An autoecological study of the central Queensland serpentine endemic plant *Neoroepera buxifolia* Muell.Arg. & F.Muell. (Picrodendraceae)

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Declaration

The work contained in this thesis has not been previously submitted, either in whole or in part, for a degree at Central Queensland University, Australia or any other tertiary institution. To the best of my knowledge and belief, the material presented in this thesis is original, except where due reference is made in text.

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The research presented in this thesis was conducted under permit numbers WISP08633110, WITK060300409 and ROC5000 issued under the provisions of the Queensland Nature Conservation Act 1992, Nature Conservation (Wildlife Management) Regulation 2006 and Forestry Act 1956 respectively.

Abstract

This thesis reports on autoecological aspects of the serpentine endemic plant *Neoroepera buxifolia* and provides a general study on the vegetation ecology of the central Queensland serpentine landscape on the east coast of Australia. The first objective of this thesis was to determine if the overlying vegetation could be used as biological indicators of the severity of the serpentine soils. The results supported this theory as negative correlations were found between the relative basal area of the endemic overstorey tree *Corymbia xanthope* and the soil calcium. The endemic and rare plants increased in abundance with soil nickel and the herbaceous species decreased with soil nickel. The species richness increased with soil boron and decreased with soil nickel. The species abundances also correlated with the landform patterns reflecting differences in soil chemistry. The overstorey tree *Eucalyptus fibrosa* subsp. *fibrosa* regulated metal uptake and was not a useful indicator.

The endemic shrub *Neoroepera buxifolia* has a high habitat specificity and is mostly restricted to the perennial and ephemeral creeks within the central Queensland serpentine landscape. It is associated with high soil magnesium and the aim was to determine if the standing volume and height was correlated with the soil magnesium and the soil Mg/Ca quotients. Whilst correlations were found between increased height and soil Mg/Ca quotients, the strongest influence on *N. buxifolia* was from the extractable soil nickel. No correlations where found between the soil magnesium and the standing volume or height of *N. buxifolia*.

Over 82% of the central Queensland serpentine landscape is covered by mining interests for economic enrichments of nickel, cobalt, iron and magnesium carbonate.

Successful restoration following mining requires the careful selection plant of species. The suitability of *N. buxifolia* to be used for phytostabilisation was assessed by determining the bioaccumulation factor of metals. *Neoroepera buxifolia* was determined to be suitable for phytostabilisation as it is a metallophyte, it does not accumulate metals and the bioaccumulation rates for nickel and cobalt did not exceed a factor of 1. However, *N. buxifolia* has narrow habitat requirements which would limit its application to high moisture areas such as tailing dams, drains and creeks.

Neoroepera buxifolia readily propagated from seed and the seeds did not require dormancy relieving techniques for successful germination. Seeds stored for three months in their fruits had high germination rates and good viability. Vegetative propagation using marcotting and ground layering techniques were also highly successful. The application of auxin promoted the development of the root ball. Propagation using cuttings was not as successful compared to the marcotting and ground layering techniques.

The final part of this thesis is an ecological risk assessment which characterised the threats to the endemic shrub *N. buxifolia*. It was determined that the greatest risks are from climate change, fire, mining, exotic species invasions, livestock grazing and habitat fragmentation. Management actions are required to mitigate and control the threats to *N. buxifolia* to reduce the risk outcomes and maintain existing populations of *N. buxifolia*.

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Most importantly I would like to acknowledge the love, support and patience from my family. I apologise to them for spending all day at work and then coming home to study part-time each night and over the weekends. My children have never known a time when mum hasn't been studying and I hope I haven't dissuaded them from tertiary education.

List of publications and presentations

Hendry RA, Wormington KR, Walsh KB (2015) An ecological study of the central Queensland serpentine endemic shrub *Neoroepera buxifolia*, Australia. *Australian Journal of Botany* (in press January 2015).

Hendry RA, Wormington KR (2014) Eucalypt forests as indicators of the gradients within the central Queensland serpentine landscape of Australia. *Australian Journal of Botany* **61**(7), 544-551.

Hendry RA, Wormington KR (2011) Eucalypt forests as indicators of the gradients within the central Queensland serpentine landscape of Australia, presentation to the 7th International Conference on Serpentine Ecology (7th ICSE), Coimbra, Portugal 2011.

Aims

The work presented in this thesis was undertaken with three principle aims:

(1) an ecological study of the serpentine vegetation

(2) an autoecological study of the vulnerable serpentine endemic plant *Neoroepera buxifolia* of central Queensland

(3) the development of an Ecological Risk Assessment to aid in the conservation management of *N. buxifolia*.

Objectives

Specifically the objectives of this study were to:

(1) Determine if the structure of the eucalypt forests and concentrations of elements in the foliage of a dominant overstorey species could be used as biological indicators of the severity of the serpentine soils.

(2) Ascertain if the standing volume and height of *N. buxifolia* is correlated with the soil magnesium and/or Mg/Ca quotients.

(3) Determine the germinability of *N. buxifolia* seeds and investigate if dormancy relieving techniques are required to enhance germination.

(4) Develop a protocol for the vegetative propagation of *N. buxifolia.*

(5) Assess if *N. buxifolia* is useful for phytostabilisation purposes by determining the bioaccumulation factor of metals in the foliage.

(6) Clarify the level of risk threats pose to *N. buxifolia* populations by undertaking an Ecological Risk Assessment and apply risk management to reduce the risk outcomes.

Overview of the thesis

This thesis addresses four broad aspects; these are ecological studies, suitability for phytoremediation, propagation and an Ecological Risk Assessment. Chapter one provides information on the geology, soils and unique serpentine vegetation both generally and with regards to central Queensland. In Chapter two the hypothesis is presented that the eucalypt forests act as indicators of the severity of the serpentine gradients. From this study it emerged that the soil Mg/Ca quotients have the greatest influence on the woody vegetation. This lead to a study in Chapter three on a serpentine endemic shrub *Neoroepera buxifolia* that is known to be associated with high soil magnesium to test the hypothesis that the woody vegetation is primarily influenced by soil Mg/Ca quotients. This chapter also considers ecological aspects of *N. buxifolia*. Chapters two and three have both been published in a peer reviewed journal. Chapter three identified correlations of *N. buxifolia* with soil extractable nickel and the Mg/Ca quotients. Chapters four and five are focused on developing propagation protocols for *N. buxifolia* to support conservation and use in phytostabilisation. Further exploration of *N. buxifolia* was undertaken in Chapter six to assess if it is useful for phytostabilisation purposes. This was achieved by examining the metal bioaccumulation factors of *N. buxifolia*. In Chapter seven, an Ecological Risk Assessment was developed to systematically determine the risk of threats to *N. buxifolia* populations, thus supporting the conservation of this endemic plant. Finally Chapter eight concludes the thesis and provides recommendations for further research. An outline of the research framework of the thesis is displayed in Figure 1-0.

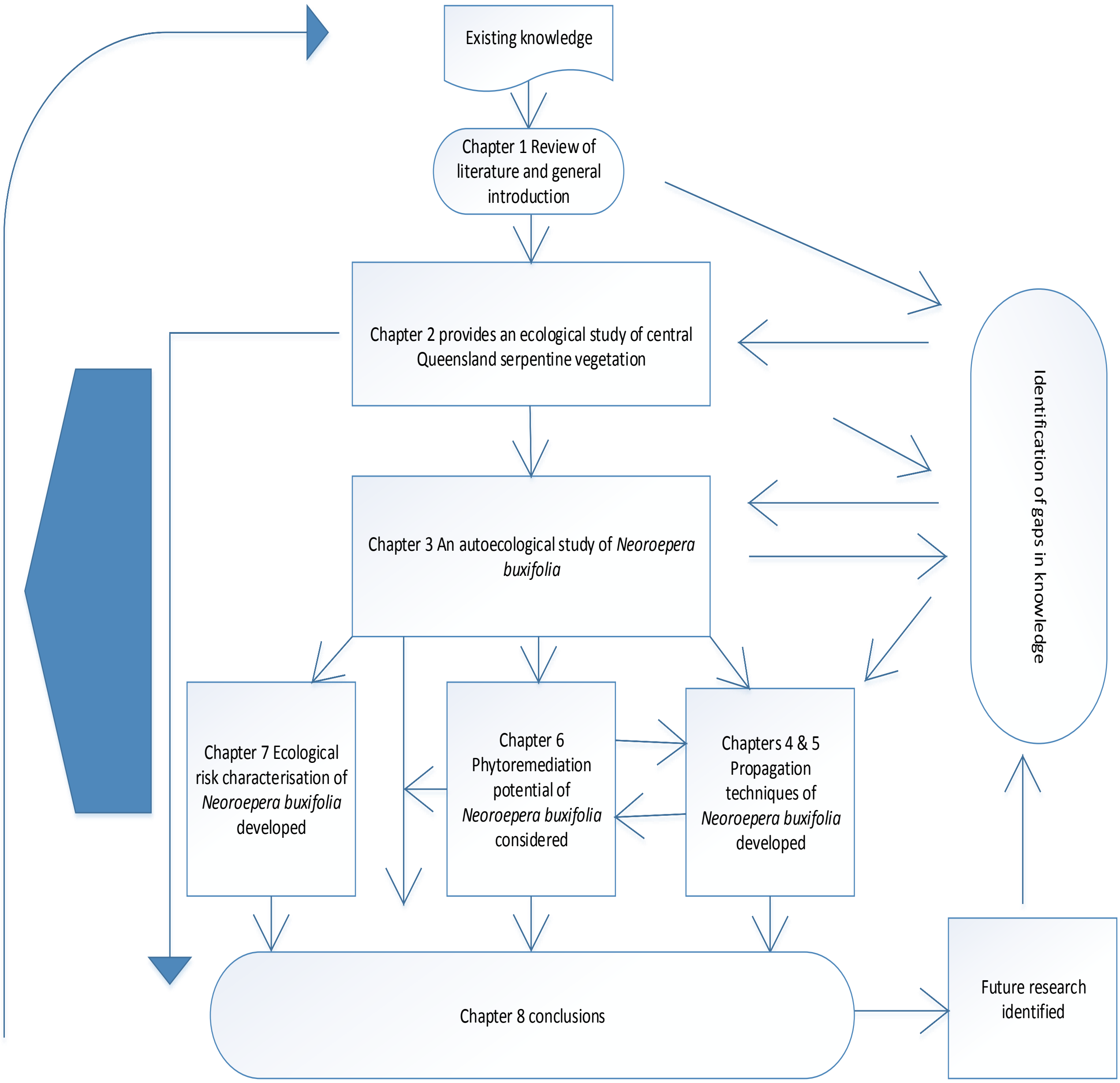

Figure 1-0 Conceptual framework of thesis.

Chapter 1 General Introduction

1.1. Serpentinite and serpentine

The central Queensland (CQ) serpentine landscape is located just north of the Tropic of Capricorn and extending from Marlborough in the north through to Canoona, and east to Bondoola, and near the mouth of the Fitzroy River to the south (Figure 1-1) (Forster and Baker 1995). There are also deposits of serpentinite on South Percy Island northeast of Rockhampton (Batianoff *et al.* 2000). The Serpentine landscape covers an area of approximately 100 000 ha.

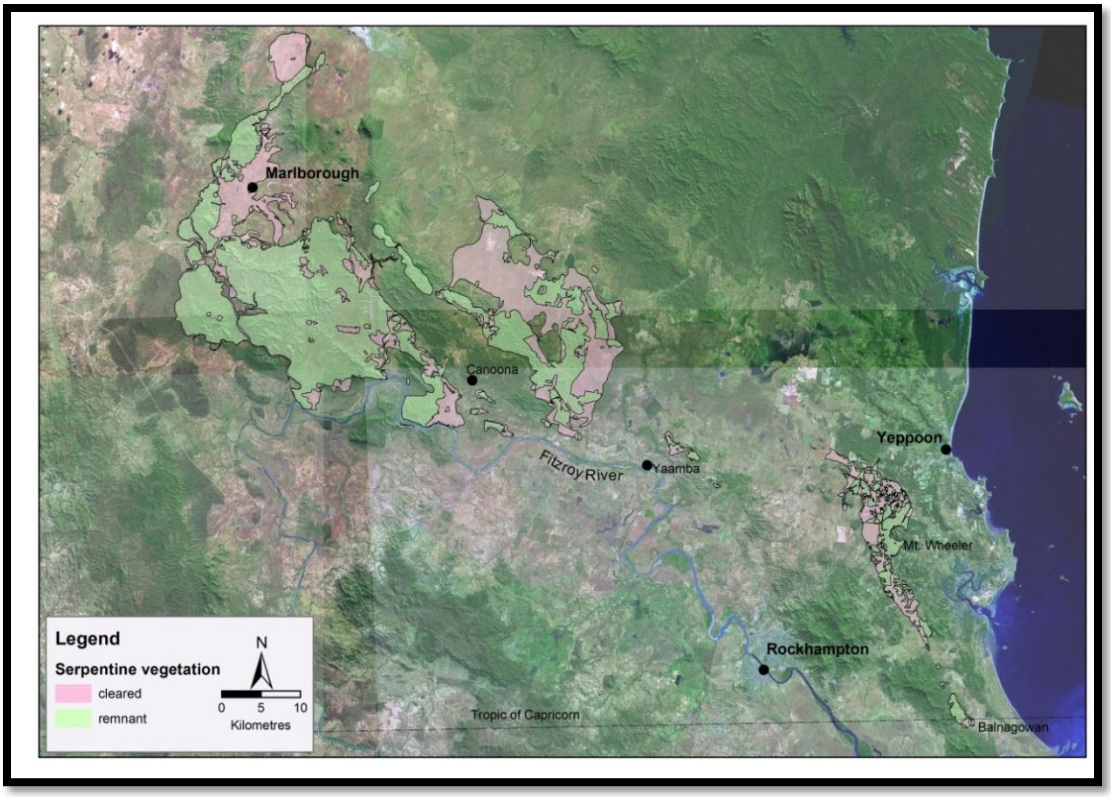

Figure 1-1 The study area with remnant and cleared (non-remnant) serpentine vegetation coverage (EPA 2009)

1.1.1 Serpentinite rock

Serpentinite is a metamorphic rock that is produced during the emplacement of mafic peridotite rock from the oceanic crust onto the continental margin. The most common peridotites are harzburgite, dunite and iherzolite. As the peridotite becomes tectonically displaced, the rocks become deformed under low pressure and at low temperatures between 100 – 300 ^{o}C with a pH of >10 and they become serpentinised. During serpentinisation of the original mafic rock, seawater becomes available along the cracks and fractures of the sheared zone and the magnesium iron and silicate minerals are altered and replaced with hydrous magnesium iron silicate minerals (Brooks 1987; Coleman and Jove 1992).

The simplified reaction of the alteration of harzburgite to serpentinite is as follows (Coleman and Jove 1992):

$$Mg_2SiO_4 + MgSiO_3 + 2H_2O = Mg_3Si_2O_5(OH)_4$$

The density of the serpentinised rocks becomes less than that of the crustal rock and as the rock undergoes folding, diapirs may form which can pierce through the overlying sediments allowing the serpentinised rock to move higher through the orogenic zones. These become the high standing serpentinite masses that have accelerated, rapid erosion and land sliding which weather into the sedimentary basins (Bhatia 2003; Brooks 1987; Coleman and Jove 1992). Serpentinite rocks have high concentrations (>70%) of mafic material (magnesium (ma) and iron (Fe) – hence the term mafic). Siderophile elements such as nickel (Ni), cobalt (Co) and chromium (Cr) may also be present (Brooks 1987).

1.1.2 Serpentinite deposits of Queensland

There are three major serpentinite deposits in Queensland and each is disjunctive to each other. In the north there is the Greenvale, Lake Lucy – Minnamoolka and Gunnawarra deposits. To the south there is the Kilkivan – Kandanga creek deposits and in central Queensland there is the Rockhampton – Marlborough and South Percy Island deposits. The central Queensland deposits are the largest of the three (Batianoff and Specht 1991).

1.1.3 Central Queensland serpentinite landscape

The original rock of the Rockhampton-Marlborough serpentinite area was olivine rich harzburgite, a type of peridotite with minor dunite and clinopyroxenite (Forster and Baker 1995; Murray 1969; Murray 2007). The harzburgite formed during geological events 562 Mya (Murray 2007). The rocks were deformed and partially to completely serpentinised when the oceanic crust was thrust into the continental crust during the late Permian period (Forster and Baker 2002). The Marlborough terrain is described as being made up of a 3 km thick sliver of serpentinised harzburgite called the Princhester serpentine. It runs in a north easterly direction and is about 60 km long and is the largest ultramafic (=serpentinite) mass in eastern Australia (Bruce *et al.* 1998; Forster and Baker 1995). There are also sparse dykes and isolated blocks of metamorphosed mafic rocks within the ultramafics (Murray 2007). The serpentinised rocks form the raised ridgelines and the surrounding low flat valleys are made up of granites and volcanic remnants (Bruce *et al.* 2000; Forster and Baker 1995; Murray 2007; Murray and Blake 2005).

1.1.4 Serpentine minerals

Serpentinite rocks contain serpentine minerals. The term serpentine refers to a group of hydrous magnesium iron minerals with the general formula $Mg_3Si_20_5(OH)_4$ (Brooks 1987). The three major serpentine minerals are antigorite, chrysotile and lizardite. Serpentine minerals are developed under a broad range of temperature and pressure conditions. Low temperature metamorphism creates mainly chrysotile and lizardite and higher temperatures create antigorite. The serpentine minerals form trioctahedral hydrous phyllosilicate structures of alternating silica and magnesium layers. Chrystolite forms as a fibrous tube-like structure, whereas lizardite and antigorite crystallize as plates. All minerals have a SiO_4 tetrahedra joined to a trioctahedral brucite layer. The dimensions between the brucite and SiO_4 tetrahedra layers can be substituted or warped. This warping or substitution is considered to be a major reason for the differences between the serpentine minerals (Coleman and Jove 1992; Kazakou *et al.* 2008).

1.1.5 Weathering of the serpentinite rock

Tropical chemical weathering of rock produces extensive laterisation and deep weathering profiles (Aristizábal *et al.* 2005). In tropical and humid zones large areas of laterites are distributed on weathered serpentine surfaces as the water has a lower pH and may be enriched with bicarbonate which is able to dissolve the serpentine minerals (Coleman and Jove 1992). Weathering of serpentinite rocks is also controlled by the composition of the original rock. Serpentine minerals are more stable than olivine and pyroxene in terms of weathering (Coleman and Jove 1992). Laterisation of the rocks causes the separation of the siderophiles such as Fe, Ni, manganese (Mn) and Co. These metals dissolve and leach through the soil profile. The laterites of the serpentinites of the tropics cover large areas and represent reserves of Ni ore deposits (Brooks 1987; Coleman and Jove 1992).

1.1.6 Regolith of the central Queensland serpentine landscape

The serpentinised harzburgite that dominates the Rockhampton - Marlborough terrain has weathered and eroded over time since the mid-Oligocene period, and is now covered in a thick weathering profile and in some areas the original weathered surface remains only as capping (Forster and Baker 2002). The weathering profile has economic enrichments of Ni, Co and chrysoprase. Chrysoprase veins are situated within the saprolite horizon in the deeply weathered serpentine and are open cut mined at Mount Slopeaway (Forster and Baker 1995; Forster and Baker 2002). The residual Ni deposits are a series of discrete enrichments that cover an area of approximately 30 – 50 km^2. The Ni deposits were discovered in 1962 by the Geological Survey of Australia (Foster and Eggleton 2002). The weathering of the serpentinite rocks has caused the groundwater flowing from the serpentine hills to become supersaturated with magnesium (Mg) which is neutralised by hydroxide (OH) and bicarbonate (HCO_3). A precipitate of magnesium carbonate ($MgCO_3$) is formed in the base-rich ground waters. Such precipitates are found deposited as high grade nodular magnesite ore in the eastern alluvial flats of Kunwarara and Yaamba (Foster and Eggleton 2002; Wilcock 1998).

