may be a reflection of the down regulation of the metabolism and growth of this species as observed during winter (Ens personal observation). In winter, the *A. longifolia* var. *sophorae* soil contained an alkane series and fewer terpenes (Ens et al. 2009). Correspondingly, there were less widespread effects of the *A. longifolia* var. *sophorae* extracts in the winter study compared to the present spring study which coincides with the detected changes in types of terpenes and alkane levels. This hypothesis is supported by other studies which have shown that terpene release follows seasonal and climatic patterns (Chou 1999; Kuhn et al. 2004; Asensio et al. 2007) and that phenols are released by plants in more humid environments (Chou 1999).

Based on our chemical interference studies to date, we suggest that *C. monilifera* spp. *rotundata* is likely to have a greater recruitment limitation effect than *A. longifolia* var. *sophorae* in winter although both species have the potential to inhibit the establishment of *A. longifolia* var. *sophorae, B. integrifolia* and *I. nodosa* in spring. All of the test species involved in the present study are likely to experience increased germination rates from winter to spring in accordance with the increasing temperatures and rainfall. This is particularly the case for *B. integrifolia* which seeds during winter and spring and has low seed bank viability (Weiss 1984). *I. nodosa* typically seeds in summer, although may seed throughout the year and

is expected to have low soil seed bank longevity (Ens personal observation). We therefore suggest that a reduction in the regeneration potential of *B. integrifolia* and *I. nodosa* is likely to occur as a result of coincidental seedling establishment times and soil chemical interference effects found for *C. monilifera* spp. *rotundata* in winter and spring and by *A. longifolia* var. *sophorae* in spring. Conversely, although the frequency of *A. longifolia* var. *sophorae* individuals in *C. monilifera* spp. *rotundata* invaded sites has been shown to be significantly lower than non-invaded sites (Mason and French 2008), the long lived seed bank (Weiss 1984) and warmer germination requirements suggest that increased interference by *C. monilifera* spp. *rotundata* against the establishment of *A. longifolia* var. *sophorae* in winter or spring is not likely. Future research on the chemical and inhibitory properties of soil extracted in summer and autumn is therefore suggested as well as the interrelationships between resource and interference competition as mechanisms of indigenous plant recruitment limitation in this system.

In spring, the differences between the hydrophobic soil chemical profile of the exotic invasive shrub, *C. monilifera* spp. *rotundata,* the indigenous *A. longifolia* var. *sophorae* and the bare sand were primarily due to the higher concentrations of 18 sesquiterpenes in the *C. monilifera* spp. *rotundata* soil extract. Therefore we propose that it is the identity of the terpenes which is likely to help explain the chemical interference observed in winter rather than the concentrations as higher concentrations of the terpenes found in *C. monilifera* spp. *rotundata* did not inhibit seedling growth any more than the lower concentrations of the same terpenes found in *A. longifolia* var. *sophorae* in spring.

Individual sesquiterpenes have been shown to have antimicrobial properties (Phillips and Croteau 1999; Melcher et al. 2003; Scher et al. 2004) which may also confer a competitive advantage to species which exude them on the sand dunes where mycorrhizal and bacterial symbioses are important for indigenous plant survival (Logan et al. 1989; Abe and Ishikawa 1999). Similarly, certain terpenes can play a role in herbivore defense (Phillips and Croteau 1999) and are therefore potentially insect deterrents which could also have indirect implications for the pollination and dispersal of indigenous species. The presence of high concentrations of sesquiterpenes found in soil associated with *C. monilifera* spp. *rotundata* in winter may therefore have indirect effects on the functioning and species composition of the indigenous ecosystem as well as the direct effects found on seedling growth (Ens et al. 2008, 2009). Therefore further investigation into the complex interactions between *C. monilifera* spp. *rotundata* derived soil compounds and the indirect effects on establishment or growth of indigenous plant species via effects on microbial symbiotic relationships, pollination success and propagule dispersal are required to facilitate full understanding of the mechanisms of *C. monilifera* spp. *rotundata* invasion and indigenous species replacement.

