Response of soil fungal richness and composition to *Lantana camara* L. infestation in the Toowoomba region, South-East Queensland, Australia

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Abstract

Infestations by the weedy perennial plant *Lantana camara* L. (lantana) cause major disruptions to biodiversity; however, the effects of lantana on soil fungal communities are poorly understood, yet may interrupt important mutualisms in natural and semi-natural ecosystems. This research is aimed at understanding the impacts of lantana on the soil fungal community of five sites on contrasting soils in the Toowoomba region of south-east Queensland. Soil samples were collected from paired lantana infested and non-infested plots and the soil fungal community ascertained via analysis of terminal restriction fragments (TRFs) of isolated fungal DNA samples. Soils from these plots were also sampled for a range of chemical characteristics. Forty-eight identifiable fungal TRFs were recovered from the T-RFLP analysis. Mean fungal taxon richness showed no significant differences between lantana infested and non-infested plots across the range of sites examined. However, one site on nutrient-poor granitic soils had significantly higher (ANOVA, $p<0.001$) fungal taxon richness than other sites on other soils. While a number of fungal taxa were ubiquitous across all sites and lantana infested and non-infested plots, some taxa were exclusively found in either infested or non-infested plots at one site, suggesting some association between fungal composition and infestation. We conclude that there are no differences in the fungal taxon richness between lantana infested and non-infested plots, but a significant difference in mean fungal taxon richness between sites representing contrasting soil types. Further analysis of fungal composition may reveal more conclusive patterns regarding the possible effects of lantana invasion on soil fungal assemblages. Further research is required to confirm compositional differences with respect to lantana infestation and determine whether such effects are the direct effects of allelopathy or indirect effects of changes to soil properties.

Key words: lantana, soil fungi, T-RFLP, allelopathy, *Eucalyptus* forest, microbial diversity.
Subject Editor: Tom May.
Published online: 5 April 2013,

Introduction

Lantana (*Lantana camara* L.) is a major pest weed in over 60 countries and is one of the 10 worst weeds worldwide (Sharma *et al.* 2005). In Australia, lantana is a weed of national significance, having invaded at least 4 million hectares of predominantly coastal and sub-coastal ranges of eastern Australia (Parsons & Cuthbertson 2001). Lantana infestations have major impacts on biodiversity both in Australia (e.g. Fensham *et al.* 1994; Gooden *et al.* 2009) and elsewhere (e.g. Bhatt *et al.* 1994; Sundarum & Hiremath 2012; Prasad 2012). While the magnitude of effects in Australia is yet to be fully determined, an interim list of species and ecological communities identified a total of 1246 native vascular plant species and 141 animal species as being at risk from lantana in New South Wales and Queensland alone (Department of Environment and Climate Change 2008).

Establishment and persistence of lantana may be partly driven by allelopathy (Mersie & Singh 1987; Achhireddy *&* Singh 1984; Gentle *&* Duggin 1997a, b). Allelopathy is a form of interference competition where a plant releases chemicals into its immediate environment that suppresses the germination and/or growth of neighbouring seedlings and which may limit their survival (Wardle *et al.* 1998). Known as the 'novel weapons' or 'allelopathic advantage against resident species' hypothesis (Bais *et al.* 2003), allelopathic chemicals released by some invasive species may be novel to resident native species. The lack of resistance or tolerance among indigenous plants provides a competitive advantage to the invader (Callaway & Ridenour 2004).

Soil fungi play a key role in ecosystems, influencing a large number of important processes including plant nutrient acquisition, carbon cycling and soil formation (van der Heidjen *et al.* 2008). Soil fungi can also be impacted by plant allelochemicals (Rose *et al.* 1983; Perry and Choquette 1987; Cote *&* Thibault 1988). Rose *et al.* (1983) found high concentrations of water soluble extracts of the litter of four shrub and three conifer species either stimulated ectomycorrhizal fungal growth, inhibited growth, or had no effect depending on both fungal and litter species. Tissue

extracts of lantana may inhibit a range of fungal species, including saprotrophic and plant pathogenic taxa, and reduce overall fungal diversity (Sharma *et al.* 2007). Contrastingly, amendment of soils with lantana leaf extracts increased the number of soil fungal species present in pot trials, though it inhibited interactions between plant roots and endophytic fungal taxa (Shaukat & Siddiqui 2001).