Foster and Eggleton (2002) give a description of the regolith architecture and the in-Situ weathering profile of the central Queensland serpentine landscape. Generally the regolith varies geochemically in Mg, Fe, Si, Ni and Co concentrations depending on the quantity of clay and saprolite (Foster and Eggleton 2002).

1.1.7 Soils derived from serpentinite rocks

Serpentine soils are the weathered product of serpentinite rocks. The characterisation of serpentine soils is broad and sometimes conflicting, and is difficult to generalise as there is a diverse range of soils produced from weathering of serpentinite rocks. However, serpentine soils can be characterise as having (Brooks 1987; Kazakou *et al.* 2008):

- a vulnerability to erosion;
- low organic material;
- low fertility;
- poor soil moisture holding capacity;
- low calcium (Ca) availability relative to Mg; and
- high concentrations of metals such as Ni, Cr and Co.

1.1.8 **Soils of central Queensland serpentine area**

Forster and Baker (1995) described and characterise the central Queensland serpentine soils of the Marlborough area. They described six major soil groups which were determined according to morphology, parent material, landscape position and chemistry. The physical properties of the six soil groups are (Forster and Baker 1995):

- Group 1: Soils overlie saprolite and are derived from strongly weathered serpentinite rocks. They are found mainly on the crests and upper slopes of the mountains and hills. The profile is a reddish brown moderate clay loam to red medium clay. Ferrous, manganiferous or ferromanganiferous nodules are usually present. These soils are well drained.
- Group 2: Soils are the stony red and brown clay loams and clays on the crests and mid to upper slopes of hills and rises. They are produced from moderately weathered serpentinite rocks. There are extensive outcrops of weathered serpentine minerals present. A honeycomb lattice created by the weathering, dissolution and re-deposition of secondary silica may be present on the rock

outcrops. The soils are shallow and very stony and the surface colour may be red or brown with a moderate to poor clay loam to medium clay subsoil structure. The nodules of Group 1 are absent and these soils are well drained.

- Group 3: Soils are found on the mid to lower slopes and footslopes of the mountains, hills and gently undulating rises. Fresh serpentinite may be exposed and there are abundant rock outcrops. The soils are shallow and stony and overlie partially weathered serpentinite, and magnesite nodules may be present. They are moderately to strongly structured soils of light to medium clay or clay loam and are black to dark brown and are moderately to well drained. Seepage zones may be present.
- Group 4: Gravely black clays at the footslopes of mountains and hills. These are depositional soils that form as colluvial and alluvial fans. They are also found at the upper reaches of small streams. The soils are shallow to moderately deep and may overlie serpentinite. The profiles are black to dark brown, moderately to strongly structured clay loams or uniform clays. Nodules are present and the soils are moderately to well drained.
- Group 5: Soils are the black and dark brown cracking clays on the alluvial fans and plains and flats adjacent to the serpentinite hills. The soils may overlie magnesite deposits. Local flooding occurs after prolonged rainfall and the soils are poorly drained. The soil profile is deep and the surface is a coarse self-mulching pedal. The light to medium clay develops cracks when dry and small manganiferous nodules may occur in the profile.
- Group 6: Poorly drained soils of the swamps, floodways and alluvial plains that are subject to prolonged flooding. They are moderately deep black cracking clays with brown subsoil over magnesite deposits. The soils grade into the soft

weathering magnesite. Broad and shallow Gilgai micro-relief may be present and the soils are self-mulching cracking clays with small manganiferous and magnesite nodules (Forster and Baker 1995).

1.1.9 Chemical characteristics of central Queensland serpentine soils

The chemical characteristics of the central Queensland serpentine soils were described by Forster and Baker (1995). It was note that in general the concentrations of exchangeable Mg increased down slope. The highest soil Mg/Ca quotients were found in group 6 soils overlying magnesite, where the quotient exceeds 500. The lowest Mg/Ca quotients of 0.4 were found in soils from Group 1 overlying saprolite. The soils with high concentrations of Mg tend to be alkaline to strongly alkaline. The alkaline soils also have an accumulation of soluble salt due to poor drainage, with a maximum electrical conductivity (EC) of 1.0 dS/m found in soils from Group 6. The cation exchange capacity (CEC) was low (13%-60%) in Groups 1 and 2 due to the high proportions of illite and kaolinite. The CEC of soil groups was high in Groups 3 and 6 (> 70%) due to high proportions of montmorillonite clay. Montmorillonite clay also has high water-holding characteristics (Forster and Baker 1995).

Soil fertility of the central Queensland serpentine soils has been found to be low, with low concentrations of phosphorus (P), extractable potassium (K) and total nitrogen (N). The organic carbon (C) and C:N ratios decrease down the landscape and are indicative of low fertility soils (Forster and Baker 1995).

The soils are high in trace elements and the mean totals of these are comparable to other serpentine landscapes worldwide. Trace elements in the soils include Ni, Co, Mn, Cr and Fe. The total concentrations of soil elements of non-serpentine and serpentine soils of Queensland are compared in Table 1.1. The highest Fe concentrations of the central Queensland serpentine soils were found in Group 1 soils and are indicative of the strong leaching environment of the illite and kaolinite clays. Nickel toxicity and availability to plants is dependent on other ions and elements such as Ca and Mg. The Ni is transported through soils from Groups 5 and 6 to the alluvial plains. The highest concentrations of total Ni in soils were found in Group 3 (0.46%-0.73%) and the lowest concentrations were found in Group 1 (0.15%-0.17%) (Forster and Baker 1995).

Bhatia (2003) described the serpentine soils of central Queensland that support populations of the Ni-hyperaccumulating plant *Stackhousia tryonii* as having similar chemical and physico-chemical characteristics as typical of serpentine soils worldwide. The clay content of the soils was high and there was a substantial proportion of sand. There were moderate concentrations of organic carbon and poor concentrations of macronutrients such as P, N, K and Ca, and high concentrations of Mn, sulphur (S), Fe, Co, Ni and Mg. The concentrations of Cr, copper (Cu) and Co were within normal ranges for serpentine soils worldwide (Bhatia 2003).

Table 1-1 Comparisons of elements in non-serpentine and serpentine soils from Queensland (mean ± standard deviation (derived from Batianoff and Specht 1992)

Element	**non-serpentine soil**		**serpentine soil**
	south Qld	central Qld	central and south Qld
Calcium (%)	0.13 ± 0.02	0.26 ± 0.15	0.61 ± 0.37
Magnesium (%)	0.47 ± 0.24	0.18 ± 0.10	8.78 ± 2.86
Mg/Ca quotient	3.5 ± 1.4	0.9 ± 0.5	6.0 ± 5.5
Cr (μg g^{-1})	58 ± 33	106 ± 51	4250 ± 2726
Co (μg g^{-1})	21 ± 6	13 ± 3	224 ± 77
Ni (μg g^{-1})	34 ± 11	34 ± 13	2960 ± 950
Mn (μg g^{-1})	830±260	458±302	1744±692

1.2 The serpentine environment and plant growth

1.2.1 General

The serpentine (ultramafic) substrate is a stressful environment for plant growth (Kazakou *et al.* 2008). Worldwide, ultramafic rocks that are rich in serpentine minerals support an unusual flora that is distinguished from surrounding flora by physiognomy and species composition (Brooks 1987; Kazakou *et al.* 2008). The flora can be divided into obligative and facultative groups, in terms of response to the serpentine soils. Serpentine obligative plants are endemic plants that grow exclusively on serpentine soils, whereas facultative plants are able to tolerate and survive on serpentine soils, but grow better elsewhere (Borhidi 1992; Brooks 1987; Kazakou *et al.* 2008).

The challenges for flora growing on serpentine soils are a high Mg : Ca ratio (Asemaneh *et al.* 2007; O'Dell *et al.* 2006), the stress of low nutrients (Chiarucci *et al.*

1998) and high heavy metal concentrations (Brooks 1987). Serpentine vegetation can be characterised by its stunted appearance that is different to the surrounding vegetation, high degree of endemism and poor plant productivity. The vegetation is commonly xeromorphic with sclerophyllous foliage and stunted growth (Borhidi 1992; Brooks 1987; Iturralde 2001).

1.2.2 Magnesium and calcium quotients and plants

Serpentine soils have high Mg/Ca quotients compared with non-serpentine soils (Table 1-1) (Brady *et al.* 2005; Brooks 1987). Calcium is essential for plant cell membrane development, stability of the plant and enzyme activation (Proctor and Nagy 1992). Low concentrations of Ca are not a primary cause of serpentine soil infertility; however, the low concentrations of Ca are exacerbated by high concentrations of Mg (Asemaneh *et al.* 2007).

Magnesium concentrations have an important influence on plants. Magnesium causes soils to become alkaline, it is toxic to plants depending on the amount of Ca present, and has complex inter-relationships with other elements (Proctor and Nagy 1992). The Mg/Ca quotient of soils is used to determine the severity of the serpentine nutrient imbalance. Well balanced soils have a Mg/Ca quotient of around 0.3 (McCarten 1986). The central Queensland serpentine soils were found to have high Mg and low Ca in the subsoil and the Mg/Ca quotients varied from 0.4 to 423 (Forster and Baker 1995). The Mg/Ca quotient has been found to have a positive effect on serpentine endemic species richness as many non-endemic competitors cannot tolerate high Mg (Grace *et al.* 2007). High leaf Ca is believed to be a key evolutionary change required for survival on serpentine soils and may be due to selective Ca transport or Mg exclusion at the root-to-shoot translocation level (O'Dell *et al.* 2006) or at the root level (Asemaneh *et al.* 2007).

1.2.3 Low nutrients and plants

Serpentine soils are infertile due to a combination of many factors such as the parent rock, steep slopes, low moisture availability and chemical composition. The metabolism of the serpentine plants is slow and there is a low production of biomass. There may also be lower seed and pollen production from plants growing on serpentine substrates compared to non-serpentine plants (Boulet 2003).

In a study of fertilizer amendment and serpentine floras O'Dell and Claassen (2006) determined that N, P, K and Ca deficiencies are the most important feature in maintaining a native serpentine plant community that is free from invasive species (O'Dell and Claassen 2006). The central Queensland serpentine soils were found to be low in P with concentrations below 7 mg kg^{-1} recorded (Forster and Baker 1995).

Specht and Batianoff (2002) determined there was a direct correlation between increased water availability and nutrients on the deeper central Queensland serpentine soils with an increase in photosynthetic potentials of mature leaves. It was noted that the density of the overstorey eucalypts increases on deeper soils and reduces in density on shallow soils (Specht *et al.* 2002).

1.2.4 Metals

Phipps (1976) in Phipps (1981) defined metals as being recognized as those elements which under biologically significant conditions exist as cations. The metals Mn, Fe, Ni, Cu and Zn are components of enzymes that are essential for the survival of plants and animals; however at high concentrations these metal ions inhibit enzyme and protein function and cause oxidative damage (Callahan 2007). Unrestricted uptake of metals results in toxicity and death (Bidwell 2000; Callahan 2007). Metal ions can also produce a deficiency of other essential ions as competition for uptake can occur with membrane transporters often acting as monovalent or divalent ion transporters (Callahan 2007). Metal ions have a high affinity for ligands, such as organic acids, amino acids, peptides and proteins which are able to bind metals.

Nickel is the metal most likely to cause toxicity to non-adapted plants in serpentine soil and it can cause a deficiency of Mg uptake (Boyd 2007; Hutchinson 1981; Kazakou *et al.* 2008). Nickel can inhibit cell division and root branching (Seregin and Kozhevnikova 2006). The uptake of Ni by plants is dependent on plant metabolism (Alloway 1995), the acidity of the soil (Callahan 2007), the presence of other metals (Alloway 1995; Chen *et al.* 2009), organic matter (Friedland 1989) and plant soil feedback relationships (Casper *et al.* 2008). Other factors that affect the amount of metals absorbed by a plant include the concentration and speciation of the metal in the soil, the movement of the metal to the root surface, the transport of the metal from the root surface to the inside of the root and the translocation of the metal from the root to the shoot. The absorption of metals by the plant can be either passive or active. Passive uptake is non-metabolic diffusion of the ions into the roots whereas active uptake is metabolic and takes place against a gradient (Alloway 1995; Baker and Walker 1990; Chen *et al.* 2009).

The uptake of ions requires mobilization of the metal at the rhizosphere (soil root zone). The pH affects the system as H^+ ions compete with metal ions for the ligand. Solubility of elements decreases at pH greater than five, however the exudation of ligands by the plants from the roots may mobilize metal ions in the soil. Interaction of microbes at the rhizosphere may also lower the pH and mobilize the metal ions (Callahan 2007).

Most organic matter in soil is comprised of humic substances and trace elements have a strong affinity for organic matter, particularly humic acids (Friedland 1989). Plants alter the soil properties which feedback to affect plant performance. Plants can alter the physical, chemical and biotic properties of soil which can positively or negatively influence plant growth (Casper *et al.* 2008).

1.2.5 Plant adaptations to metals

Plants have a number of physiological features to cope with the high metal content of the serpentine soils. Three types of plant-soil relationships have been described by Baker (1981), these are indicators, excluders and accumulators (Figure 1-2) (Baker 1981; Baker and Walker 1990). Indicator plants are those whose internal metal concentrations reflect that of the soil substrate. They have a linear relationship to metals in the soil, and as the soil concentration of metals increases so does the metal concentrations within the plant (Baker 1981; Kazakou *et al.* 2008; Nkoane *et al.* 2005).

Excluders are plants that have low metal concentrations in their tissues over a wide range of soil concentrations. Metal exclusion may occur at the roots and transport to the shoots is restricted. However, if the concentrations of metals in the soil become toxic, unrestricted transport will occur. Avoidance of the metals can be achieved by the release of metal complexing compounds. Restriction of metals can also be undertaken by mycorrhizas in the rhizosphere (Baker 1981; Bidwell 2000; Boulet 2003).

Accumulators are those plants that concentrate metals in their above-ground parts to levels higher than that of the soil. Accumulator species are able to translocate metals to the shoots and the concentration of metals in the leaves becomes higher than the roots. The metals are compartmentalized and stored in the plant organs (eg. into the vacuoles, cell walls and epidermal cells) (Baker 1981; Kazakou *et al.* 2008).

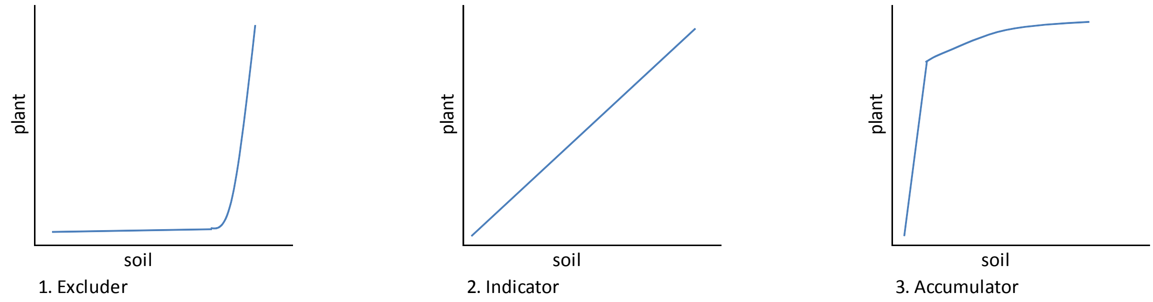

Figure 1-2 Three strategies of metal accumulation by plants in relation to substrate concentrations (Baker 1981)

1.2.6 Nickel hyperaccumulation

Some plants have evolved to survive in metal-rich soils by collecting and storing large amounts of metals in their above-ground tissues (Brooks 1987). Brooks (1987) described these plants as hyperaccumulators. Hyperaccumulator plants are able to translocate and accumulate metals in the above-ground parts to levels higher than that of the soil without toxic symptoms. The threshold value of hyperaccumulation for Ni in plant foliar dry matter is 1000 mg kg^{-1}, and for Co and Cr is 100 mg kg^{-1}. A number of theories regarding the advantages of metal hyperaccumulation have been put forward including metal disposal (Basic *et al.* 2006), drought resistance (Bhatia *et al.* 2005), interference with neighbouring plants (Morris *et al.* 2009) and defence against predators (Boyd 2007; Palomino *et al.* 2007). Metals may also be inadvertently taken up by plants due to a lack of mycorrhizal fungi (Baker 1981; Boyd 2007; Kazakou *et al.* 2008).

Nickel is the most commonly hyperaccumulated metal element (Boyd 2007). Worldwide more than 360 species have been recognised as being able to hyperaccumulate Ni. These species represent 90 genera and 40 families that exhibit Ni concentrations of 1000 mg kg^{-1} or more. Most of these species are endemic to serpentine soils; however some occur on a variety of soils but only hyperaccumulate metals on serpentine soils (Kazakou *et al.* 2008).

Two species, *Stackhousia tryonii* Bailey (Stackhousiaceae) and *Pimelea leptospermoides* F. Muell. (Thymelaeaceae) have been recognized as being Ni hyperaccumulators of the central Queensland serpentine landscape (Batianoff *et al.* 1990; Bhatia 2003; Bidwell 2000). *Stackhousia tryonii* was found to hyperaccumulate between 3800 to 8000 mg kg^{-1} of Ni and a specimen with a Ni concentration of 41,300 mg kg^{-1} has been recorded from Percy Island (Batianoff *et al.* 1990; Batianoff and Specht 1991; Bhatia 2003; Kazakou *et al.* 2008).

The plant *Pimelea leptospermoides* was found to be a moderate but variable hyperaccumulator of Ni with a wide range of Ni concentrations from less than 100 mg kg^{-1} to greater than 1000 mg kg^{-1} (Bidwell 2000; Reeves 2002). Bidwell (2000) found there were two distinct groups of *P. leptospermoides*, with one group showing a correlation with soil EDTA-extractable Ni and the other having no correlation. Bidwell (2000) explains the differences between the groups could be due to the ages of the plants sampled or leaf age or differences between the genetic make-up of the plants (Bidwell 2000). The mean concentrations of Ni found in *P. leptospermoides* are listed in Table 1-2.

Table 1-2 Concentrations of nickel (μg g^{-1}) measured in *Pimelea leptospermoides* from 12 sites on central Queensland serpentine soils (Bidwell 2000).

Mean	SD	Min.	Max.
720	613	56	2010

Bidwell (2000) identified Australia's first recorded hyperaccumulator of Mn, *Gossia bidwillii* Benth. (Myrtaceae) in a study to locate new metal hyperaccumulators within the central Queensland serpentine landscape (Bidwell 2000).

Although interesting, hyperaccumulators account for a small proportion of serpentine flora and they do not provide for an understanding of the more general role of Ni on vegetation. The majority of plants exclude metals and hyperaccumulators represent a small subset of metal-tolerant plants (Batianoff *et al.* 1990; Bhatia 2003; Bidwell 2000).