Not only does the addition of compounds by an exotic plant have the potential to affect the resident vegetation community, the absence of key compounds in invaded systems may also drive species compositional shifts through both direct and indirect mechanisms. In this study, the *A. longifolia* var. *sophorae* soil extracts were distinguished by significantly higher amounts of an unidentified phenolic compound compared to the bare sand and *C. monilifera* spp. *rotundata* soil extracts. The presence of phenolic compounds in the indigenous vegetated system may be integral to the indigenous community as various phenolics are known to affect litter decomposition (Hattenschwiler and Vitousek 2000), nutrient cycling (Hattenschwiler and Vitousek 2000), certain microbes (Inderjit and Dakshini 1991; Hattenschwiler and Vitousek 2000; Souto et al. 2000; Seneviratne and Jayasinghearachchi 2003), and to act as allelopathic (Inderjit and Dakshini 1991; Leu et al. 2001; Chon et al. 2002) and anti-herbivore (Buchsbaum et al. 1984) agents. Thus, mechanisms of interference by indigenous dominant species may be different from that of exotic species, however, dominance by either may rely on chemical interference.

Additionally, the chemical profile of the bare sand was characterised by high concentrations of n-hexadecanoic (palmitic) acid and 9-hexadecenoic acid (Table 1; Fig. 1). Palmitic acid is a common plant (Liu and Huang 2004) and fungal (Ruess et al. 2005; Trepanier et al. 2005) fatty acid, which is transferred to small animals, such as collembola, via fungi (Ruess et al. 2005). Bacteria such as *Aspergilli* spp. are inhibited by palmitic acid (Altieri et al. 2007). The presence of palmitic acid in bare sand patches suggests the absence or lack of small animal activity and possibly an altered microbial community compared to vegetated areas of the coastal sand dune systems studied.

One of the advantages of this method is that any hydrophobic compounds in the soil will be adsorbed to the resin. Hydrophobic soil compounds may include root exudates, compounds washed from leaves, plant decomposition products, microbially transformed plant products, and in this case, marine derived compounds and any subsequent biotic or abiotic transformations of these compounds. However the current method only adsorbed compounds that came into contact with the resin bag itself, including mobile compounds which move through the soil profile across the plane of the resin bag. Hence, the method presented does have limitations associated with the depth and location of resin bag burial. To emulate potential field based allelopathy, the resin bags should be buried at a depth where seeds are likely to germinate or where seedling roots occur. The present research was based in a sand dune system where the sand surface is dynamic and seeds are likely to drop down into the sand based on high sand porosity and continual movement of sand grains. Based on prior experiences in this system, a depth of 10 cm was chosen to bury the resin bags as we suspect that this is where plant derived compounds and seedling roots are likely to co-occur. Resin bag burial depths should be selected based on soil surface stability and germination and/ or seedling growth depths. This method delivers a "snapshot" of compounds as collected by the resin bags during a specific time period which can then tested for bioactivity in the laboratory. Further limitations may arise here as a result of continued breakdown and transformation of compounds in the laboratory environment and possible lack of replenishment of the original compounds. Modifications of this method could be developed to continually trap products in line with methods developed by Tang and Young (1982), or to trap products of specific origin, such as root exudates. Additionally, repeated application of this method throughout different seasons and years could better elucidate temporal dynamics in soil chemical profiles.

This study demonstrated that different species are associated with distinct soil chemical signatures and that plant-soil chemistry has the potential to influence plant community composition. Exotic invasive plants can potentially have a deleterious effect on indigenous plant communities if they create a soil chemical environment which differs greatly from the pre-invasion condition. We found that although *C. monilifera* spp. *rotundata* had a unique soil hydrophobic chemical signature compared to indigenous *A. longifolia* var. *sophorae*, in the laboratory setting these two extracts had similar effects on the seedling growth of several indigenous species. We therefore suggest that the identity rather than the concentration of the sesquiterpenes detected in the present study is likely to influence seedling growth and that the concentration of sesquiterpenes exuded from plants follows temporal changes. Moreover, seedlings of two test species grew better when exposed to unvegetated soil hydrophobic extracts than vegetated soil hydrophobic extracts, suggesting that sesquiterpenes, which were ubiquitous in the plant extracts, influence indigenous species establishment in this system. Further application of this rapid soil chemical capturing technique and pot trials would elucidate the seasonal and spatial dynamics of the soil chemistry in invaded and non-invaded dunes. Additional bioassays of other important system components, such as mycorrhizal fungi and soil microbial populations, coupled with longer term growth studies, may help clarify the effects of soil chemicals in this system.

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