This research is aimed at understanding the possible impacts of lantana infestation on the soil fungal community of 5 sites on contrasting soil types in south-east Queensland. We specifically test the hypothesis that soil fungal richness and composition are different across lantana infested and non-infested plots. In this study, we conducted an analysis of terminal restriction fragments of isolated fungal DNA samples from soils of paired lantana infested and non-infested plots to determine patterns in fungal species composition and diversity. Further, to assist with our interpretation of the molecular analyses, a range of soil chemical characteristics were also determined and compared across site and lantana treatments.

Materials and methods

Field sites and acquisition of soil samples

The study was undertaken in the Toowoomba region, south-east Queensland (Fig. 1). Five study sites of *Eucalyptus* open forest/woodland vegetation on contrasting soil types, consisting of a lantana-infested plot and a nearby undisturbed (non-infested) plot were selected. The distance between paired plots ranged between 50 and 150 m. Lantana infested plots contained between 65-80% foliage projective cover of lantana; non-infested plots contained <2% foliage projective cover of lantana and most contained no lantana. Site one (Upper Flagstone Creek) was located on sandstone soil, site three (Crows Nest National Park) on granitic soil, while sites two (Duggan Park) and four (Geham National Park) were on basaltic red soils (Fig. 1). Site five was located on alluvium black soil at Felton, south west of Toowoomba (Fig. 1). At each site a 10 x 10 m quadrat was established. Ten 1 g soil samples for T-RFLP fungal analysis were obtained randomly (using random numbers) with a 5 cm diameter steel soil corer to a maximum depth of 10 cm from within each of the plots. Three soil samples were randomly collected within each plot, bulked and stored at room temperature prior to chemical analysis.

T-RFLP analysis of soil fungal communities

Fungal DNA was extracted from 0.25 g of soil using a GeneworksPowersoil DNA extraction kit (Geneworks, Thebarton, South Australia) as per the manufacturer's instructions. PCR amplification of fungal ITS DNA involved 50 μl reaction volumes, each containing 38 μl sterile distilled H_2O, 5 μl 10X buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100; Invitrogen Australia, Mt Waverley, VIC, Australia), 2.5 μl 50 mM $MgCl_2$ (Invitrogen

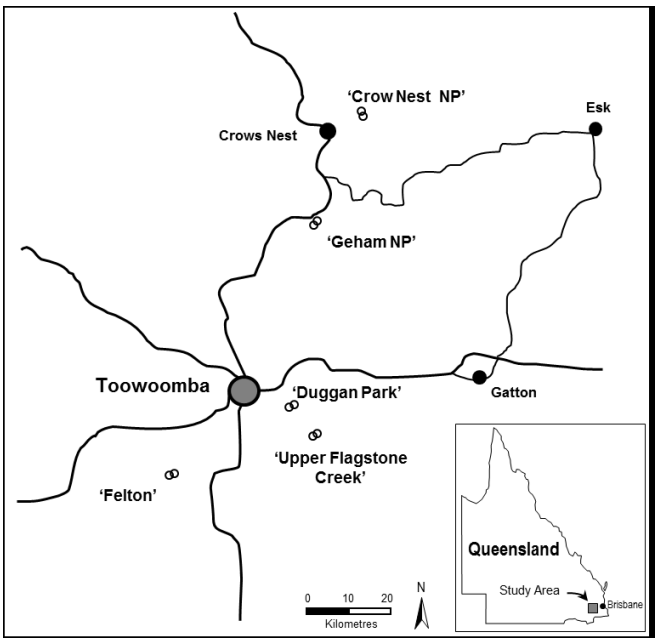

Fig 1. Map of study area showing location of study sites.