1.2.7 Vegetation of the central Queensland serpentine landscape

Remnant vegetation covers 68% of the central Queensland serpentine landscape (Table 1-3) (EPA 2007; EPA 2009). It is believed that Ni in the soil has resulted in a reduced diversity of the overstorey species on the serpentine uplands. The dominant overstorey species are *Eucalyptus fibrosa* F. Muell. subsp. *fibrosa* (Myrtaceae) and *Corymbia xanthope* A.R. Bean & (Myrtaceae) (Specht *et al.* 2006; Specht *et al.* 2002). The vegetation varies from a complex of *E. fibrosa* subsp. *fibrosa* and *C. xanthope* woodlands on the serpentinite hills, with a variable shrub layer of species such as

Acacia leptostachya Benth. (Mimosaceae), *Hakea trineura* F.Muell. (Proteaceae), *Xanthorrhoea* sp. (Xanthorrhoeaceae) and *Macrozamia serpentina* D.L. Jones & P.I. Forst. (Zamiaceae). Rainforest elements may also be present in the understorey. The alluvial plains and drainage lines overlying magnesite deposits of the lowlands are covered in *Eucalyptus tereticornis* Sm (Myrtaceae), *Melaleuca viridiflora* Sol. ex Gaertn. (Myrtaceae), *Corymbia tessellaris* F. Muell. (Myrtaceae), *E. fibrosa* subsp. *fibrosa* woodlands. Riverine wetlands and fringing riverine forests and woodlands occur on the drainage lines derived from serpentinite. Small areas of semi-evergreen vine thicket on serpentinite are also present (EPA 2007).

Table 1-3 Pre-cleared and remnant areas of serpentinite regional ecosystems (re) and their conservation status under VMA 2005 (EPA 2009). (Pre-cleared area = vegetation prior to tree clearing, VMA = Vegetation Management Act Queensland 2005, remnant veg. = not cleared).

Regional ecosystem code	Vegetation Description	Area of vegetation pre- clearing (ha)	Area of vegetation remaining (remnant veg.) (ha)	Conservation status of vegetation (VMA 2005)
11.3.38	*Eucalyptus tereticornis, Melaleuca viridiflora, Corymbia tessellaris, E. fibrosa* subsp. *fibrosa* woodlands on alluvial plaints and drainage lines derived from serpentinite. Overlying magnesite deposits	38 677	11 156	endangered
11.11.7	*Eucalyptus fibrosa* subsp. *fibrosa* and *Corymbia xanthope* woodland on serpentinite	60 093	55 566	not of concern
11.11.25 (c)	Riverine vegetation on drainage lines derived from serpentinite	2085	718	not of concern
11.11.21	Semi evergreen vine thicket on serpentinite	331	271	of concern
total :		**101 186**	**67 711**	

The structural and floristic characteristics of the central Queensland serpentine vegetation communities in relation to the soils have been described by Specht *et al*. (2002); and Specht *et al*. (2006). Batianoff *et al*. (2000) listed a total of 634 vascular plants growing on the central Queensland serpentine and of these 17 plants have been identified as either being endemic or closely associated with the central Queensland serpentine habitats (Table 1-4) (Aola 2014; Batianoff *et al.* 2000; Jackes 2005):

Table 1-4 Vascular plants identified as being endemic or closely associated with the central Queensland serpentine landscape.

Species	**Endemic**
Bursaria reevesii L.Cayzer, Crisp & I.Telford (Pittosporaceae)	y
Capparis humistrata F.Muell (Capparaceae)	y
Corymbia xanthope A.R. Bean & Brooker (Myrtaceae)	y
Cycas ophiolitica K. D. Hill (Cycadaceae)	y
Eucalyptus fibrosa F. Muell. subsp. *fibrosa* (Myrtaceae)	n
Hakea trineura F.Muell.(Proteaceae)	y
Leucopogon cuspidatus R. Br (Epacridaceae)	n
Lissanthe brevistyla A. R. Bean (Ericaceae)	y
Macrozamia serpentina D.L. Jones & P.I. Forst. (Zamiaceae)	y
Marsdenia brevifolia Benth. P.I.Forst. (Apocynaceae)	n
Melaleuca sp. (Marlborough creek G.N.Batianoff+ MC9108006) (Myrtaceae)	y
Myrsine serpenticola Jackes, sp. *nov*. (Myrsinaceae)	y
Neoroepera buxifolia Muell.Arg. & F.Muell. (Picrodendraceae)	y
Olearia macdonnellensis D.A.Cooke. (Asteraceae)	n
Pimelea leptospermoides Benth (Thymelaeaceae)	n
Pultenaea setulosa Benth.(Fabaeceae)	y
Stackhousia tryonii Bailey (Stackhousiaceae)	y

1.2.8 Plants as an indicators of bioavailable metals and soil fertility

Plants act as ‘phytometers’ of site quality and indicator plants are useful in classifying soil moisture and nutrient availability. Plants integrate many growth related factors that are difficult to measure directly (Wang 2000). In a study of the vegetation along a gradient from rainforest to dry sclerophyll forest, Neave *et al*. (1995) found the foliage nutrient content of the eucalypt trees reflected the soil fertility. It was determined that the A horizon of the soil was most likely to influence plant nutrition as the fine roots of the plants are found within the first 30 cm of the soil profile (Neave *et al.* 1995). The concentrations of nutrients in eucalypt foliage reflect differences in species’ ability to take up nutrients and differences in soils (Foulds 1993; Lambert and Turner 1983; McColl and Humphreys 1967). The concentrations of foliar nutrients in eucalypts were found to be greater in plants growing on high nutrient soils (Braithwaite *et al.* 1984; Foulds 1993; Hobbie and Gough 2002).

The central Queensland serpentine plants *E. fibrosa* subsp. *fibrosa*, *C. xanthope* and *Marsdenia brevifolia* have been found to have high concentrations (150-250µg g^{-1}) of Ni in their foliage. These concentrations of Ni detected in the foliage are not high enough for these species to be considered hyperaccumulators (Specht *et al.* 2002). The foliar concentrations of the nutrients N, P, K and Ni increased in *E. fibrosa* subsp. *fibrosa* as the stand height increased from the skeletal to the deep lateritic soils (Specht *et al.* 2006; Specht, *et al.* 2002). It is thought that the deeper lateritic soils have more available soil moisture, and as more water becomes available phosphate, ammonium and Ni ions are taken up into the transpiration stream, and the photosynthetic potentials in the mature leaves increase. As the photosynthetic potential increases, more leaves are produced per foliage shoot and the number of leaves per hectare (leaf area index) and stand height increases (Specht *et al.* 2006; Specht *et al.* 2002).

Positive correlations have been determined between endemism and soil Ni concentrations in serpentine communities. As the Ni concentrations in the soil increase so do the number of endemic species. Conversely, as the Ni in the soil increases the overall species richness declines (Batianoff and Singh 2001). The relationships between the overlying vegetation and the serpentine soils will be further investigated in this thesis to determine if the structure of the forests can be used as biological indicators of the severity of the serpentine soils.

Chapter 2 Eucalypt forests as indicators of the gradients within the central Queensland serpentine landscape of Australia

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Plate 2-1 Serpentine vegetation of central Queensland

2.1 Abstract

The eucalypt forests of the central Queensland serpentine landscape on the eastern coast of Australia are dominated by two overstorey species. These are *Eucalyptus fibrosa* F.Muell. subsp. *fibrosa,* the most dominant tree occurring throughout the landscape, and *Corymbia xanthope* A.R.Bean & Brooker, a serpentine endemic species which has a more restricted distribution. We hypothesised that the structure and foliage elements of the eucalypt forests could be used as biological indicators of the severity of the serpentine soils. This was tested by surveying 30 plots (50 × 20 m) within the upland landform patterns of the central Queensland serpentine landscape. The structure of the forests and abundance of the species were recorded and foliage samples from the dominant tree *E. fibrosa* subsp. *fibrosa* were collected and analysed for metal and nutrient content. Soil samples from each site were collected and analysed for major cations, extractable metals and fertility. Analysis of the data showed that there are significant correlations between the structure of the eucalypt forests and the landform patterns and soil chemistry. The relative basal area of *C. xanthope* is a useful measure of the severity of the serpentine soils and

correlates with the soil Mg/Ca quotients. The tree *E. fibrosa* subsp. *fibrosa* was found to regulate its uptake of soil elements and cannot be used as an indicator of soil elements.

2.2 Introduction

The serpentinite deposit of central Queensland is the largest ultramafic mass of eastern Australia, covering an area of ~100 000 ha (Bruce and Niu 2000; Murray 2007). The central Queensland serpentine soils are comparable to serpentine soils worldwide and have low fertility, low phosphorus, low extractable potassium, low total nitrogen, high magnesium : calcium quotients and high concentrations of trace elements. In addition to this, the central Queensland serpentine soils are shallow and stony, with a limited water-holding capacity (Forster and Baker 1995). The vegetation displays the typical 'serpentine syndrome' of a short stature, low species richness and high concentrations of endemism. It is believed that the combination of water stress, the uptake of nickel and low nutrients of the serpentine soils has reduced the diversity of the overstorey trees to two species of eucalypts (Specht *et al*. 2002, 2006). These are *Eucalyptus fibrosa* F.Muell. subsp. *fibrosa* (a broad-leaved iron bark) and the serpentine endemic *Corymbia xanthope* A.R.Bean & Brooker (Glen Geddes bloodwood) (Specht *et al*. 2002, 2006). *E. fibrosa* subsp. *fibrosa* is the dominant tree occurring throughout the landscape, whereas *C. xanthope* is subdominant and occurs in the subcanopy.

The objective of the current study was to investigate the relationships between the overlying vegetation and the extractable metals in the soil. We hypothesised that the structure of the eucalypt forests and concentrations of elements in the foliage of a dominant eucalypt species could be used as biological indicators of the severity of the serpentine soils. To test this hypothesis, we

(1) measured the structure of the forests and the abundances of the species;

(2) collected and analysed soil samples;

(3) collected and analysed *E. fibrosa* subsp. *fibrosa* foliage samples; and

(4) analysed the soil, foliage samples and species assemblages for correlations.

2.3 Materials and methods

2.3.1 Study area

The central Queensland serpentine landscape is located north of the Tropic of Capricorn and extends from Canoona, north to Marlborough, east to Bondoola and south to near the mouth of the Fitzroy River (Figure 2-1); (Forster and Baker 1995; EPA 2007, 2009). There are also deposits of serpentinite on South Percy Island north-east of Rockhampton (Batianoff *et al*. 2000). The current study considered the area between Canoona and Marlborough only.

2.3.2 Climate

The climate of the study area is classified as subtropical. The mean monthly maximum temperatures at Rockhampton, south of the study area, range from 23.1°C to 32.1°C, with average minima of 10.5–22.5°C. The average annual rainfall at Marlborough is 1262 mm, with the highest falls occurring in the months December–March. The dry season is from June to September (BOM 2009; Price and Morgan 2010).

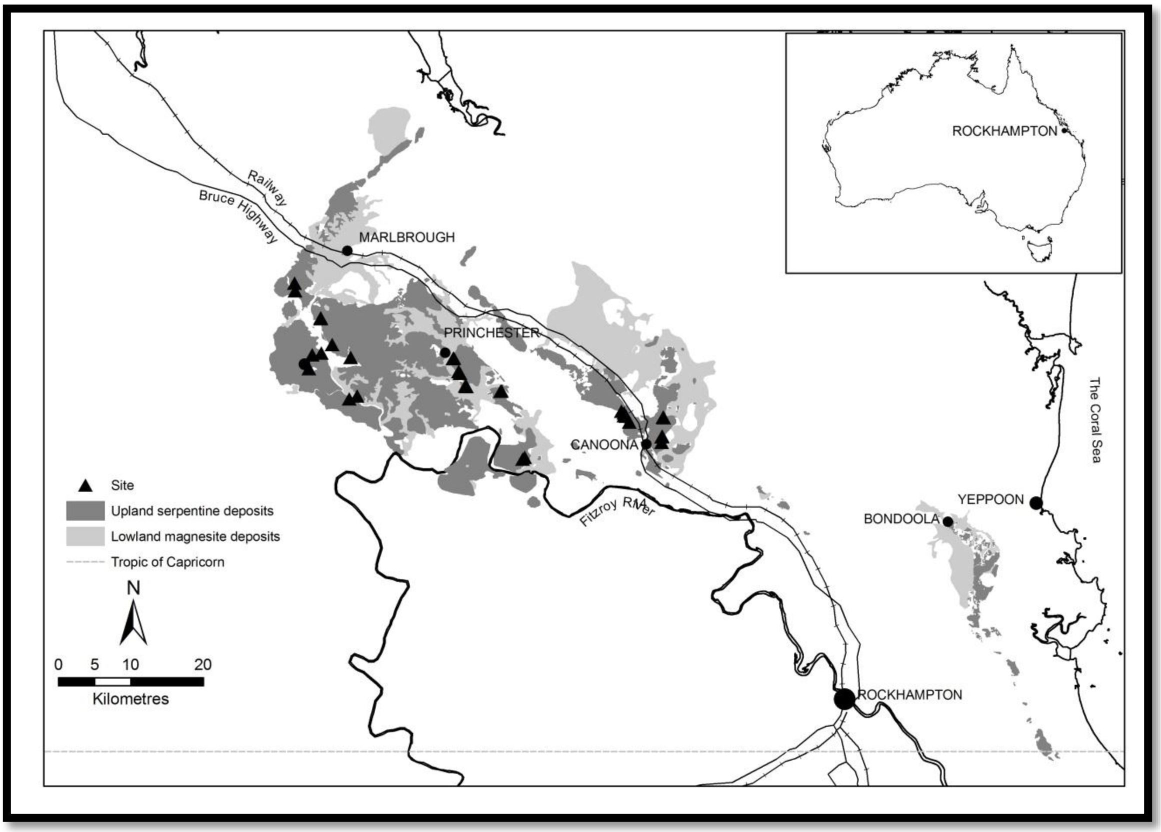

Figure 2-1 Locality map showing the distribution of the central Queensland serpentine landscape and the plot locations (derived from EPA 2009)

2.3.3 Survey methods

A series of 1000-m^2 survey plots (50 × 20 m) were used to assess the vegetation structure, and collect soil and foliage samples. Thirty survey plots were placed within remnant eucalypt forests in the serpentine landscape between March and October 2010. The position of the plots was recorded with a Garmin XL GPS. The plot locations were chosen by ease of access to private property and close proximity to vehicle tracks (within 500 m). Topographic information, combined with relief and modal slope, was used to determine the landform pattern for each site (Table 2-1).

The basal areas of the trees greater than 10-cm basal-diameter were measured at each plot with a diameter tape at breast height. The basal-area measurements for each tree were converted to relative basal area (the proportion of total basal area of a species). Relative basal area better reflects the competiveness of individuals when encountering other individuals in the same forest community than does absolute size (basal area) (Luo and Chen 2011).

The percentage cover for the overstorey and understorey was estimated using the line-intercept method along a 50-m tape (Neldner *et al*. 2005). The height of the overstorey stratum, slope class (Table 2-1) and the aspect were determined. The plant assemblages at each plot were also recorded. The abundance of each species present was scored on a scale of 1–7, with 1 for species occurring only once and 7 for dominant species (Melzer 2004). Plant species that could not be identified in the field were sent to the Queensland Herbarium for identification.

Table 2-1 Landform-pattern, slope-class and growth-form descriptions for the survey plots

Parameter	Description
Landform pattern	
Riverine	Stream channel
Alluvial plain	Alluvial plain characterised by frequent active erosion by overbank stream flow
Rise	Very low relief 9–30 m, with very gentle to steep slopes
Low hill	Low relief 30–90 m. Gentle to very steep slopes
Hill	High relief 90–300 m. Gently inclined to precipitous slopes
Mountain	Very high relief greater than 300 m. Moderate to precipitous slopes

Parameter	Description
Slope class (average degrees)	
Level	0°20′
Very gentle	1°
Gentle	3°
Moderate	10°
Steep	23°
Very steep	37°
Growth form	
Grasses and sedges	Herbaceous monocots
Herbs	Herbaceous or slightly woody annuals or perennials in the ground stratum
Shrubs	Woody plants. Multi-stemmed at the base, or if single-stemmed less than 3 m tall within the lower mid-stratum
Tall shrubs/small trees	Woody plants. Multi-stemmed at the base or single-stemmed within the mid-stratum to upper mid-stratum
Trees	Woody plants. Usually with a single stem in the canopy or sub-canopy

The plant species were grouped according to their height, growth characteristics and functional group as grass or sedge, herb, shrub, tall shrub/small tree or tree (Table 2-1). Species were recorded as being either endemic or rare according to EPA (2010). Three soil samples from the top 10 cm were collected within the survey plots using a hand held soil auger. The soil cores were sealed into plastic bags and transported back to the laboratory for processing. Foliage samples from three *E. fibrosa* subsp. *fibrosa* canopy trees were collected from each plot with a 3-m extended tree pruner, which when held gave a cutting height of 5 m. This allowed foliage samples to be taken consistently from the same height at each site.

2.3.4 Soil analysis

The three soil samples per plot were pooled together for analysis (due to financial constraints). The soil samples were oven-dried at 40°C for 24 h and screened to 2 mm. The soil was analysed for Colwell P, and nitrate N (NO_3) and ammonium N (NH_4-N), S, extractable metals (B, Co, Cu, Fe, Mn, Ni, and Zn) and major cations (Mg, Ca, Al, K, Na) by using the Mehlich No. 3 method and inductively coupled plasma–optical emission spectrometry (ICP–OES). Total N was analysed using the Kjeldahl method and soil pH (1 : 5 water) was analysed according to the methods of Rayment and Lyons (2010) (4A1). The soil analysis was undertaken by ChemCentre Laboratory Western Australia.

2.3.5 Foliage analysis

Intact leaves were used for analysis and were rinsed with MilliQ water and oven-dried at 40°C to constant weight. The samples were ground to a fine powder with a stainless-steel coffee and spice grinder. Concentrations of dissolved metals and major cations (B, Ca, Cu, Fe, K, Na, Ni, Mg, Mn, S, Zn) were determined by acid digesting the samples in HNO_3 with H_2O_2, filtering and using ICP–atomic emission spectrometry (AES) for elemental assay (Jones 2001). Total N was determined by Kjeldahl digestion for N. The analysis by ICP–AES and Kjeldahl digestions were undertaken by CSBP Soil & Plant Laboratory Western Australia.

2.3.6 Statistical analysis

The data were analysed using PRIMER 6 and SPSS 19. Principal component analysis (PCA) was performed on the soil elements and also on the *E. fibrosa* subsp. *fibrosa* foliage elemental data. Elemental data were square-root transformed and normalised and dissimilarity was measured using Euclidean distance.

The species-abundance data were square-root transformed and compared using the Bray–Curtis similarity index (Clarke and Gorely 2006).

Non-metric multidimensional scaling (MDS) was employed to construct scatter plots of the species abundance data using factors. The factors were landform pattern (Table 2-1) and landform elements (slope, aspect, position). Stress factors of <0.05 give an excellent representation, with no prospect of misinterpretation. Stress factors of >0.1 and <0.2 still give potentially useful two-dimensional representation (Clarke and Warwick 2001; Clarke and Gorely 2006).

Analysis of similarity (ANOSIM) tests the null hypothesis that there are no differences among the sites by a single factor. The Global *R* and corresponding pairwise comparisons (*R*) measure the degree to which the sites differ. If there are no differences among sites by factor, the Global R and pairwise R will be near zero. The significance of the tests are determined via randomisation tests (n = 999 permutations; Clarke and Warwick 2001; Clarke and Gorely 2006). ANOSIM was performed on soil elements, foliage elements and species-abundance data. The factors were landform pattern and landform elements.

Multiple stepwise linear regression modelling using SPSS 19 was performed on structural data, species richness, species diversity and site elements. Proportional data were arcsine transformed and natural log-transformation was applied to continuous data. Log + 1 transformations were used if the data contained zero values. Data were transformed to best satisfy linearity and assumptions of normal distribution and homoscedastic variance. The significance of the statistical tests was set at 5% probability.

2.4 Results

2.4.1 Site elements

The soil chemistry was consistent with serpentine soils worldwide, with high concentrations of Ni, high Mg/Ca quotients and low fertility (Table 2-2). The mountain sites appeared to separate from the other landform patterns (Figure 2-2) and the soil fertility decreased from the mountains to the plains (particularly for NH_4-N, K, B, Mn and S). The differences in soil elements between the landform patterns were significant and pairwise comparisons found differences between the mountains and alluvial plains, low hills, rises and hills (R = 1.00, P = 0.029, R = 1.00, P = 0.008, R = 0.659, P = 0.029, R = 0.522, P = 0.003, respectively; Global R = 0.253, P = 0.016). There were also differences between the rises and low hills and between the alluvial plains and rises (R = 0.856, P = 0.008, R = 0.704, P = 0.029, respectively). Nickel was highest on the hills and low hills and the Mg/Ca quotient was highest on the alluvial plains (Table 2-2).

The soil elements did not vary across the landform elements (slope, aspect, or slope type).

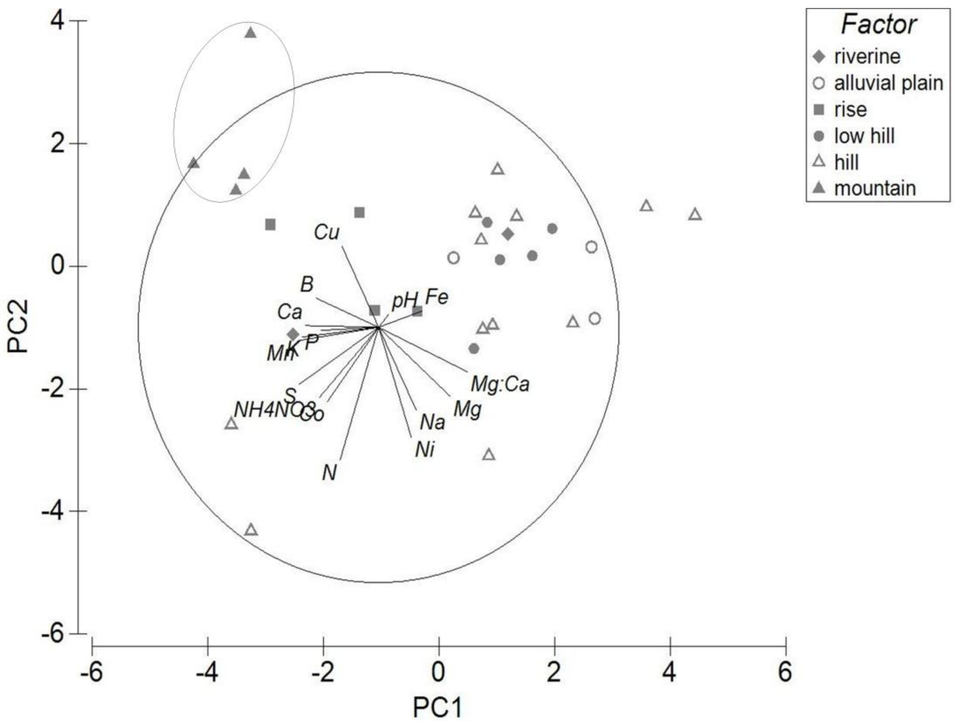

Figure 2-2 Principal component analysis (PCA; 77% variation captured) of the soil elements for each of the landform patterns, with the mountain sites separated out graphically

Table 2-2 Summary of soil elements across the landform pattern types. Means followed by min–max range in parentheses

Parameter	Riverine	Alluvial plains	Rises	Low hills	Hills	Mountains
Concentration of NH_4-N (mg L^{-1} DW)	8.00 (1–15)	8.33 (6–12)	15.00 (8–21)	6.80 (5–9)	14.17 (1–40)	12.25 (11–15)
Concentration of NO_3 (mg L^{-1} DW)	0.50 (0–1)	1.00 (1–1)	4.50 (1–8)	2.20 (1–4)	4.75 (0–11)	2.25 (0–5)
Concentration of P (Colwell) (mg L^{-1} DW)	12.00 (7–17)	5.00 (1–8)	8.50 (7–11)	6.00 (1–9)	6.75 (1–19)	9.75 (7–14)
Concentration of K (Colwell) (mg L^{-1} DW)	200.50 (191–210)	113.67 (99–135)	168.25 (123–273)	114.80 (77–144)	101.75 (54–182)	179 (145–223)
Exc. Al (mEq 100 g^{-1})	0.01 (0–0.01)	0.01 (0.01–0.01)	0.01 (0–0.01)	0.01 (0.01–0.02)	0.02 (0–0.04)	0.02 (0.01–0.05)
Exc. Ca (mEq 100 g^{-1})	14.06 (11.34–16.78)	9.80 (8.7–10.7)	11.75 (8.69–14.9)	9.85 (7.42–12.72)	8.70 (3.88–18.99)	13.51 (10.22–15.54)
Exc. Mg (mEq 100 g^{-1})	22.07 (14.67–29.47)	24.98 (17.33–31.87)	14.33 (10.57–19.11)	19.94 (16.07–23.97)	17.41 (10.06–24.85)	4.99 (3.73–7.28)
Exc. K (mEq 100 g^{-1})	0.42 (0.41–0.43)	0.28 (0.24–0.34)	0.39 (0.28–0.66)	0.27 (0.16–0.31)	0.24 (0.13–0.44)	0.42 (0.38–0.47)
Exc. Na (mEq 100 g^{-1})	0.17 (0.15–0.18)	0.28 (0.13–0.36)	0.13 (0.1–0.17)	0.17 (0.13–0.24)	0.19 (0.09–0.28)	0.11 (0.08–0.16)
Extractable concentration of B (mg L^{-1} DW)	0.75 (0.6–0.9)	0.37 (0.3–0.5)	0.53 (0.2–0.8)	0.28 (0.1–0.5)	0.28 (0.1–0.8)	0.78 (0.4–1.1)
Extractable concentration of Cu (mg L^{-1} DW)	0.65 (0.4–0.9)	1.10 (0.7–1.7)	1.23 (0.5–1.9)	0.96 (0.6–1.3)	1.15 (0.4–2.5)	6.50 (1.1–20)
Extractable concentration of Fe (mg L^{-1} DW)	145.00 (140–150)	106.67 (100–110)	108.00 (72–130)	112.00 (100–130)	104.83 (52–140)	95.75 (78–110)
Extractable concentration of Mn (mg L^{-1} DW)	130.00 (110–150)	92.67 (43–140)	165.00 (120–200)	114.00 (100–140)	132.08 (48–250)	227.50 (200–250)
Extractable concentration of Ni (mg L^{-1} DW)	94.00 (91–97)	89.67 (55–120)	79.00 (46–120)	109.20 (96–130)	122.92 (61–250)	65.75 (11–120)
Extractable concentration of S (mg L^{-1} DW)	8.00 (7–9)	4.67 (4–5)	7.25 (5–9)	4.80 (3–6)	6.42 (3–15)	8.75 (7–12)

Parameter	Riverine	Alluvial plains	Rises	Low hills	Hills	Mountains
Extractable concentration of Zn (mg L^{-1} DW)	1.30 (1–1.6)	1.17 (0.8–1.5)	2.38 (1.1–4.6)	1.62 (1.4–1.8)	2.26 (0.9–12)	4.30 (1.6–11)
Extractable concentration of Co (mg L^{-1} DW)	6.30 (4.7–7.9)	4.63 (2–7.3)	7.23 (5.3–9.5)	6.08 (5.2–7.4)	6.83 (2.1–10)	7.60 (2.7–10)
pH	5.44 (5.12–5.76)	5.55 (5.36–5.69)	5.67 (5.57–5.87)	6.82 (5.33–9)	5.95 (5.15–6.55)	6.01 (5.24–6.37)
%N	0.35 (0.26–0.43)	0.26 (0.23–0.28)	0.31 (0.28–0.35)	0.27 (0.21–0.37)	0.32 (0.13–0.63)	0.24 (0.2–0.27)
Mg / Ca	1.74 (0.87–2.6)	2.59 (1.62–3.19)	1.27 (0.88–1.67)	2.11 (1.42–3)	2.48 (0.98–6.4)	0.40 (0.24–0.71)

2.4.2 Floristic structure

Eucalyptus fibrosa subsp. *fibrosa* was the most dominant tree throughout the landscape and was found at every site, whereas the endemic tree *Corymbia xanthope* was subdominant. The relative basal area of *E. fibrosa* subsp. *fibrosa* did not vary significantly according to the landform patterns (Global R = 0.010, P = not significant); however, it was found to decrease weakly with the soil Mg/Ca quotient (Table 2-3). Significant differences were found in the relative basal area of *C. xanthope* according to the landform patterns (Global R = 0.191, P = 0.031). The relative basal area of *C. xanthope* was greatest on the low hills and hills (Table 2-4) and increased with the soil Mg/Ca quotients (Table 2-3). *C. xanthope* was negatively correlated with soil Ca (Table 2-3), but not correlated with soil Mg.

The canopy height varied across the slope types and was greater at the level sites than at the steep sites (R = 0.349, P = 0.009; Global R = 0.132, P = 0.041). The canopy height did not vary according to the soil elements or landform pattern.

There were no significant relationships between the canopy cover and the soil elements, landform elements or soil types.

2.4.3 Species richness and assemblages

The overall species richness increased as the soil became more acidic (Table 2-3). The species richness also increased with soil B and decreased with soil Ni (Table 2-3). The Shannon–Wiener diversity (H') decreased with the soil Mg and soil acidity (Table 2-3). No other correlations were evident between species richness and the other soil elements, landform elements or landform patterns. However, significant differences were found in the occurrence of the endemic and rare flora in the understorey according to the landform patterns.

There was a greater presence of endemic and rare flora on the hills than on the rises and low hills (*R* = 0.429, *P* = 0.009, *R* = 0.302, *P* = 0.014, respectively; Global *R* = 0.279, *P* = 0.006) (Table 2-4). The presence of endemic and rare flora did not vary according to landform elements (aspect, slope) or soil type. The landform patterns also influenced the grass and sedge assemblages. There was a greater assemblage of grasses and sedges on the mountains than on the rises, hills and low hills (*R* = 0.630, *P* = 0.029, *R* = 0.452, *P* = 0.001, *R* = 0.513, *P* = 0.016, respectively; Global *R* = 0.287, *P* = 0.002). The assemblages of the grasses and sedges were greater on the rises than on the low hills and on the hills than on the low hills (*R* = 0.438, *P* = 0.032; R = 0.216, *P* = 0.038) (Table 2-4).

Table 2-3 Multiple linear regression model results with floristic structure and foliage elements as the dependent variables and soil elements as the independent variables

Dependent variable	Independent variable	B	SE	β	*P*-value	R^2
Relative basal area *E. fibrosa*	Constant	1.350	0.105		0.000	
	Ln(Mg : Ca)	–0.216	0.098	–0.397	0.036	0.158
Relative basal area *C. xanthope*	(Constant)	0.120	0.101		0.248	
	Ln(Mg : Ca)	0.286	0.095	0.509	0.006	0.260
Relative basal area *C. xanthope*	(Constant)	1.067	0.235		0.000	
	Ln(Ca)	–0.292	0.101	–0.491	0.008	0.241
Species richness	(Constant)	11.688	15.339		0.453	
	Ln(B)	12.067	5.366	0.343	0.034	0.189[A]
	Ln(Ni)	–5.789	2.018	–0.446	0.008	0.122[A]

Dependent variable	Independent variable	B	SE	β	*P*-value	R^2
	pH	6.488	2.518	0.399	0.017	0.149^A
					0.002	0.392^B
Species *H*’	(Constant)	2.386	0.059		0.000	
	Ln(Mg)	–0.037	0.007	–0.796	0.000	0.469^A
	pH	–0.019	0.009	–0.308	0.042	0.083^A
					0.000	0.516^B
Herb S	(Constant)	29.457	6.720		0.000	
	Ln(Ni)	–4.896	1.328	–0.675	0.001	0.245^A
	Ln(B)	9.038	3.101	0.459	0.008	0.124^A
	Ln(Cu)	–3.042	1.423	–0.410	0.043	0.101^A
					0.001	0.404^B
Herb d	(Constant)	5.736	1.250		0.000	
	Ln(Ni)	–0.816	0.262	–0.474	0.005	0.292^A
	Ln(B)	1.935	0.717	0.412	0.012	0.165^A
					0.001	0.412^B
Ln(foliage Na)	(Constant)	6.392	0.147		0.000	
	Ln(Na)	4.115	0.860	0.684	0.000	0.448

^{A}Partial R^2; ^{B}Adjusted R^2.

The assemblages of herbs also varied according to the landform pattern and there was a greater assemblage of herb species on the mountains than on the alluvial plains, rises, hills and low hills (R = 0.778, P = 0.029, R = 0.740, P = 0.029, R = 0.531, P = 0.003, R = 0.616, P = 0.008, respectively; Global R = 0.277, P = 0.008). The hills had a greater assemblage than did the low hills (R = 0.202, P = 0.043) (Table 2-4). When examining the soil elements, the following correlations were detected: the total number of herb species decreased with increasing soil Ni and Cu and increased with increasing soil B

(Table 2-3); and the species richness of the herb species decreased with increasing soil Ni and increased with soil B (Table 2-3).

Shrub species assemblages grouped according to the landform pattern. The assemblages of the shrubs were greatest on the hills, compared with the mountains, and increased on the hills compared with the low hills (*R* = 0.673, *P* = 0.001, *R* = 0.358, *P* = 0.001, respectively; Global *R* = 0.358, *P* = 0.001) (Table 2-4). Similarly, the tall shrubs/small trees were clearly distinguished according to the landform pattern, particularly for the mountain sites (Figure 2-3). The assemblages of the tall shrubs and small trees were greater on the mountains than on the alluvial plains and rises and low hills (*R* = 0.759, *P* = 0.029, *R* = 0.854, *P* = 0.029, *R* = 0.844, *P* = 0.029, respectively; Global *R* = 0.380, *P* = 0.003) (Table 2-4). Yet the landform pattern did not influence the assemblages of the canopy trees (Global *R* = 0.169, *P* = 0.089) (Table 2-4). Refer to Appendix 1 for the complete species list for each plot.

2.4.4 Foliage elements of *Eucalyptus fibrosa* subsp. *fibrosa*

Overall, 65.8% of the variance in foliage elemental composition of *E. fibrosa* subsp. *fibrosa* among sites was captured with a PCA. PC1 explained 35.2% of the variation and there were positive loadings of the foliar elements K, Cu, P, N and Na and negative loadings of B, Ca, Mg, Ni and Mn. The foliage elements from PC1 did not vary with the landform pattern, aspect, slope or soil type (Global *R* = 0.120, *P* = not significant, Global *R* = 0.003, *P* = not significant, Global *R* = 0.030, *P* = not significant, Global *R* = 0.057, *P* = not significant, respectively). There were no significant relationships between the foliage elements of PC1 and the total basal area of *E. fibrosa* subsp. *fibrosa*, or the percentage cover of the canopy or the canopy height. With the exception of soil and foliage Na, which were positively correlated (Table 2-3), no further correlations between the foliage and soil elements were evident. The mean foliage element concentrations of *E. fibrosa* subsp. f*ibrosa* are listed in Appendix 2.

Figure 2-3 Multidimensional scaling (MDS) of the assemblages of the tall shrubs/small trees grouped by landform pattern for each site with the mountains separated graphically

Table 2-4 Summary of the floristic structure for each of the landform-pattern types. Data are means, followed by minimum–maximum range in parentheses

Parameter	Riverine	Alluvial plains	Rises	Low hills	Hills	Mountains
Relative basal area of *C. xanthope*	0.15 (0.40–0.25)	0.05 (0.00–0.10)	0.10 (0.03–0.16)	0.27 (0.60–0.55)	0.28 (0.08–0.55)	0.05 (0.00–0.14)
Relative basal area of *E. fibrosa*	0.72 (0.70–0.75)	0.95 (0.90–1.00)	0.86 (0.78–0.97)	0.73 (0.45–0.94)	0.71 (0.45–0.92)	0.93 (0.82–1.00)
Foliage cover (%)	51.00 (45.60–56.40)	77.13 (43.00–97.40)	50.50 (34.80–71.80)	62.20 (38.00–79.00)	53.40 (24.40–91.20)	63.50 (48.00–79.40)
Canopy height (m)	17.00 (15.90–18.10)	17.00 (17.00–17.00)	15.90 (13.70–18.00)	15.73 (14.30–17.00)	15.93 (11.67–20.30)	17.26 (13.20–20.21)
Species richness	26.00 (25.00–27.00)	23.67 (19.00–30.00)	31.50 (23.00–39.00)	27.00 (23.00–30.00)	25.30 (17.00–40.00)	32.50 (27.00–40.00)
Total no. of species (S)						
Endemic flora	4.00 (4.00–4.00)	2.00 (0.00–3.00)	2.70 (1.00–5.00)	3.40 (2.00–5.00)	3.70 (2.00–5.00)	2.50 (1.00–4.00)
Grass	3.00 (3.00–3.00)	6.67 (5.00–8.00)	7.00 (7.00–7.00)	6.2 (5.00–7.00)	6.10 (3.00–12.00)	7.25 (4.00–11.00)
Herb	9.00 (8.00–10.00)	6.70 (2.00–15.00)	10.50 (9.00–15.00)	5.80 (3.00–10.00)	6.50 (1.00–13.00)	10.50 (7.00–14.00)
Shrub	7.50 (6.00–9.00)	5.70 (5.00–6.00)	7.75 (6.00–10.00)	6.80 (3.00–9.00)	7.00 (5.00–10.00)	6.00 (4.00–8.00)
Tall shrub/small tree	2.50 (2.00–3.00)	3.00 (3.00–3.00)	3.75 (2.00–5.00)	3.20 (0.00–7.00)	3.33 (2.00–8.00)	4.00 (3.00–5.00)
Tree	3.50 (2.00–5.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	2.00 (2.00–2.00)	2.25 (1.00–4.00)

2.5 Discussion

Plants can exhibit the effects of metals and reveal information on the soil quality that is difficult to measure using direct soil analysis (Lyon *et al*. 1971*a*; Rayment and Lyons 2010; Bauer *et al*. 2011). Indeed, our hypothesis that the eucalypt forests of the upland central Queensland serpentine landscape could be used as biological indicators of the severity of the serpentine soils was supported by the results. The relative basal area of the endemic tree *C. xanthope* increased as the availability of soil Ca decreased. Correlations between the soil Mg/Ca quotients and the biomass of woody species have been found on serpentine landscapes worldwide. Soil Mg/Ca quotients are more likely than trace elements to influence woody serpentine plant species (Alexander 1988; Bauer *et al*. 2011; O'Dell *et al*. 2006).

The soil chemistry varied according to the landform pattern, and these differences were similar to patterns found by Forster and Baker (1995) and Batianoff and Singh (2001). The soils of the mountains had the highest Ca and lowest Mg and the concentrations of Mg increased further down the landscape. The mountains had the lowest concentrations of extractable Ni and the hills had the greatest concentrations. Plant growth also varied according to the landform pattern, reflecting the differences in the soil chemistry. The assemblages of the species were greater on the mountains than on the sites further down the landscape, possibly reflecting the greater availability of Ca, lower Mg and lower concentrations of extractable Ni. Our results showed that the overall diversity of species was negatively correlated with the soil Mg. The ability of serpentine-adapted species to take up and translocate Ca in the presence of high concentrations of Mg may be a more important adaptation than heavy-metal resistance (O'Dell *et al*. 2006).

The canopy height was greater on the level sites than on the steeper sites, probably because of higher water availability. Previous studies on the central Queensland serpentine landscape have found that the flow of water increases from the skeletal soils of the slopes to the deeper soils of the plains (Specht *et al*. 2002, 2006) and steeper slopes retain less water than do flatter sites (Cooke and Leishman 2011; Schutz *et al*. 2002; Reeves 2003; Wormington *et al*. 2007).