Australia), 1μl 10 mM dNTP (Invitrogen Australia), 1 μl of each of a fluorescein-labeled ITS1F primer (Gardes & Bruns 1993) and a ITS4 (White *et al.* 1990) primer, 0.5 μl of *Taq*DNA polymerase (Invitrogen Australia) and 1 μl of extracted genomic DNA. PCR was carried out in a Thermo Hybaid PCR Express thermocycler (Integrated Sciences, Willoughby, NSW, Australia) with 35 cycles of 95°C for 1 min, 50°C for 1 min and 72°C for 1 min, and a final incubation at 72°C for 10 min. PCR reactions were duplicated and negative controls were included without DNA. PCR products were electrophoresed in 2% (w/v) agarose gels with ethidium bromide and visualised under UV light. Purification of ITS-PCR products involved use of a DNA purification kit (Macherey-Nagel, Cheltenham, Australia) as per the manufacturer's instructions.

For T-RFLP analysis 100-200 ng PCR product (estimated visually from the electrophoretic gel) was placed in a microcentrifuge tube with approximately 13 μl sterile distilled H_2O, 1 μl of Taq1 restriction enzyme (Promega Australia, Annandale, NSW), 2 μl of 10x buffer (Promega) and 2 μl 0.1% BSA (Promega). Sample tubes were incubated for 2 hours at 37°C and the digest stopped by increasing temperature to 80°C for 5 min. To purify the digests, 18 μl of restricted DNA sample was added to 4 μl 3 M NaOAc (pH5.2) and 18 μl isopropanol. After mixing by inversion, samples were incubated for 10 min at room temperature then centrifuged at 13,200 rpm for 10 min. Following supernatant removal, 40 μl of 70% ethanol was mixed into the sample and the tubes were incubated for 5 min at room temperature. After centrifugation at 13,200 rpm for 5 min, the supernatant was removed from the samples and the tubes were air dried. Next, 20 μl of sterile distilled H_2O, was added to the tubes and, after re-suspension of the DNA samples, a 10 μl aliquot was removed and sent to the Melbourne

node of the Australian Genome Research Facility for fragment analysis.

The resultant terminal restriction fragments were used to determine fungal species ('entities'). Fragments were considered to be separate entities if they were greater than 1.5 base pairs (bp) difference in size. To remove background 'noise', fragments less than 25 bp and above 510 bp were omitted. The number of fungal entities was compared between paired lantana infested and non-infested plots across sites through a two-factor analysis of variance using SPSS Version 15 (SPSS Inc. 2006).

Soil chemical analyses

Soil chemical characteristics were determined from bulked soil samples by SGS Agritech, Toowoomba using standard methods: pH, nitrate, ammonium, potassium, calcium, magnesium, sodium, chlorine, cation exchange capacity (Rayment & Higginson 1992), phosphorus (Colwell 1963), and organic carbon (Walkley and Black 1934). Soil chemical characteristics were compared between paired lantana infested and non-infested plots across sites through a two-factor analysis of variance and independent samples T-tests (SPSS Version 15, SPSS Inc. 2006).

Results

T-RFLP analysis of soil fungal communities

Across all samples, 48 identifiable fungal terminal restriction fragments (TRFs) were recovered from the T-RFLP analysis (Table 1). A maximum of 17 fungal TRFs was found in a single Crows Nest sample, while five samples contained only a single fungal species. The highest total number of fungal TRFs (35 entities) was in the Crows Nest lantana infested treatment, while the second highest total number (28 entities) was in the Crows Nest non-infested treatment (Table 1). The Felton and Duggan Park lantana infested treatments contained the lowest total number of fungal TRFs (9 entities) (Table 1).