Overall, the assemblages of the endemic and rare species are greatest on the hills, reflecting high concentrations of extractable Ni and a high Mg/Ca quotient and skeletal soils with low moisture-holding capacity. Within these edaphic serpentine conditions, differences in soil elements influence the individual endemic species. The relative importance of the soil chemistry varied from one functional group to another. We found that the abundance of the herbaceous species was strongly negatively influenced by extractable Ni, supporting the findings of Lazarus and Richards (2011). Interactions between the total species richness and soil acidity, B and Ni were found and it is likely that B and Ni are more available to the plants in acidic soils. The total species richness increased with soil acidity and soil B. Boron is not considered to be a characteristic element of serpentine soils but it may have a greater influence on serpentine flora than previously considered (Sambatti and Rice 2007). Boron is an essential micronutrient to plants and some functions of B are related to N, P, K and Ca uptake (Williamson and Johnson 1981; Bradshaw 1997; Cornara *et al*. 2007). The species richness decreased with increasing soil Ni, supporting previous findings (Batianoff *et al*. 1997, 2000).

The uptake of Na by *E. fibrosa* subsp. *fibrosa* may act to reduce the uptake of Mg. The maintenance of the Na ions in the foliage may reduce the uptake of more toxic ions (Crooke 1956; Russell 1970; Chiarucci *et al*. 1998). No other relationships were found between the foliage and soil nutrients and metals or landform patterns, indicating that *E. fibrosa* subsp. *fibrosa* is able to regulate the uptake of the other measured elements.

The canopy tree *E. fibrosa* subsp. *fibrosa* is the most successful tree on the central Queensland serpentine landscape and generally outcompetes *C. xanthope.* Only when the serpentine gradients becomes severe, exhibiting high Mg/Ca quotients and potentially lower soil-water potentials, does the endemic *C. xanthope* increase in relative basal area. The structure of the eucalypt forests of the central Queensland serpentine landscape varies with the landform patterns, reflecting differences in the soil chemistry. In particular (or most notably), the relative basal area of *C. xanthope* is a useful measure of the severity of the serpentine soils because it was found to be positively correlated with the soil Mg/Ca quotients. The foliage of the *E. fibrosa* subsp. *fibrosa* cannot be used as an indicator of the soils because it appears that the eucalypt is able to regulate uptake of the soil elements, with the exception of Na.

Chapter 3 An ecological study of the central Queensland serpentine endemic shrub Neoroepera buxifolia, Australia

(A slightly modified version of this chapter has been accepted for publication as: Hendry RA, Wormington KR, Walsh KA (2015) An ecological study of the central Queensland ultramafic endemic shrub *Neoroepera buxifolia* Muell.Arg. & F.Muell. (Picrodendraceae), Australia, Australian Journal of Botany – in press)

Plate 3-1 *Neoroepera buxifolia* shrubs

3.1 Abstract

The serpentine endemic shrub *Neoroepera buxifolia* has high habitat specificity and is mostly restricted to the perennial and ephemeral creeks and drainage lines of the central Queensland serpentine landscape. It has an association with high magnesium concentrations in the soil and this study seeks to determine if there is a correlation between populations of *N. buxifolia* and the extractable magnesium and/or Mg/Ca quotients in the soil. This was achieved by measuring the standing volume, height and seedling counts of *N. buxifolia* against the soil elements and other plot characteristics. Whilst correlations were found between increased height of *N. buxifolia* and the Mg/Ca quotients, extractable soil nickel had the greatest influence. The standing volume of *N. buxifolia* also increased with soil extractable nickel. The standing volume of *N. buxifolia* was greatest in the upper tributaries of the 1st order ephemeral creeks and presence of permanent water of the perennial creeks did not influence the standing volume or height of *N. buxifolia*.

3.2 Introduction

Serpentine landscapes worldwide are ecologically significant and support large numbers of endemic flora. Serpentine soils can be characterised as being vulnerable to erosion, and having low organic material, low fertility, poor moisture holding capacity, low calcium (Ca) availability relative to magnesium (Mg) and high concentrations of trace elements such as nickel (Ni) and chromium (Cr) (Brooks 1987; Kazakou *et al.* 2008). Serpentine vegetation can be characterised as having a high degree of endemism, poor plant productivity and a stunted appearance (Brooks 1987). The serpentine landscape of central Queensland on the east coast of Australia is ecologically significant and supports about 17 endemic flora species (Aola 2014; Batianoff *et al.*; Batianoff *et al.* 19972000; Jackes 2005). One such endemic species is *Neoroepera buxifolia* Muell.Arg. & F.Muell. (Picrodendraceae) a monoecious microphyll shrub to small tree that grows up to 7m tall. Its form, flowers, leaves and fruit are described in detail by Airy Shaw (1980) and Henderson (1992). *Neoroepera buxifolia* is classified as vulnerable under the Australian Government Environmental Protection and Biodiversity Conservation Act (1999) (EPBC 1999) and the Queensland State Government Nature Conservation Act (1992) (NCA 1992) due to its localised distribution and lack of representation on conservation reserves (Threatened Species Scientific Committee 2008). *Neoroepera buxifolia* occurs in the ephemeral (water flows during a storm or rain event but not for extended periods afterwards) and perennial (the water flows beyond storm and rain events) creeks, riverbanks, terraces and drainage lines of the central Queensland serpentine landscape and is associated with high concentrations of Mg in the soil (Batianoff *et al.* 2000).

Within the central Queensland serpentine landscape weathering of the serpentinite rock causes the groundwater flowing from the hills to become supersaturated in Mg. This precipitates out in the groundwater as magnesium carbonate ($MgCO_3$) and deposits of magnesite ore have developed on the alluvial flats (Forster and Baker 1995; Forster and Baker 2002; Wilcock 1998). Magnesium causes soils to become alkaline and is toxic to plants depending on the amount of Ca present (Proctor and Nagy 1992). The Mg/Ca quotient of soils is used to determine the Mg gradient or severity of the serpentine soils (McCarten 1986).

Neoroepera buxifolia has an association with high soil Mg (Batianoff *et al.* 2000) and this study seeks to determine if the population density of *N. buxifolia* is correlated to the Mg and/or soil Mg/Ca quotients. Correlations between soil Mg/Ca quotients and the biomass of woody species have been found on serpentine landscapes worldwide (Alexander 1988; Bauer *et al*. 2011; O'Dell *et al*. 2006) including within central Queensland (Hendry *et al.* 2014, Chapter 2). The habitat requirements for *N. buxifolia* including the site characteristics such as perennial or ephemeral creek, stream order, floristic assemblages and soil elements will be assessed to determine the influence extractable soil elements and site characteristics have on *N. buxifolia* populations. This will be achieved by:

1. exploring the influence of soil elements, site characteristics and floristic assemblages on the standing volume of *N. buxifolia*;
2. comparing the soil, site characteristics and floristic assemblages between perennial and ephemeral creeks within the serpentine landscape with and without *N. buxifolia* present.

3.3 Methods and materials

The ephemeral and perennial creeks within the serpentine landscape in close proximity to vehicle tracks (within 1km) between Yaamba and Marlborough were searched (on-foot and by vehicle) for the presence of *N. buxifolia* (Figure 3-1). A total of twenty-five locations with *N. buxifolia* present were located and assessed for vegetation structure and to collect soil samples. A further ten plots without *N. buxifolia* present for at least 500m up and downstream were assessed and used as comparison plots. The sample area at each plot was

1000m^2 (50 x 20m) and a 50m tape was laid down along the centre of the creek or across the contour of the hill (Neldner *et al.* 2005). If there was water present in the creek the tape was placed along the creek bank close to the edge and if there was a large body of water present such as in a permanent water course only one side of bank was assessed.

The position of the plot within the catchment was determined using stream order defined according to Strahler (1957), the upper reaches of the tributaries on the hills and mountains were designated as order 1 channels, and where two 1st order channels join an order 2 channel is formed. When two 2nd order channels join the new segment is an order 3 channel and so on. The highest order stream has the most water discharge (Strahler 1957).

The standing volume within the plot area was calculated as m^3 per plot (1000 m^2) by measuring the height, length and width of each *N. buxifolia* shrub or tree.

A species assemblage list was recorded for each plot, using abundance ratings of 1 to 7, with 1 only occurring once within the plot and 7 being dominant (Melzer 2004). Species were identified in the field or, if unable to be identified, a specimen was collected and sent to the Queensland herbarium for identification.

Three soil samples from the top 10 cm were collected within 1 metre from underneath the canopy of *N. buxifolia* trees within the plot using a soil auger. In the plots where there was no *N. buxifolia* present the soil was collected from beneath the canopy of the understory. The soil cores were sealed into plastic bags and transported back to the laboratory for processing. The soil samples were oven-dried at 40^{o}C for 24 hours and screened to 2 mm. The three soil samples per plot were pooled together for analysis (due to financial constraints). The soil was analysed for extractable elements (Al, B, Ca, Co, Cu, Fe, Mg, Mn, Ni, P, K, Na, S, Si, Zn) using Mehlich 3 extraction and ICP-MS. Soil pH (1 : 5 water) and soil conductivity were determined using the methods of Rayment and Lyons (2010) (4A1 and 4B1; respectively). Analysis was undertaken by the Environmental Analysis Laboratory, Southern Cross University.

The number of *N. buxifolia* seedlings was counted at each plot by placing 1 m^2 quadrats every 5 m along the centre transect line to give a total of ten quadrats. The quadrat was recorded as being either under or outside a *N. buxifolia* canopy. When the quadrat was placed outside a *N. buxifolia* canopy the distance from the edge of the nearest canopy was measured in 1m intervals.

3.3.1 Data analysis

The data were analysed with a number of statistical methods using PRIMER 6 (Clarke and Gorely 2006) and SPSS 20 (IBM Corp. Released 2010). ANOSIM (analysis of similarity) tests were performed on soil elements, standing volume of *N. buxifolia*, species assemblage data and seedling counts. ANOSIM tests allow for a test of the null hypothesis that there are no differences between the plots by a single factor (Clarke and Gorely 2006; Clarke and Warwick 2001). The factors were perennial or ephemeral creek, stream order, *N. buxifolia* and non-*N. buxifolia* present, assemblage data, aspect and seedling counts (Clarke and Gorely 2006).

SIMPER (similarity percentages) routines are non-statistical, exploratory analysis used to identify the species responsible for either clustering patterns or differences between sets of samples (Clarke and Warwick 2001). SIMPER routines were performed on the species assemblage data using type of creek (either perennial or ephemeral) as the factor. Similarity was measured using a Bray-Curtis similarity index.

Multiple linear regressions were used to construct models from the data with the soil elements serving as the independent variables and the volume and height of *N. buxifolia* as the dependent variables (SPSS v20). The data were log +1 transformed where necessary to best satisfy linearity, and the assumption of normal distribution and homoscedastic variance of the variables in the multiple linear regression models. The significance of the statistical tests was set at 5% probability.

The soil elements for the *N. buxifolia* and non-*N. buxifolia* plots and comparisons of the species richness between plots were compared using non-parametric Mann-Whitney U tests for 2 independent samples and Kruskal-Wallace with chi-square for multiple independent samples (IBM Corp. Released 2010). Non-parametric tests were performed as the data did not satisfy the assumptions of normal distribution.

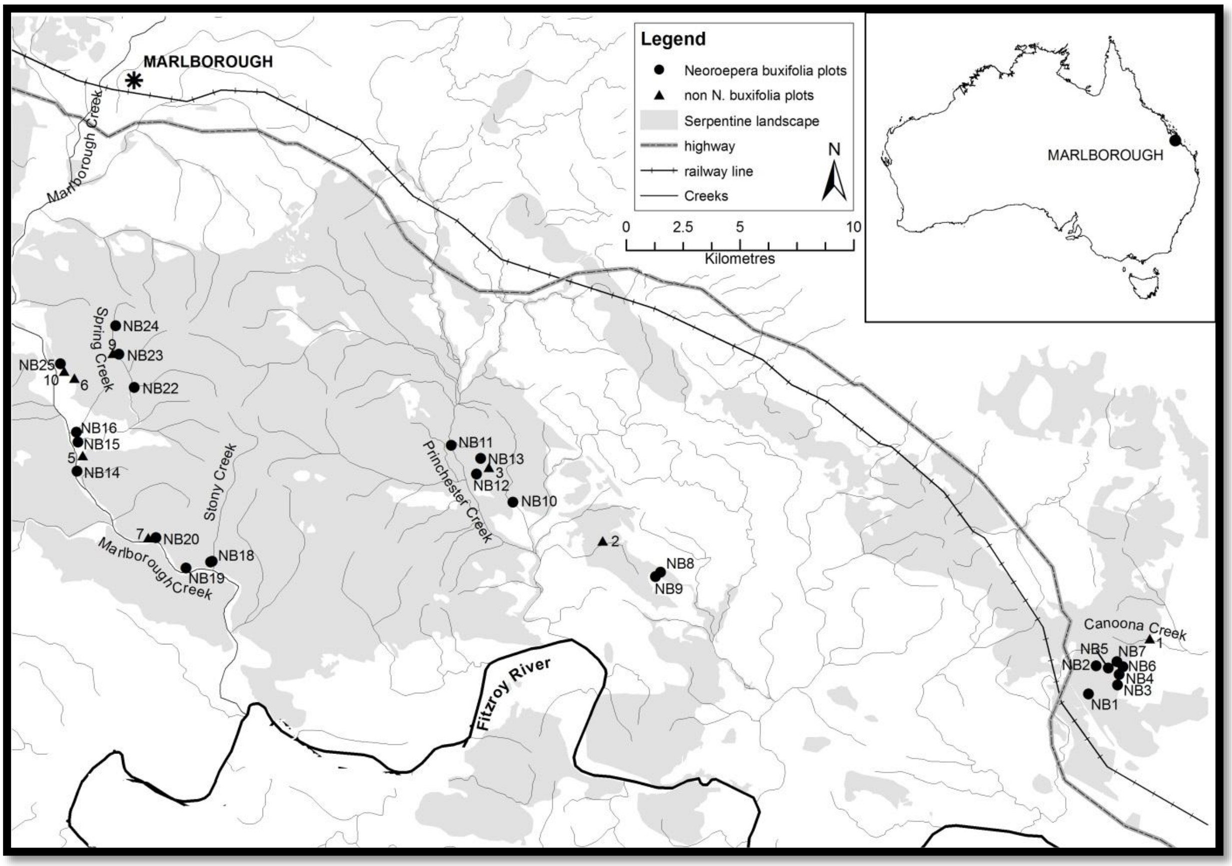

Figure 3-1 Locality map of the plots in relation to the central Queensland serpentine landscape.

3.4 Results

A total of twenty-five plots with *N. buxifolia* present and ten plots without *N. buxifolia* present within the serpentine landscape were surveyed (Figure 3-1). Of the twenty-five plots with *N. buxifolia* present, seventeen were ephemeral and 7 were perennial sites. One *N. buxifolia* plot was located on a hillside at Glen Avon Station forming a closed forest. The ten plots without *N. buxifolia* present were ephemeral and all the perennial creeks surveyed had *N. buxifolia* present.

3.4.1 Soil elements

The extractable Ni and Si increased (r^2 = 0.427, β = -0.654, p = <0.010; r^2 = 0.770, β = -0.878, p = <0.010; respectively) as the soils became less alkaline, and as the soil became alkaline the extractable Mg increased (r^2 = 0.491, β = 0.701, p = <0.010). Extractable Ni (χ^2 (3) = 8.432, p = <0.050) was highest in the order 1 channels and lowest in the order 5 channels. Extractable Cu and S had greater mean rankings in the non *N. buxifolia* plots (Cu =24.22, S = 23.22; respectively) compared to the *N. buxifolia* plots (Cu = 15.08, S = 15.44; respectively) (χ^2 (1) = 4.042, p = <0.050, χ^2 (1) = 5.578, p = <0.050; respectively). There were no other significant differences in measured soil elements between the plots according to channel order or between the non-*N. buxifolia* and *N. buxifolia* present plots (Table 3-1).

Table 3-1 The mean soil properties and elements for the *Neoroepera* and non-*Neoroepera buxifolia* plots. (p = <0.05 indicated with *)

Properties and Element	*N. buxifolia* plots Mean (min - max)	Non-*N. buxifolia* plots Mean (min - max)
conductivity $\mu s\ cm^{-1}$	74.84 (28-168)	64.89 (33-130)
extractable aluminium mg kg^{-1}	249.55 (165-391)	302.77 (199-428)
extractable boron mg kg^{-1}	2.16 (0.7-5)	1.95 (0.7-4)
extractable calcium mg kg^{-1}	940.75 (315-1797)	1161.93 (568-2285)
extractable cobalt mg kg^{-1}	8.92 (4-14)	7.75 (4-15)
extractable copper mg kg^{-1}	1.47 (0.7-3)*	2.09 (1-3)*
extractable iron mg kg^{-1}	168.54 (115-478)	143.70 (108-260)
extractable magnesium mg kg^{-1}	2882.66 (1495-5772)	3339.03 (1780-5028)
extractable manganese mg kg^{-1}	121.69 (77-194)	109.84 (43-238)
extractable nickel mg kg^{-1}	87.62 (28-196)	65.29 (32-115)
extractable phosphorus mg kg^{-1}	3.89 (1-15)	2.89 (1-6)
extractable potassium mg kg^{-1}	71.52 (36-141)	78.53 (34-184)
Extractable silicon mg kg -1	454.49 (173-865)	442.48 (289-585)
extractable sodium mg kg^{-1}	37.52 (13-87)	36.70 (13-77)
extractable sulphur mg kg^{-1}	24.07 (3-49)*	38.78 (2-63)*
extractable zinc mg kg^{-1}	2.30 (0.7-12)	1.70 (0.7-4)
Mg/Ca	3.67 (1-11)	3.55 (1-9)
pH	7.13 (6-9)	7.20 (7-8)

3.4.2 Standing volume and height of *Neoroepera buxifolia*

The standing volume of *N. buxifolia* increased with soil extractable Ni (r^2 = 0.408, β = 0.638, p = <0.001). The height of *N. buxifolia* increased with soil extractable Ni, K and Mg/Ca quotients (r^2 = 0.405, β = 0.800, p = <0.001, r^2 = 0.125, β = 0.513, p = <0.021, r^2 = 0.124, β = 0.124, p = <0.010, adj. r^2 = 0.633; respectively).

The height of *N. buxifolia* was greatest in the upper tributaries of the 1st order streams (χ^2 (3) = 10.02, p = 0.018). However, the standing volume did not significantly differ between the stream orders. There were no significant correlations in height or standing volume of *N. buxifolia* between the ephemeral or perennial stream types. There were no other significant correlations between the remaining soil elements (including extractable Mg) and the standing volume and height of *N. buxifolia*. The mean standing volume and height for *N. buxifolia* are given in Table 3-2.

Table 3-2 Mean total species within each plot, volume and height of *Neoroepera buxifolia* followed by minimum and maximum in parentheses.

Measurement	Mean (min-max)
Total species per plot	24 (14-39)
volume of *N. buxifolia* (m^3 plot $1000m^2$)	1110 (20-7000)
Height of *N. buxifolia* (m)	2.64 (0.95-7.00)
N. buxifolia seedling densities per plot ($1000m^2$)	2606 (0-13 833)

3.4.3 Species assemblages

The overall species richness did not significantly differ between the stream orders or between the perennial and ephemeral creek types however there were differences in the characteristic species according to creek type. The characteristic woody species of the perennial creek plots with *N. buxifolia* present contributing to 50% of the abundances in order of contribution were *N. buxifolia*, *Casuarina cunninghamiana* Miq. (Casuarinaceae), *Amorphospermum antilogum* F.Muell. (Sapotaceae) and *Melaleuca* sp. (Marlborough Creek G. N. Batianoff +MC9108006) (Myrtaceae) forming a fringing riverine open forest to woodland (average similarity = 43.84). The *N. buxifolia* at these plots occurred in the mid-stratum on the creek banks and white precipitate of magnesium carbonate was frequently observed on the edges of the water (pers. obs).