Five TRFs (1, 2, 3, 17 and 19) were ubiquitous and found across all sites and lantana treatments and a further two TRFs (10 and 18) were found in nine of the ten site/treatment combinations (Table 1). Twenty-eight TRFs were exclusive to Crows Nest samples, of which nine TRFs were found exclusively in Crows Nest lantana infested samples (TRFs 21, 22, 36, 38, 39, 41, 43, 45 and 47) and nine were exclusive to Crows Nest non-infested samples (TRFs 28, 29, 31, 33, 35, 42, 44, 46, 48; Table 1). One TRF (25) was present in four of the five lantana infested sites; although this TRF was also found in a single sample in the Duggan Park non-infested site. Apart from two TRF single occurrences (TRFs 20 and 26) in one Felton non-infested sample, there were no other TRFs that were exclusive to sites other than the Crows Nest sites. Twenty-five samples spread across all sites (except Geham) and both lantana infested and non-infested plots contained no fungal TRFs.

The mean number of identifiable fungal TRFs from T-RFLP analysis of soils across lantana treatments in the study sites is shown in Figure 2. Two-factor analysis of variances comparing numbers of identifiable fungal TRFs showed there was no significant difference between lantana infested and non-infested plots ($p>0.05$); however, the Crows Nest plots contained a significantly higher number of fungal entities than other sites ($p<0.001$; Table 2; Fig. 2).

Soil chemical analyses

A summary of soil attributes across study sites and lantana treatments is shown in Table 3. Soil chemical characteristics were highly variable across study sites and lantana treatments. Two-factor analyses of variance showed the interaction term to be significant for many soil variables, preventing formal statistical comparisons of main effects (site and treatment). Paired comparisons within sites showed no significant differences between lantana infested and non-infested plots (T-tests; $p>0.05$). Non-homogeneity of variances prevented statistical contrasts across sites; however, the Crows Nest soils contained generally lower concentrations of phosphorus, calcium, magnesium, potassium and lower cation exchange capacity (Table 3).

Discussion

There were no significant differences in the mean number of fungal entities (taxon richness) between lantana infested and non-infested treatments, suggesting the presence or absence of lantana does not influence fungal taxon richness across the broad range of soils examined. However, fungal richness was significantly higher in the nutrient-poor granitic soils (Crows Nest sites), regardless of lantana infestation. The soil at this site contained particularly low levels of magnesium and calcium, and a low cation exchange capacity, but also generally lower potassium and phosphorus. This is consistent with a number of studies that have shown a relationship between soil fungal richness and soil fertility with low levels of soil nutrients, including cations, increasing soil fungal populations, particularly ectomycorrhizal taxa (Toljander *et al.* 2006; Midgely *et al.* 2007). Increased soil fungal diversity is speculated to be critical to the productivity of nutrient poor natural landscapes (van der Heidjen *et al.* 2008).

In addition, over 58% of the fungal entities detected were exclusive to the Crow Nest samples, suggesting some specificity in relation to low fertility soils. Host-fungal specificity has been suggested as a mechanism leading to a plant-soil feedback where particular host species influence the composition of soil fungal communities (e.g. Mangan *et al.* 2010). Other studies have found that edaphic factors, such as soil nutrients, can also be important in determining fungal species composition (e.g. Kernaghan & Harper 2001; Martinez-Garcia *et al.* 2010). However, due to the lack of replication at the soil type level, the current study is

Table 1. Summary of terminal restriction fragments (TRFs) for fungal 'identifiable entities' across study sites and lantana treatments.