The overlying vegetation at the ephemeral creek plots was *Eucalyptus fibrosa* F.Muell. subsp. *fibrosa* (Myrtaceae) forest to woodland with or without *Corymbia xanthope* (A.R.Bean & Brooker) K.D.Hill & L.A.S.Johnson (Myrtaceae) and the *N. buxifolia* at these plots grows in the mid-stratum on the banks or within the creek beds. The characteristic woody species at the ephemeral creek plots contributing to 50% of the species abundances in order of contribution where *E. fibrosa* subsp. *fibrosa, Acacia leptostachya* Benth. (Mimosaceae) and *N. buxifolia* (average similarity = 34.48).

The vegetation at the ephemeral creek plots without *N. buxifolia* present was dominantly *E. fibrosa* subsp. *fibrosa* forest to woodland with mid-stratum of *A. leptostachya*. Other characteristic woody species in the understory included A. *antilogum, Alphitonia excelsa* (Fenzl) Benth. (Rhamnaceae), *Pimelea leptospermoides* F.Muell. (Thymelaeaceae) and *Dodonaea triquetra* J.C.Wendl. (Sapindaceae). The species richness at these plots was significantly lower compared to the plots with *N. buxifolia* present (Mann-Whitney U = 34.5, $p = <0.05$).

The one hillside plot located at Glen Avon station was a low forest dominated by *N. buxifolia* occurring in dense patches interspersed with *Acacia disparrima* M.W.McDonald & Maslin subsp. *disparrima* (Mimosaceae), *A. leptostachya* with emergent *C. xanthope*. This patch of *N. buxifolia* low forest occurs beneath powerlines and has been disturbed by line maintenance.

Exotic species at the time of the surveys were at low frequency, *Cryptostegia grandiflora* R.Br. (Apocynaceae) (Rubber vine) was only recorded at 2 plots whereas *Lantana camara* L. (Verbenaceae) (Lantana) was recorded at 9 plots. Refer to Appendix 1 for the plant species list for each of the plots. During the surveys, cattle were observed using the *N. buxifolia* creeks for water and shade and a local grazier has observed cattle using *N. buxifolia* as drought fodder (pers. comm. Scott 2011). *Neoroepera buxifolia* shrubs were observed to be re-sprouting successfully after fire.

3.4.4 Regeneration

Neoroepera buxifolia uses ballistochory for seed dispersal and an explosive enmasse release of seed was observed at the Marlborough locality on the 1 February 2011 during the summer monsoon season. It was observed that the seed capsules opened explosively and the seeds were fired out and dispersed up to 1 m from the canopy of the parent plant covering the ground. The first author was unable to locate any remaining unreleased fruit/seeds on the trees in the area. The seed release immediately preceded a flash flood event caused by unsettled weather from cyclone Yasi (2011) north of the study area, and due to the dangerous conditions the researcher was unable to make a quantitative assessment of the seed densities, although the easily observable seeds thickly covered the ground. All the ephemeral and perennial creeks surveyed in the following July and August period showed some evidence of flash flooding (*ie*. debris in bases of trees, scouring of river bed). The seedlings at this time tended to be found in protected micro-relief sites, particularly where the soil had built up around the bases of the *N. buxifolia* shrubs. For all the plots there were no seedlings observed outside of 1 m from beneath the canopy of a *N. buxifolia* shrub. The mean seedling density was 2606 seedlings plot^{-1} (1000m^2). No correlations were found between the number of seedlings and the soil elements, aspect or slope.

During the July/August field survey vegetative growth and coppicing were observed in *N. buxifolia* shrubs that had been pushed over and buried in sediment by flash flooding. These were developing new root systems and sprouting new growth.

3.5 Discussion

The endemic *Neoroepera buxifolia* occupies a narrow range of habitats and has a patchy distribution within the central Queensland serpentine landscape (Batianoff *et al.* 2000). When it does occur it becomes locally abundant sometimes forming dense stands fringing the ephemeral creeks and gullies and perennial creek banks derived from serpentinite. Whilst there were correlations found between the height of *N. buxifolia* and the soil Mg: Ca quotients, the strongest relationship was between the height of *N. buxifolia* and soil extractable Ni. The volume of *N. buxifolia* also increased with soil extractable Ni. There were no correlations between *N. buxifolia* and the soil Mg. These findings support those from Hendry and Wormington (2014) which found the assemblages of endemic and rare species throughout the central Queensland serpentine landscape reflects high Mg/Ca quotients and extractable Ni in the soils. Studies on serpentine vegetation worldwide have also found relationships between soil Mg/Ca quotients, soil Ni and endemic flora (Brooks 1987; Ghasemi and Ghaderian 2009; Lazarus *et al.* 2011; Robinson *et al.* 1996).

The standing volume of *N. buxifolia* was greatest in the upper tributaries of 1st order stream channels and the concentrations of extractable Ni were also greatest in the upper channels. Forster and Baker (1995**)** found that Ni in the central Queensland serpentine landscape is transported in the soils towards the alluvial plains. It is likely that the Ni is concentrating in the drainage lines and gullies as it is transported toward the alluvial plains. Silicon and Ni in the soil increased as the soils became more acidic. Silicon is not often counted as an essential mineral in plants. However, it may have a role in reducing metal toxicity and drought stress (Doncheva *et al.* 2009; Epstein 1999).

Neoroepera buxifolia tolerates a very high Mg/Ca quotient with up to 11.05 recorded for one plot. At some perennial creeks the water was supersaturated and Mg was observed precipitating out of the solution. Many serpentine-adapted plants have a high requirement for Mg, and both Mg and Ca are capable of partially alleviating metal toxicity by displacing metal cations from the cell walls and outer plasma membrane binding sites (Brady *et al.* 2005; Goodwin-Bailey *et al.* 1992; Kinraide 1994; Proctor and McGowan 1976).

The positive correlation between soil extractable K and the height of *N. buxifolia* in this study could be due to a plant-soil feedback relationship as serpentine soils are characterised by low fertility. Plants have been documented to alter the soil characteristics that feedback to affect the performance of that species or others (Casper *et al.* 2008; Ehrenfeld *et al.* 2005; Kulmatiski *et al.* 2008). Positive feedbacks may be important on serpentine soils where abiotic stresses are severe as it increases the performance of the species inducing the change (Casper *et al.* 2008; Casper and Castelli 2007). Increasing soil Ni could also affect the performance of other non-adapted plants, thus decreasing competition from other plant species. Plant – soil feedback and competition on serpentine soils are difficult to separate and their importance should be considered together (Casper and Castelli 2007). It is difficult to predict if abiotic soil conditions have been altered by the overlying plant community composition (Pernilla Brinkman *et al.* 2010) and the differences in soil conditions may be caused by abiotic or other biotic factors, for example feedback could be mediated by arbuscular mycorrhizal (AM) fungi (Bever 2002), or soil nematodes (Van Der Stoel *et al.*

2002) or invertebrate fauna (Jouquet *et al.* 2006). The evidence for plant-soil feedback caused by *N. buxifolia* in this study is limited as the soil elements between the *N. buxifolia* plots and non-*N. buxifolia* plots only differed slightly.

The majority of *N. buxifolia* populations observed in this current study were growing in perennial and ephemeral drainage lines, gullies, and creek banks derived from serpentinite, with only one hillside plot surveyed. The vegetation of the perennial creeks was a more developed riparian open-forest with deeply rooted trees that have access to permanent water in the channel compared to the eucalypt woodlands of the ephemeral creeks. Whilst the presence of permanent channel water did not influence the standing volume or height of *N. buxifolia*, ephemeral drainage lines and gullies generally have humid microclimates and provide topographic protection against fires and droughts and have an enhanced moisture supply from the soil (Bowman 2000; Mac Nally *et al.* 2000). Networks of dry creek beds and gullies continue to accumulate lateral flows of water during and after rain events (Bowman 2000).

No correlations were found between the number of seedlings and the soil elements, aspect or slope. Seed dispersal of *N. buxifolia* is by ballistochory with an explosive on masse release of seed. Whilst it is possible the seeds are also dispersed via hydrochory and seedlings were observed in niches and around the bases of *N. buxifolia* trees, there were no seedlings found further than 1 metre from underneath a *N. buxifolia* canopy. Long distance seed dispersal in endemic plants is rare and episodic and many species use vegetative reproduction (Wolf 2001). *Neoroepera buxifolia* was observed to be able to reproduce vegetatively, with new plants forming from those that had been knocked over by flash flooding. Stems and branches that were buried under sediment were observed to be rooting and new shoots were sprouting.

The soil elements at the *N. buxifolia* plots and non-*N. buxifolia* plots only differed in the S and Cu content and there were no other significant differences in measured soil elements between the plot types. Importantly there were no significant differences in soil Mg/Ca quotients or extractable Ni between the plot types. It is possible that the lack of *N. buxifolia* at these sites is related to differences hydrology, fire regimes, differences in geology or historical usage or lack of seed dispersal. Cattle grazing is a major land use in central Queensland (Wormington and Houston 2008) and the majority of *N. buxifolia* habitat is on privately owned land subjected to livestock grazing. Cattle have been observed to be grazing on *N. buxifolia* during drought (pers. comm. Scott 2011) and livestock can affect natural ecosystems by causing soil compaction, erosion, pugging and nutrient deposition (Lunt *et al.* 2007). *Neoroepera buxifolia* shrubs were observed to be re-sprouting following fire. However, further research is required to determine if *N. buxifolia* is able to maintain its population with frequent fire intervals and what are the minimum intervals required between fires before seed production starts. Riparian zones are susceptible to weed invasions (Grice 2004) and whilst during the surveys *C. grandiflora* and *L. camara* were observed growing in low abundances these weeds have the potential to establish and threaten *N. buxifolia* populations.

Limited seed and propagation dispersal may be a great influence on the distribution of *N. buxifolia* populations. The development of propagation methodologies would allow the introduction of *N. buxifolia* into suitable habitats such as the non-*N. buxifolia* plots thus expanding its population which could improve conservation of this endemic plant. The soil elements are considered further in Chapter 6 of this thesis when the plant-soil relationships of *N. buxifolia* are explored.

Chapter 4 Seed germination of the endangered serpentine endemic shrub Neoroepera buxifolia

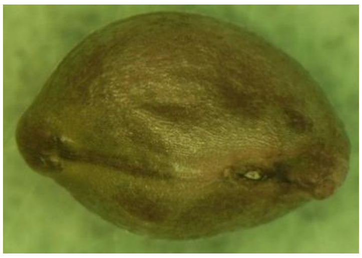

Plate 4-1 ***Neoroepera buxifolia*** **seed**

4.1 Abstract:

Consideration of the ecological aspects of the species is useful in elucidating the types of dormancy present. The aim of this study was to determine the germinability of the seeds of the serpentine endemic shrub to small tree *Neoroepera buxifolia*. This was achieved by examining the viability of the seeds and investigating dormancy relieving techniques. The seeds of *N. buxifolia* stored in their fruit for 6 months were viable (100%) and had a high germination rate (up to 87.5% depending on treatment). It was determined that dormancy relieving techniques were not necessary to initiate germination.

4.2 Introduction:

Neoroepera buxifolia Muell.Arg. & F.Muell. (Picrodendraceae) is an endemic shrub to small tree that has a limited distribution within the central Queensland serpentine landscape on the east coast of Australia (Hendry *et al.* 2015 in prep.). It has a restricted distribution along the ephemeral and perennial creeks and it is associated with high magnesium in the soil (Batianoff *et al.* 2000). There is a potential for *N. buxifolia* to be used in phytoremediation within the central Queensland serpentine landscape particularly around tailings dams and drainage lines following mining and mineral exploration (Chapter 6). There is currently limited published information on the germination characteristics of *N. buxifolia*. Propagation from seed is generally the most simple and effective means of establishing new plants. Yet there is a need to determine when seed should be collected, how seed should be stored, if there are dormancy mechanisms to overcome and the viability of the seed (Cochrane *et al.*

2002). Breaking dormancy is one of the most important factors to improve germination rates (Bell *et al.* 1993b; Cochrane *et al.* 2002). A consideration of the ecological aspects of the species is required to predict the cues to break dormancy and initiate germination (Baskin and Baskin 1998b; Bell *et al.* 1993b; Cochrane *et al.* 2002).

The aim of this study is to determine the germinability of *N. buxifolia* seeds and investigate if dormancy relieving techniques are required to enhance germination. This will be achieved by:

1. Determining permeability of the seeds

2. Investigating the viability of *N. buxifolia* seeds

3. Performing germination tests on seeds

4.3 Methods and materials

Mature fruit (Plate 4-1) of *N. buxifolia* were collected late January and early February 2011 from creeks along Corumburra Rd, Marlborough station (Lat. 22^{o}55’31”E, Long 149^{o}51’42”S) and Hams Road Canoona (Lat. 23^{o}2’59”E,Long. 150^{o}16’46”E). The collected fruits were sealed in paper bags and stored for 6 months in a dry store cupboard.

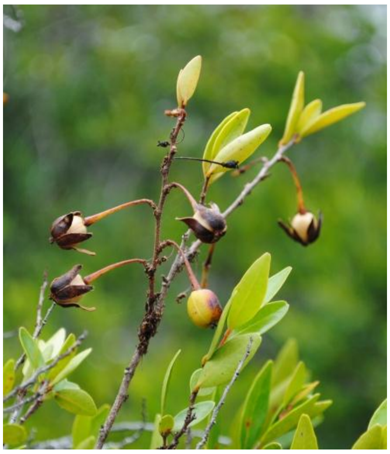

Plate 4-2 Mature fruit of *Neoroepera buxifolia*

4.3.1 Seed preparation

Neoroepera buxifolia fruit have been observed to release seeds via ballistochory in the field (pers. obs). To allow the fruit to open in the laboratory, the collected fruit were placed into paper bags and placed in a drying oven at 40°C until the seeds were released (up to 12 hours).

4.3.2 Seed weight, size and water imbibition test

Water imbibition tests were conducted according to the methods of Baskin and Baskin (1998a). Three replicates of 20 seeds were measured and weighed and the average seed dimensions and weight were calculated. The seeds were placed onto filter paper moistened with distilled water in petri dishes. After 5 minutes the seeds were removed, blotted dry with paper towel and transferred to a plastic boat for weighing. The seeds were returned to the moist filter paper and re-weighed every hour for 6 hours, then again at 12 hours, 24 hours, 48 and 72 hours.

4.3.3 Viability tests

A fresh batch of 30 seeds was selected and imbibed in water for 12 hours, then nicked and soaked in 1% tetrazolium chloride solution for 12 hours. The seeds were then bisected and checked for staining. Those that were stained pink were assessed as being viable (Peters 2000).

4.3.4 Germination tests

Seedling emergence from sand germination tests was performed in 100 ml plastic containers ¾ filled with washed river sand. The sand was moistened with distilled water to 2 cm below the surface. Thirty holes were pushed into the sand to a depth of 5 mm. One prepared seed was place per hole and lightly covered in sand and the container was fitted with a perforated lid. Four replicate containers of 30 seeds were used for each treatment. The treatments were:

- Control (no treatment);
- Soaked –seeds were placed in petri dishes and covered in distilled water for 24 hours;
- Leached –seeds were placed in a fine meshed sieve bags and placed under running water for 24 hours.

The experiment was conducted in a temperature controlled growth cabinet set at 25/25ºC 12 hour day/night cycle. The containers were arranged in a random design and moved around every 7 days. Seed germination was recorded weekly for a period of 56 days after which the ungerminated seeds were removed from the sand and were visually assessed.

4.3.5 Data analysis:

The time taken to reach 50% germination (T_{50}) was calculated based on the final germination percentage according to the following equation (Coolbear *et al.* 1984; Farooq *et al.* 2005):

$$T_{50} = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{(n_j - n_i)}$$

Where N is the final number of germinated seeds, n_i and n_j are the cumulative number of seeds at time T_i and T_j. Time T_i and T_j are the times adjacent to $\frac{N}{2}$ ($T_i < \frac{N}{2} > T_j$).

The germination rate (GR) was calculated as the number of germinated seeds after the germination time period. GT is the sum of the number of germinated seeds after t days, and Dt is the number of days of the trial.

GR = Σ GT/DT

The percentage germination (% G) was calculated as:

% G = (The number of germinated seeds/the number of seeds) x 100

The germination rate, percentage germination and T_{50} were each compared between treatments using a one-way analysis of variance (ANOVA) using IBM SPSS ver. 20 followed with a *post hoc* Tukey test between the treatments. The percent germination data was arcsine transformed before analysis. Significance was set at 95% probability.

4.4 Results

The mean width and length of the seeds was 2.48 mm w and 4.77 mm l respectively and the mean weight was 11.21 mg (Table 4-1). The viability of the seeds was very high with 100 % of the seeds staining pink with tetrazolium chloride solution (n = 30). The seeds were observed to have a white covering that turned into sticky mucilage when soaked. The percent water imbibition doubled in the first 3 hours and stopped increasing by 5 hours. The mucilage coating began to come off the seeds after 5 to 6 hours of soaking possibly causing a decrease in seed weight (Figure 4-1). A brown leachate was observed coming from the seeds that were soaked in water.

Table 4-1 Mean seed measurements followed by minimum and maximum values in parenthesis (n = 60).

Width (mm)	Length (mm)	Weight (mg)
2.48 (1.6 - 3.3)	4.47 (3 - 5.4)	11.21 (10.77 - 11.63)

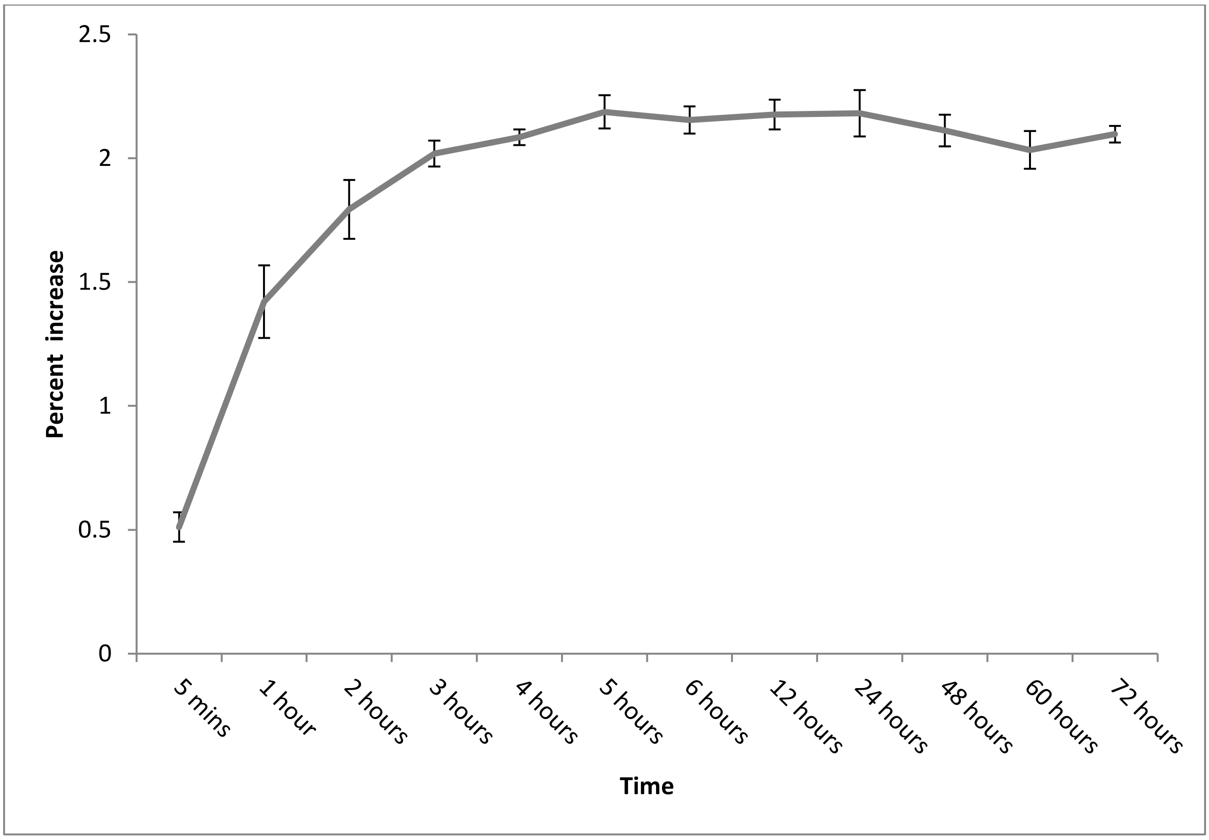

Figure 4-1 Mean rate of water imbibition per seed expressed as percent increase compared to the initial dry weight with standard error bars (n = 60).

4.4.1 Germination

The time to 50% germination (T_{50}) for all treatments was around 18 days and did not vary significantly between the treatments ($F(2, 9) = 0.153$, p = ns). There were no new germinates after 49 days and at 56 days the ungerminated seeds were inspected for viability. The remaining ungerminated seeds were found to be decayed and unviable. Overall the control treatments had the highest percent germination with a mean of 87.5 %, while the leached treatment had the least success with 67.7%. The soaked treatment had a mean of 79.2% germination. However, these differences were not significant ($F(2, 9) = 3.154$, p = ns). The rates of germination were significantly different between week 5 ($F(2, 9) = 7.891$, $p = 0.01$) and week 6 ($F(2, 9) = 7.329$, $p = 0.013$), as the control treatments had a greater rate of germination compared to the leached treatments (Figure 4-2).

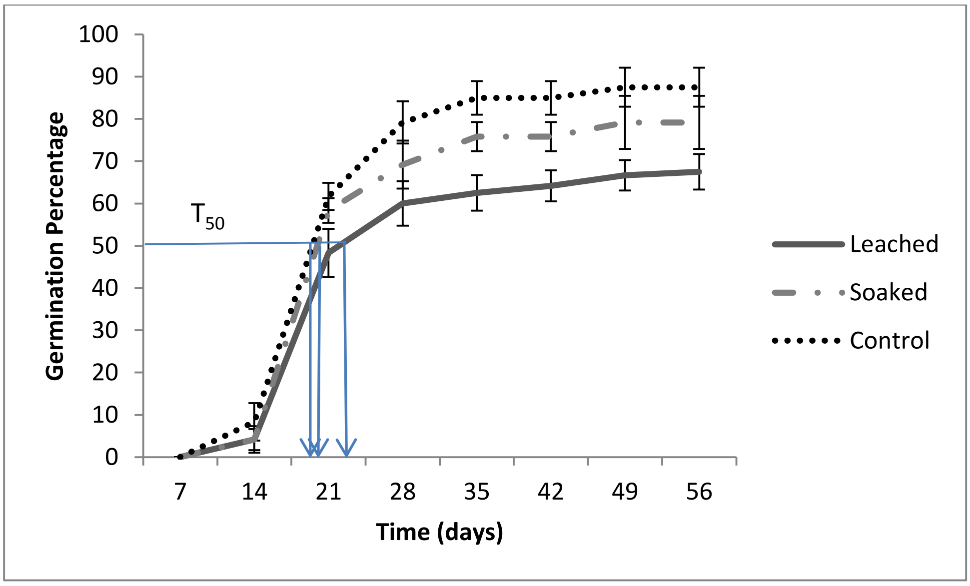

Figure 4-2 Mean germination percentages over 56 days for each treatment type with T50 indicated (SE bars indicated).

4.5 Discussion

The seed viability and germination of *N. buxifolia* seeds stored in their fruits for 6 months were excellent and the results indicate that no pre-treatments are necessary to achieve high germination success and no additional treatments are required to relieve embryo dormancy. Indeed, the leached treatment resulted in a lower rate of germination compared to the control treatments. These results support the findings of Bell *et al.* (1993a) and Cochrane *et al.* (2002) who found canopy-stored seeds require few pre-treatments to stimulate germination.

The seeds of *N. buxifolia* have been observed to release explosively enmasse in the summer monsoon (January to February) when it is likely that the conditions are most favourable for germination (*ie.* high rainfall) (Hendry *et al.* 2015). The seeds were found to be permeable to water and when imbibed the seeds produce a mucilage coating that enveloped the entire seed. Mucilage plays many ecological roles in the adaptation of plants to diverse environments. Physiological roles for seed coat mucilage may include promoting water uptake (Bansal *et al.* 1980), adhesion to soil (Gutterman and Shem-Tov 1997), fixing trace elements (Melo and D'Souza 2004) and as a nutrient reserve to alleviate germination stress (Yang *et al.* 2012).

Chemicals, such as phenols and abscisic acid in the seed can act as germination inhibitors and can be leached out in water (Zare *et al.* 2011). The seeds that were soaked for 24 hours in distilled water turned the water a dark colour indicating the presence of substances leached from the seeds. As the leaching and soaking of seeds did not result in a greater germination rate or percentage the chemicals are either not an inhibitor of germination or were leached out of the control seeds anyway when the seeds came in contact with the wet sand.

In conclusion, no treatments are required to break seed dormancy or enhance seed germination of 6 month old *N. buxifolia* seeds. The seeds are readily permeable to water and it is likely that the recruitment of new germinates relies on the coincidence of seed fall with wet season rains and flooding. Fruit should be collected in late December and early January and more work is required to investigate the viability of long-term storage of seeds; particularly in regards to storing the seed in the fruit capsule until required.

Chapter 5 Vegetative propagation of Neoroepera buxifolia by marcotting, ground layering and cuttings.

Plate 5-1 a). Successful cutting of *Neoroepera buxifolia.* b). Marcotting c). Developed root ball and d).Ground layered branch.

5.1 Abstract

The aim of this study was to develop protocols to vegetatively propagate an endemic serpentine shrub to small tree *Neoroepera buxifolia* that has a restricted distribution. The use of vegetative cuttings, marcotting and ground layering techniques were investigated. *Neoroepera buxifolia* responded well to marcotting and ground layering techniques, but the cuttings had a low strike rate. This suggests *N. buxifolia* benefitted from receiving metabolites from the parent tree. The application of honey and the auxin IBA promoted rooting success, whereas IAA/NAA treatment was inhibitory to root development.

5.2 Introduction

Neoroepera buxifolia Muell.Arg. & F.Muell. (Picrodendraceae) is an endemic shrub to small tree that is mostly restricted to the ephemeral and perennial creeklines of the central Queensland serpentine landscape on the east coast of Australia (Chapter 3). It is one of only two species belonging to the genus *Neoroepera,* the other is *N. banksii* Benth. which occurs in far north Queensland (Henderson 1992). *Neoroepera buxifolia* grows in a restricted geographical range and occupies a specialised habitat and is at threat from localised extinction due to pressures from mining, grazing and weed invasion (Threatened Species Scientific Committee 2008). Riparian plants often have reproductive adaptations, such as the ability to reproduce vegetatively that allow them to survive frequent disturbances caused by flooding (Naiman *et al*. 1998). *Neoroepera buxifolia* has been observed to be growing vegetatively following disturbances caused by flash flooding (Chapter 3) suggesting this species would be an ideal candidate to propagate asexually.

To date, there is no published data on the vegetative propagation of *N. buxifolia*, yet these data are essential for the conservation and restoration of this species. In addition, there is a potential to use *N. buxifolia* for land rehabilitation and phytostabilisation following mining and mineral exploration within the central Queensland serpentine landscape, as it is able to tolerate high concentrations of Ni and high Mg/Ca quotients yet maintain a high biomass provided it has adequate soil moisture (Chapter 6).

Although the seed germination of *N. buxifolia* is excellent (up to 87.5% success rate) (Chapter 4), plants grown from seed may take years to grow and develop, and due to its restricted distribution and vulnerability the over-collection of seed could reduce natural succession. Vegetative propagation using cuttings has been used extensively for woody species worldwide to rapidly multiply plants. A number of studies have shown that the application of auxins increases root development (Abu-Zahra *et al*. 2011; Pop *et al*. 2011), however high concentrations of auxins may be inhibitory (Ofori *et al.* 1996). The rooting medium used is also critical to rooting success (Tchinda *et al*. 2013) as well as the type of cuttings used (Khan *et al*. 1988) and the time of year (Blakesley *et al.* 1991). Marcotting or air layering has been found to be a successful technique for the propagation of woody species

(Bell 2001; Kabata-Pendias 2004; Mertens *et al*. 2004) and together with ground layering may provide reliable methods of propagation (Pulford and Watson 2003) which would allow *N. buxifolia* to be mass produced quickly and efficiently.

This study aimed to develop a protocol for the vegetative propagation of *N. buxifolia* by investigating three approaches: 1). The use of cuttings, 2). Marcotting, and 3).Ground layering. These techniques were combined with the application of auxins and in the case of the cuttings different rooting media and cutting types to determine how *N. buxifolia* responds to vegetative propagation and which technique is the most successful.

5.3 Materials and Methods

5.3.1 Cuttings

Cutting material was collected from 30 healthy mature *N. buxifolia* plants with an average height 2.5 ± 0.3 m growing alongside ephemeral creek beds on Hams Road Canoona in September 2011 (spring). Two branches per donor plant were collected at breast height (approx. 1.3 m). Only branches capable of harvesting at least 15 cuttings were collected. The collected material was placed into plastic bags, sprayed with water to maintain humidity and stored in a cooler before being transferred to the laboratory for processing. The cutting material was stored in a cold room overnight before being processed.

Three types of cuttings were prepared from the collected material. These were tip cuttings, heel cuttings and stem cuttings. Each cutting was approximately 10 cm long with two upper leaves left in place. The basal ends of the cuttings were dipped into water and then the treatment and placed into the media to a depth of about 3 cm.

Commercial plant growth regulators and honey were tested for their effects on the rooting success of the cuttings. Honey was found to promote primary root formation in cuttings of the central Queensland metallophyte *Stackhousia tryonii* Bailey and may act as an osmotic agent (Bhatia 2003) and has antiseptic qualities (Balabushka 1985; Danehloueipour *et al*. 2006). The treatments were:

1 Control – water only

2 3 IBA (3g kg^{-1} Indole-3-butyric acid (IBA) powder (Richgro))

3 8 IBA (8g kg^{-1} Indole-3 butyric acid (IBA) powder (Richgro))

4 3 IBA gel (3 g L^{-1} Indole-3-Butyric acid gel (clonex purple) (Yates))

5 Honey (Capilano Honey Ltd.)

6 IAA/NAA (0.05 g L^{-1} indole acetic acid and 0.05g L^{-1} Naphthalene acetic acid - Multicrop Plant Starter (Multicrop (Aust.) Pty. Ltd.)) - cuttings put in 2cm of plant starter for 24hrs before planting.

Three types of rooting media were used:

1. Sand – coarse sand;

2. Serpentine soil mix consisting of 3 parts of 2 mm screened serpentine soil collected from ephemeral creeklines mixed with 2 parts of perlite and 2 parts of coarse sand;

3. Commercial cutting mix (from Orams Nursery, Rockhampton) consisting of 60% perlite, 15% peatmoss, 5% vermiculite, 1% lime, 19% polystyrene foam).

The media were placed into 6-celled free draining pots.

Ten replicates were used for each of the three rooting media and six treatments and three cutting types giving a total of 540 cuttings. The pots with the cuttings were placed on growth benches in a misthouse under a natural photoperiod and equipped with a misting system set to keep the humidity between 95 and 100%. Bottom heating was applied with the temperature at root level set to 25ºC. The cuttings were maintained for 13 weeks.

Survival of the cuttings was determined by development of roots and shoots. The length and number of the roots were also measured.

5.3.2 Marcotting

In-situ marcotting including aerial and ground layering was conducted in an ephemeral creek at Canoona, along Hams Road on lot/plan 1278/LN2914 (lat. 23º 2' 36"S, long. 150º 16' 49" E) in December 2011. The study sites were located at the top of the creek line within dense *N. buxifolia* low forest at least 500 m from the vehicle track. The aerial layering treatments were conducted on vertical branches that were the width of a thumb (approx. 2 cm). A ring of bark approximately 2.5cm wide was removed from the branch using multi-grips exposing the woody tissue. The treatments were a control (no hormone), 1 IBA, 3 IBA and 8 IBA powder and 3 IBA gel (clonex purple, Yates) and were applied using a small paint brush. The wound was covered in sphagnum moss that had been soaked in water and squeezed to remove excess water. The area was covered with polyethylene film, wrapped with aluminium foil and left for 3 months. There were 10 replicates per treatment giving a total of 50 branches. After 3 months the number, diameter and length of the roots were recorded. The marcotted branch was recorded as successful if the branch was alive and new roots had formed. For this study the branches were not removed (due to permitting restrictions) and the newly formed roots were carefully scrapped off and the branches and tree were allowed to resume normal growth.

Thirty vertical layered stems were prepared by selecting flexible low branches from *N. buxifolia* shrubs and bending them to the ground and laying them in a hollow. The branches were anchored with a plastic peg designed to secure irrigation hose. The growing tip was held upright with a wooden stake and loosely secured with wire ties. There were no auxin treatments and the hollow was backfilled with soil and watered to saturation. The layered stems were left unattended for the duration of the experiment (3 months). At the conclusion of the experiment the formation of roots was checked by gently digging around the buried stem. The vertical layering was deemed successful if roots were present holding the branch in place and shoots were present. The plastic pegs and wooden stakes were removed and the newly formed plants were allowed to remain in place.

5.3.3 Statistical analysis

The mean ranks of the length and number of roots for each of the cuttings were analysed using a Kruskal-Wallis Test using root length and number of roots as the dependent variables and the grouping variables were cutting type, media type or treatment type (StatSoft, Inc, Statistica 12). A non-linear logistic regression model (Logit) was used to examine the effects of cutting type, medium used and treatment on the percentage success rate of the cuttings (StatSoft, Inc. Statistica 12). Comparisons of the means were used to determine differences among the cutting types, media and treatments.

For the marcotting experiment one-way between-groups multivariate analysis of variance was performed to investigate the differences on the development of the roots according to the treatments applied. Three variables were used and these were, number of primary roots, length of primary roots and the diameter of primary roots; the independent variable was treatment. Percent success for the treatments was analysed using a non-linear logistic regression model (Logit). The data were normally distributed and did not require transformation.

5.4 Results

5.4.1 Cuttings

The overall success rate for the cuttings was low with only 13% of the 540 cuttings successfully striking. There were significant interactions between the cutting and treatment type on success rates of the cuttings (χ^2 (2) = 11.463 p = 0.003). However, the medium type did not significantly influence the success rate. The stem cuttings treated with honey had the greatest success rate (30% , Figure 5-1) and 20 % or greater success rates were achieved with 3 IBA tip cuttings, 3 IBA heel, 8 IBA heel cuttings and the control stem cuttings. Overall, the plant starter treatments were the least successful with only a 2% success rate (Table 5-1). It can also be observed the tip cuttings were the least successful at 8.3% and the stem cuttings were the most successful at 16.7% (Table 5-1, Plate 5-1a).

The root length of the successful cuttings varied according to the cutting type (H (2) =6.02 p = <0.05). The mean rank of the stem cuttings (280.24) was higher compared to the tip cuttings (257.41). The medium type did not influence the root length (H (2) = 4.33 p >0.05). The treatment type significantly influenced the root length (H (5) = 13.50 p = <0.05) as the plant starter treatment had the lowest mean ranking compared to the other treatment types.

The number of roots varied according to cutting type (H (2) = 6.74, p = <0.05) with the stem cuttings having more roots and tip cuttings have least roots. Medium type did not influence the number of roots (H (2), = 3.55 p = ns). The number of roots was greatest for the 3 IBA treatments and least for plant starter treatment. (H (5) = 15.45 p = <0.05).

Table 5-1 Effect of cutting, medium and treatment types on the % success and root length and number of *Neoroepera buxifolia* cuttings.

	% success	Mean root length (mm) ±SE	Mean number of roots (mm) ±SE
Cutting type			
A	8.3±0.2	0.4±0.2	0.1±0.0
B	13.9±0.2	3.9±1.5	0.3±0.1
C	16.7±0.2	4.8±1.7	0.4±0.1
Medium type			
S	15.0±0.2	4.2±1.8	0.3±0.1
M	8.8±0.2	0.3±0.1	0.1±0.0
C	15.0±0.3	3.4±1.2	0.3±0.1
Treatment type			
1	14.4±0.4	4.0±0.3	0.4±0.1
2	18.9±0.4	3.0±1.3	0.3±0.1
3	14.4±0.3	3.1±2.2	0.3±0.1
4	11.1±0.3	2.3±1.5	0.2±0.1
5	16.7±0.4	4.3±2.0	0.3±0.1
6	2.2±0.2	1.6±1.6	0.1±0.1

(A = tip cutting, B = Heel cutting, C = Stem cutting, S = Sand, M = Serpentine soil mix, C = commercial cutting mix, 1 = control, 2 = 3 g kg^{-1} IBA, 3 = 8 g kg^{-1} IBA, 4 = 3 g L^{-1} IBA gel, 5 = Honey and 6 = IAA/NAA liquid plant starter).

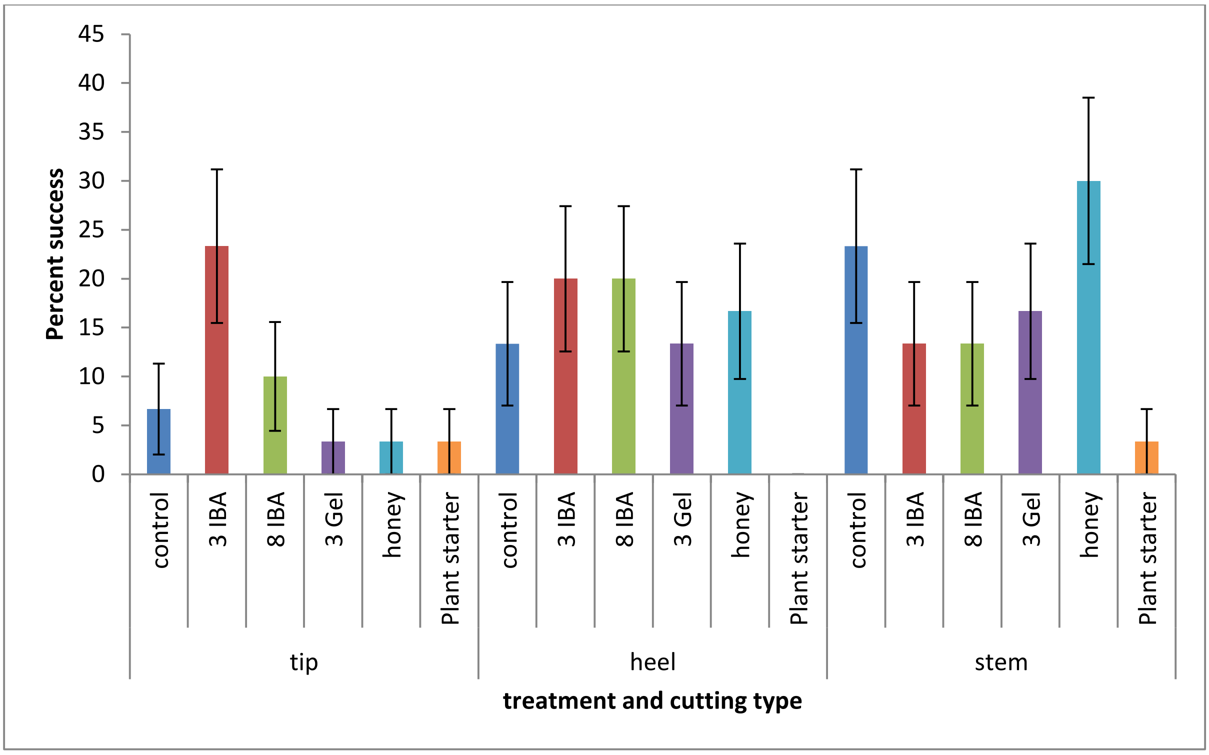

Figure 5-1 The percent success rates for the *Neoroepera buxifolia* cuttings for each of the treatment and cutting types with standard error bars.