Site	Treatment	TRFs present	Total number of TRFs present
Crows Nest	No Lantana	1, 2, 3, 6, 7, 8, 9, 10, 16, 17, 19, 23, 24, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 40, 42, 44, 46, 48	28
	Lantana	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 25, 27, 30, 32, 34, 36, 37, 38, 39, 40, 41, 43, 45, 47	35
Felton	No Lantana	1, 2, 3, 4, 10, 11, 13, 16, 17, 18, 19	11
	Lantana	1, 2, 3, 10, 11, 17, 18, 19, 25	9
Geham	No Lantana	1, 2, 3, 4, 5, 9, 10, 11, 13, 15, 16, 17, 18, 19, 24	15
	Lantana	1, 2, 3, 4, 10, 11, 16, 17, 18, 19, 24, 25	12
Upper Flagstone	No Lantana	1, 2, 3, 4, 10, 11, 14, 15, 17, 18, 19, 20, 26	13
	Lantana	1, 2, 3, 4, 5, 9, 15, 16, 17, 18, 19, 24	12
Duggan Park	No Lantana	1, 2, 3, 4, 9, 10, 11, 14, 15, 16, 17, 18, 19, 24, 25	15
	Lantana	1, 2, 3, 10, 16, 17, 18, 19, 25	9

unable to distinguish between host plant specificity or soil type specificity in these *Eucalyptus* systems and further research is required.

Despite a lack of difference in soil fungal richness between lantana infested and non-infested plots across all sites, fungal composition, as inferred from TRF 'identifiable entities', suggests a number of patterns. While there are a number of fungal entities that were ubiquitous to all sites and lantana treatments, a number of entities were only found in either the Crows Nest lantana infested plot or the non-infested plot, suggesting that lantana infestation does influence fungal composition, at least at this site. This result is not consistent across all soil types examined and suggests that lantana infestation has a more significant effect on soil fungal community composition in the nutrient poor granitic soils. However, this study did not replicate across soil type and it is therefore not possible to conclude that patterns observed for the Crows Nest site are generalisable across all granitic soils more broadly. Further research is required to more conclusively test this hypothesis.

No significant differences in soil chemical properties between lantana infested and non-infested plots in the Crows Nest site, or other sites, were detected here. Osunkoya and Perrett (2011) report some soil properties, such as pH, calcium, organic carbon and total nitrogen to be elevated in lantana infested soils in south-east Queensland, although they also report site effects (related to vegetation type and surrounding land use) were more frequently observed than effects due to lantana infestation. Other studies have also found altered soil properties beneath lantana (e.g. Bhatt *et al.* 1994; Gentle & Duggin 1998; Sharma & Raghubanshi 2011). The discrepancy of these findings with the current study may be related to the history of lantana disturbance at the sites. The pairing of infested and non-infested plots in this study meant that the infested plots most likely coincided with the invasion front of lantana and may represent a more recent disturbance history which had yet to exhibit altered soil properties.

While there have been a number of studies that have reported ex situ allelopathic effects of extracts from various weed species on soil microbes, including fungi (e.g. Rose *et al.* 1983; Sharma *et al.* 2007), and the effects of lantana more broadly on above-ground plant communities in Australia (e.g. Fensham *et al.* 1994; Gooden *et al.* 2009), there is no published information on the allelopathic impacts of lantana on specific soil fungi. Further research is needed to determine whether the suggested changes in fungal composition observed here can be attributed to direct allelopathic effects of lantana or indirect effects through changes to soil properties.

In this study, the number of fungal identifiable entities (taxa) as TRFs in each sample was usually fewer than 10, with 25% of individual 0.25 g soil samples containing no fungal DNA fragments, or at least too few to be detected. This may be due to the inherent heterogeneity of fungal assemblages in these soils or possibly an artefact of the methodological approach. A study by Midgley *et al.* (2007) using the same T-RFLP approach showed that agricultural and natural soils in northern New South Wales had an average of between 8 and 29 terminal restriction fragments (identifiable entities) per sample. Midgley *et al.* (2011) further reported an average of between 15 and 36 TRFs per sample in a study comparing woodland reserves and grazed grasslands with cropped sites in northern-central New South Wales. These studies extracted fungal DNA from

Table 2. Summary of two-factor analyses of variance comparing mean fungal TRFs (identifiable entities) across study sites and lantana treatments.

Source	df	F	p	Tukey's test
Site	4	5.96	<0.001	CN[a] F[b] DP[b] G[b] UF[b]
Treatment (No lantana/Lantana)	1	0.12	0.73	n/a
Site*Treatment interaction	7	0.37	0.83	n/a
Total	73			

CN = Crows Nest; F = Felton; DP = Duggan Park; G = Geham; UF = Upper Flagstone Creek.
Homogeneity of variances was indicated (Levene's test, $p>0.05$); sites sharing the same superscript are not significantly different (Tukey's test; $p<0.05$).

1.5 g of soil, six times the soil volume than the present study, which may explain the relatively low numbers of fungal entities identified here. Ranjard*et al.* (2003) assessed soil sample sizes of between 0.125 g and 4 g and found that for some soils, samples less than 1 g exhibited high variability in fungal diversity. While there is no generally accepted standard for soil sample volumes, the spatial heterogeneity of fungal assemblages in soils (Franklin & Mills 2003; Kirk *et al.* 2004) necessitates some caution when extrapolating between studies. However, this should not preclude comparisons of relative fungal diversity and composition between specific treatments. Indeed, Ranjard *et al.* (2003) point out that smaller soil sample sizes encompassing greater heterogeneity may be preferable to determine the maximum extent of fungal diversity.

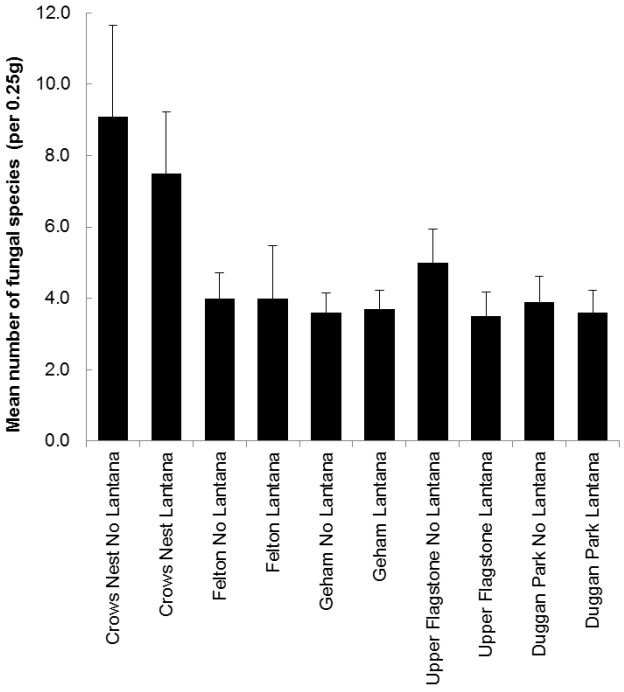

Fig 2. Mean number of fungal TRFs across study sites and lantana treatments. Error bars are standard errors.

In the present study, we compared paired lantana infested and non-infested plots across a range of soil types. The results suggest that the effects of soil type on fungal species richness, at least for the Crows Nest site (granitic soil), is stronger than any effects of the presence/absence lantana in the overstorey. However, some effect of lantana on fungal composition, as inferred from fungal TRFs, is indicated on the low fertility granitic soils of the Crows Nest site. We conclude that soil fungal diversity across open forest/woodlands of the Toowoomba region may be influenced by soil type and that the introduced shrub, lantana, may have an effect on soil fungal community composition within the Crows Nest site. Further research is needed to test whether lantana impacts on fungal communities may be more pronounced in low fertility soils. Characterisation of the terminal restriction fragments obtained in this study to better determine the species complement and functional types is a logical next step to fully characterise the possible impacts of lantana on indigenous soil fungal communities.

Acknowledgements

We thank Dr. Kate Reardon-Smith, Megan Batterham, and Peter Wagner for assistance with field work. We also thank the landholders who allowed access to their properties and made the research possible: Ken Cunliffe, Bernie McGovern, and Dr. Kate Reardon-Smith. The Australian Centre for Sustainable Catchments provided both logistic and some funding support for this project for which we are grateful. We also acknowledge the assistance of Toowoomba Regional Council, particularly Veronica Newberry, for their support of the work within council areas. Finally, we acknowledge the support of Drs Peter Turner and Paul Downey (NSW Department of Environment and Climate Change) for advice and financial support for components of this work.

Table 3. Mean (and standard error) of soil variables across study sites and lantana treatments.

Site No.	1		2		3		4		5	
Site Name	Upper Flagstone Creek		Duggan Park		Crows Nest NP		Geham NP		Felton	
Treatment	No Lantana	Lantana	No Lantana	Lantana	No Lantana	Lantana	No Lantana	Lantana	No Lantana	Lantana
pH	5.5 (0.10)	5.9 (0.13)	5.9 (0.04)	6.1 (0.04)	5.2 (0.23)	5.5 (0.10)	6.0 (0.02)	6.1 (0.18)	7.4 (0.08)	7.8 (0.01)
Ammonium (mg/kg)	0.7 (0.16)	2.1 (0.36)	2.9 (0.34)	2.2 (0.82)	1.4 (0.60)	1.9 (0.17)	2.7 (0.31)	3.6 (0.45)	1.0 (0.13)	2.2 (0.63)
Nitrate (mg/kg)	1.0 (0.50)	1.5 (0.50)	12.3 (2.03)	3.3 (2.75)	4.3 (0.67)	4.3 (0.88)	5.3 (1.45)	8.0 (1.53)	5.0 (0.58)	14.7 (2.33)
Potassium (mg/kg)	180 (7)	209 (21)	269 (25)	682 (163)	128 (13)	137 (10)	128 (15.8)	160 (13.1)	719 (65)	1268 (108)
Phosphorus (mg/kg)	5.0 (2.52)	2.7 (0.33)	57.3 (3.48)	56.7 (11.0)	1.7 (0.33)	1.7 (0.33)	4.3 (0.33)	13.0 (8.54)	78.0 (5.5)	210.3 (54.8)
Calcium (mg/kg)	557 (23)	831 (63)	3486 (257)	4015 (86)	440 (52)	517 (61)	2083 (158)	3016 (352)	5160 (241)	13277 (818)
Magnesium (mg/kg)	447 (31)	534 (23)	1020 (80)	1432 (26)	100 (6)	118 (13)	1080 (35)	1086 (96)	1371 (52)	1254 (76)
Organic Carbon (%)	1.4 (0.06)	1.2 (0.06)	4.0 (<0.01)	4.0 (<0.01)	1.3 (0.20)	1.1 (0.03)	4.0 (<0.01)	4.0 (<0.01)	3.1 (0.07)	4.0 (<0.01)
Chloride (mg/kg)	10.0 (3.2)	11.3 (2.4)	17.3 (5.2)	31.5 (3.5)	13.3 (4.9)	12.3 (0.9)	20.3 (4.8)	15.3 (1.9)	13.3 (1.7)	50.0 (24.0)
Sodium (mg/kg)	23.3 (5.0)	22.7 (7.5)	71.7 (9.2)	94.0 (1.0)	21.3 (2.1)	23.0 (4.0)	80.0 (17.7)	73.7 (16.3)	25.3 (7.5)	47.0 (12.4)
CEC (MEq/100g)	7.1 (0.2)	9.2 (0.5)	26.9 (1.9)	34.2 (0.8)	3.5 (0.4)	4.0 (0.4)	20.1 (1.0)	24.9 (2.5)	39.8 (1.37)	80.3 (4.7)

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