5.4.2 Marcotting

Marcotting of *N. buxifolia* was highly successful with an overall success rate of 92%. There were significant differences in success rate between the treatments (χ^2 (4) = 17.162 p = <0.001). The 8 IBA treatment produced the lowest success rate of 60% compared to the other treatments which had 100% success (Table 5-2, Plate 5-1 b & c).

There were significant differences in the number, length and diameter of the roots according to the treatments (F (12, 100.83) = 2.0325, p = <0.05). The 3 IBA gel treatment resulted in the greatest number of primary roots, whereas the 8 IBA powder produced the least amount of roots (p = <0.01) and smallest diameters and shortest lengths of the primary roots compared to the other treatment means (Table 5-2).

Table 5-2 Effect of auxin treatments on marcotted *Neoroepera buxifolia* branches.

Auxin treatment	% success	mean number of primary roots (±SE)	mean length primary root (mm) (±SE)	mean diameter of primary roots (mm) (±SE)
control	100	8.6±1.7	87.5±7.0	2.2±0.4
1 IBA (1g/kg IBA)	100	7.9±1.9	90.9±4.5	2.2±0.2
3 IBA (3g/kg IBA)	100	8.2±1.3	101.5±5.8	2.0±0.2
8 IBA (8g/kg IBA)	60	3.3±1.1	71.1±11.0	1.4±0.2
3 IBA gel (3 g/L IBA)	100	13.9±1.8	97.5±3.9	2.3±0.1

5.4.3 Ground layering

The ground layering method had a 100 % success rate with the branches taking root beneath the soil (Plate 5-1 d). Two of the vertical layering branches could not be relocated at the end of the experiment and it was assumed the markers had been washed away during flash flooding. These branches were excluded from the analysis.

5.5 Discussion

The marcotting and ground layering techniques proved to be successful methods of propagation of *Neoroepera buxifolia*. Whilst marcotting was successful without the application of hormone treatments, the use of 1 or 3 IBA treatments increased root development. The response varied with the concentration of hormone treatment and the 8 IBA powder was inhibitory resulting in a reduced success rate and also produced the smallest root ball with the smallest diameter roots compared to the control (no treatment) and other auxin treatments. In comparison, the cuttings had a low strike success rate after 3 months. The success of the cuttings varied according to the cutting type and treatment used as the stem cuttings with honey treatment had the greatest striking success and the best root development compared to the tip and heel cuttings. The Plant Starter treatment had the least success rate and lowest root development.

Adventitious rooting capabilities are dependent on various factors such as genetic control and critical balances between internal substances such as hormones, carbohydrates, nitrogenous compounds and hydromorphics (de Almeida *et al.* 2010; Agulló-Antón *et al.* 2011; Pop *et al*. 2011; da Costa *et al*. 2013). Rooting is facilitated when reserve substances and growth promoters are in abundance (Agulló-Antón *et al*. 2011). The low strike rate from the cuttings compared to the Marcotted branches may be because the experiments were conducted during different seasons or that either the cuttings required more metabolites or the mist house did not provide a desirable environment for rooting. Seasonal changes in carbohydrates and hormones in the vegetative shoots could affect the rooting ability of the cuttings (Denaxa *et al.* 2012). The material for the cuttings experiment was collected in spring whereas the marcotting and layering experiments were conducted over the summer period. Denaxa *et al.* (2012) found that the rooting ability of some cultivars of *Olea europaea* L. (Olive tree) was greater in summer compared to spring due to the mobilisation of carbohydrates and auxins in summer, whereas other cultivars did not show differences in rooting ability between the two seasons.

The marcotted branches formed roots without the application of auxins suggesting the branches were well supplied with endogenous auxins. The branches were receiving metabolites from the parent tree, whereas the cuttings were isolated and dependent on their own reserves. The application of auxin concentrations higher than those found in plant tissues may cause death (Hartmann 2014) and the 8 IBA treatment had an inhibitory effect on the rooting of the marcotted branches. The 3 IBA gel treatment was more successful compared to the 3 IBA powder application and it would be expected that the gel be more likely to stay on the exposed tissue whereas the powder would come off.

The stem cuttings were more successful than the tip cuttings. Stem cuttings may hold more endogenous stored carbohydrates and auxins compared to the terminal tip cuttings and the mature tissue may restrict water loss (Haissig 1989; Day and Loveys 1998). Exogenous hormones applied to the base of cuttings have been found to influence carbohydrate utilization for the synthesis of essential compounds such as proteins for the development of roots (Agulló-Antón *et al*. 2011). The cuttings treated with 3 IBA produced more roots compared to the other treatments. Low concentrations of auxin are promotory but high concentrations can be inhibitory (Ofori *et al.* 1996; De Klerk *et al.* 1997). The IAA/NAA treatment had an adverse effect on rooting success and survival. NAA may be more persistent in the plant tissues compared to other auxin types which have an inhibitory effect on root formation (De Klerk *et al.* 1997). The IBA and IAA auxins are generally destroyed by auxin-oxidase when exposed to light before becoming inhibitory after the roots have started to form (Smulders *et al.* 1990; De Klerk *et al*. 1995; De Klerk *et al*. 1997). The honey treatment resulted in the greatest success rate. Honey is thought to have antiseptic qualities (Balabushka 1985; Danehloueipour *et al*. 2006) and may work as an osmotic agent (Bhatia *et al*. 2002).

Once the root ball has developed, the marcotted branches can be removed from the parent tree and placed into a pot to allow the root system to develop. The advantage of marcotting is that large, sun hardened plants are produced ready for transplanting. The ground layering was also highly successful with large plantlets ready for transplanting with a developed root system already in the ground. Mist propagation systems have relatively high capital and running costs (Leakey *et al*. 1990) and the cuttings required regular attention throughout the experiment and had a low strike rate. It is recommended that either marcotting with the application of 3 IBA gel or ground layering techniques be applied if vegetative propagation of *N. buxifolia* is required. However, as propagation from plant cuttings has the potential to produce large numbers of new plants, it is further recommended that more investigations into the differences in seasonal success be conducted.

Chapter 6 Elemental composition of Neoroepera buxifolia along the serpentine gradient and its potential role in phytoremediation.

6.1 Abstract

Plate 6--2 *Neoroepera buxifolia* foliage ready for processing

A large area of the central Queensland serpentine landscape is under mining exploration permit or mining lease. Mining laterite deposits of nickel is a destructive process and careful planning is required to select species suitable for the successful revegetation and restoration of the landscape. Endemic species such as *Neoroepera buxifolia* have specialised physiological mechanisms to tolerate the edaphic serpentine conditions and are also adapted to the local climate. *Neoroepera buxifolia* propagates readily from seed, marcotting and ground layering. The aim of this study was to investigate the phytoremediation potential of *N. buxifolia* as a phytostabiliser. This was achieved by determining the plant-soil relationships of populations of *N. buxifolia*. *Neoroepera buxifolia* did not accumulate trace elements and the bioaccumulation factor for the elements Ni and Co did not exceed 1. This indicates that *N. buxifolia* could be useful for phytostabilisation. However, it has narrow habitat requirements which would limit its use to areas of high soil moisture such as drains and tailing dams.

6.2 Introduction

Because of their geological origins and chemical composition, serpentine landscapes contain commercially valuable minerals and metal ores (O'Dell and Claassen 2009; O'Dell and Claassen 2011). There are economic enrichments of nickel (Ni), cobalt (Co) iron (Fe), magnesium carbonate ($MgCO_3$) and chrysoprase within the weathering profile of the central Queensland serpentine landscape (Forster and Baker 1995; Forster and Baker 2002). A precipitate of $MgCO_3$ is formed in the base-rich ground waters from the serpentine hills, which is deposited as high grade nodular magnesite ore in the eastern alluvial flats of Kunwarara and Yaamba (Wilcock 1998; Foster and Eggleton 2002). Over 85% of the serpentine landscape is covered under Mining Leases, Mining Development Lease (MDL), Exploration Permits for Mineral (EPM), or Exploration Permit for Coal (EPC) covenants (Table 6-1). Mining laterite deposits for metals is a destructive process. During the mining and construction process the serpentine topsoil is stripped to the bedrock. This results in the loss of biological features of the overburden topsoil including water-holding capacity, cation exchange capacity (CEC), organic matter, nutrients, seeds, and mycorrhizal communities (O'Dell and Claassen 2009; O'Dell and Claassen 2011). Mined serpentine areas are susceptible to becoming barren, erosive landscapes with no natural revegetation, causing elevated sedimentation and heavy metal transport into watersheds. There is a risk of windborne trace elements and possible chrysotile (asbestos) pollution impacting on adjacent ecosystems and human health (O'Dell and Claassen 2009; Padmavathiamma and Li 2009). Revegetation and ecosystem recovery on serpentine soils following mining disturbance is difficult due to the edaphic conditions and the effects of mining and mineral exploration may be long lasting and cause dramatic changes (De Grood *et al.* 2005). An increase in global demand for Ni will place pressure on serpentine landscapes and careful planning is required for revegetation and restoration to be successful (O'Dell and Claassen 2009).

Table 6-1 The total area of the central Queensland serpentine landscape covered by mining and exploration tenures and the areas covered by each of the tenure types (DNR 2014)

	Area (ha)
Area of serpentine landscape	101 186
#Total area covered by mining/exploration tenure	86,804
Tenure type	
Mining lease	12,342
Mining development lease	10,447
Exploration permit for mineral	81,035
Exploration permit for coal	7,309

many of the tenure types overlap in area so the combined areas of mining/exploration tenures is greater than total area covered by the tenure types.

Successful revegetation of disturbed substrates following metal extraction requires physical site stabilisation, substrate amendment and correct plant selection (Bradshaw and Chadwick 1980; Williamson and Johnson 1981; Bradshaw 1997; O'Dell and Claassen 2009). Serpentine landscapes have a high proportion of endemics that have specialised physiological mechanisms to tolerate high concentrations of metals and low nutrients. Generally these species have a high root : shoot biomass ratio, slow growth rate, regulation of Ca and Mg uptake, and maintenance of tissue Ca and Mg ratios (Kazakou *et al.* 2008). Local species in the vicinity of the potential revegetation site are adapted to local climate, tolerate the edaphic soil conditions and are ideal candidates for rehabilitation (O'Dell and Claassen 2009; O'Dell and Claassen 2011). Hendry *et al.* (2015 in prep.) found that the biomass of *N. buxifolia* increased with soil extractable Ni and soil Mg/Ca quotients and this endemic plant is well adapted to the serpentine conditions. Furthermore *N. buxifolia* is readily propagated both vegetatively and from seed (Chapters 4 & 5).

Plants have a number of physiological features to cope with the high metal content of the serpentine soils and three types of plant-soil relationships have been described by Baker (1981); these are accumulators, indicators and excluders (Baker 1981; Baker and Walker 1990) Accumulators are those plants that concentrate metals in their above-ground parts regardless of whether the soil metal concentrations are high or low, and hyperaccumulators are those that store large amounts of metals in their above-ground tissues (Brooks 1987, Baker 1981; Kazakou *et al.* 2008). Previous studies have determined that *N. buxifolia* is not a hyperaccumulator of Ni (*ie*. Bidwell 2000, Reeves 2002). Indicator plants are those whose internal metal concentrations have a linear relationship to metals in the soil and as the soil concentration of metals increases so does the metal concentrations within the plant (Baker 1981; Kazakou *et al.* 2008; Nkoane *et al.* 2005). Excluders are plants that have low metal concentrations in their tissues over a wide range of soil concentrations. However, if the concentrations of metals in the soil become toxic, unrestricted transport into the plant tissue can occur (Baker 1981; Bidwell 2000; Boulet 2003).

Vegetation cover reduces leaching and soil erosion and it is a feasible, practical method for remediating soils (Padmavathiamma and Li 2009). The two major types of phytoremediation are phytoextraction and phytostabilisation. The aim of phytoextraction is to remove the metals from the soil using vegetation, which is harvested and removed from the site (McGrath and Zhao 2003). *Neoroepera buxifolia* is not a candidate for phytoextraction of metals as the ideal plant species is a hyperaccumulating plant with a high biomass and fast growth rate (McGrath and Zhao 2003). Plants that are useful for phytostabilisation should be metallophytes that do not accumulate metals in their foliage (Mendez and Maier 2008a; Mendez and Maier 2008b). The purpose of phytostabilisation is to stabilize the soil and reduce the flow of heavy metal contaminants into the environment. The efficiency of phytoremediation is determined by the metal bio-concentration factor. The bio-concentration factor (BF) is the quotient of metal concentration in the plant foliage and metal concentration in the soil (McGrath and Zhao 2003). The bioaccumulation factor quotient for trace element concentration in the aerial biomass is usually >>1 in hyperaccumulators, >1 in accumulators, and <1 in excluders (McGrath and Zhao 2003; Cornara *et al*. 2007). Plants useful for phytoextraction would have a BF value of > 1 whereas those suitable for phytostabilisation should not exceed a BF value of 1.

The primary aims of this study were to:

1). Determine the bioaccumulation factor of metals in the foliage of *N. buxifolia* and;

2). Assess if *N. buxifolia* is useful for nickel phytostabilisation purposes.

This was achieved by assessing the soil and foliar elements of *N. buxifolia* shrubs and trees growing within the central Queensland serpentine landscape.

6.3 Methods

The methodology for the selection of the plots and the collection and analysis of the soil samples is detailed in Chapter 3 of this thesis "An ecological study of the central Queensland serpentine endemic shrub *Neoroepera buxifolia*".

Foliage samples from three *N. buxifolia* trees directly above the three soil sample cores were collected from each plot at the time of the surveys. Three branches at breast height were collected from each sample tree. The branches were placed in labelled plastic bags and transported in a cooler containing ice to the laboratory. Prior to analysis, the leaves were separated from stems and were carefully washed in running reverse osmosis water. The leaves with petioles intact were dried in an oven at 40ºC to constant weight and ground to a fine powder using a stainless steel coffee/spice grinder. The samples were microwave digested with nitric acid and analysed using ICP-MS (Environmental Analysis Laboratory SCU). The elements analysed were B, C, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, N, P, S, Si and Zn.

6.3.1 Data analysis

Stepwise multiple linear regressions were used to construct models from the data with the soil elements serving as the independent variables and the foliage elements as the dependent variables (SPSS v20). The data were log +1 transformed to best satisfy linearity, and the assumption of normal distribution and homoscedastic variance of the variables. The significance of the statistical tests was set at 5% probability.

In order to assess the efficiency of *N. buxifolia* for phytoremediation, the bioaccumulation factor (BF) was calculated for the metals. BF = foliage element / soil element.

6.4 Results

6.4.1 Foliage element concentrations of *Neoroepera buxifolia*:

The mean concentrations of the foliage micronutrients Co, Fe, Mn, and Ni were lower than soil concentrations on a % dw basis, whereas foliage concentrations of micronutrients B, Cu, Mo, Na, Si and Zn and macronutrients Ca, Mg, K, P and S were higher than the soil (Table 6-2). The BF of some elements was high despite low concentrations of these elements in the soil. The highest BF values for the plant micronutrients were B, Na and Zn (>1). Copper, Mn and Fe ranged from being excluded to slightly accumulated (<1 - >1), whereas Co and Ni were excluded (<1). The macronutrients K, P, S were highly accumulated, followed by Ca and Si (>1). Magnesium ranged from being excluded to slightly accumulated (<1 to >1) (Table 6-2).

The concentrations of the metals Co, Ni, Fe, Mn and Mg in the foliage of *N. buxifolia* were low compared to the mean foliage elements of the central Queensland serpentine foliage (Bidwell 2000) (Table 6-2). The concentrations of foliar macronutrients N, P and K were similar to those found elsewhere in native Australian flora (Table 6-3). The elemental concentrations in *N. buxifolia* foliage increased with soil metal concentration, with notable correlations (Table 6-4). For example, foliage Ca was negatively correlated with soil Mg (Table 6-4). Foliage Ni was positively correlated with soil Ni but was also negatively correlated to soil Ca (Table 6-4). The foliage Cu was positively correlated with soil Cu, and with soil Mo. Foliage K was correlated with soil K and soil Co and negatively correlated with soil Si. The remaining foliage and soil relationships are listed in Table 6-4.

Table 6-2 Mean elemental concentrations of *Neoroepera buxifolia* foliage, the extractable soil elements, the foliage bioaccumulation factors (BF) and the mean concentrations of elements measured from central Queensland serpentine areas (minimum and maximum in parentheses following each mean unless otherwise stated).

Element	foliage elements	soil elements	BF	serpentine flora of Central Queensland mean foliage concentration followed standard deviation #
B (µg g^{-1})	51.68 (24-82)	2.17 (0.68-5.05)	29.44 (8.45-77.94)	
Co (µg g^{-1})	0.23 (0.1-1)	8.92 (4.31-13.7)	0.03 (0.01-0.13)	2.6 (±1.6)
Cu (µg g^{-1})	2.88 (1-6)	1.47 (0.68-2.97)	2.12 (0.79-3.95)	na
Fe (µg g^{-1})	77.44 (12-484)	168.54 (115.07-477.73)	0.49 (0.04-2.89)	184 (±268)
Mn (µg g^{-1})	85.12 (22-371)	121.69 (77.39-193.57)	0.80 (0.12-4.79)	154 (±261)
Na (µg g^{-1})	268.12 (113.23-810.96)	37.52 (12.70-87.19)	8.78 (2.75-32.10)	na
Ni (µg g^{-1})	9.48 (1.2-25.1)	87.62 (28.13-195.62)	0.12 (0.03-0.53)	28 (±34.9)
Zn (µg g^{-1})	16.60 (8-31)	2.30 (0.70-12.23)	10.66(1.49-25.81)	na
C (µg g^{-1})	441.6 (430.1-455.8)	-	na	na
Ca (mg g^{-1})	5.5 (2.5-11.2)	9.4 (3.1-17.9)	7.90 (1.91-31.13)	na
K (mg g^{-1})	3.75 (1.61-7.11)	0.07 (0.04-0.1)	57.07 (26.13 - 93.39)	na

Element	foliage elements	soil elements	BF	serpentine flora of Central Queensland mean foliage concentration followed standard deviation [#]
Mg (mg g^{-1})	3.07(1.18-6.82)	2.9 (1.5-5.8)	1.11 (0.53-1.90)	4.5 (±3.9)
N (mg g^{-1})	9.98 (7.77-12.50)	-	na	na
P (mg g^{-1})	0.43 (0.30-0.58)	0.04 (0.01-0.15)	142.03 (25.31-253.22)	na
S (mg g^{-1})	1.10 (0.83-1.36)	0.02 (0.002-0.05)	79.43 (16.86-396.36)	na
Si (mg g^{-1})	1.10 (0.58-1.71)	0.45 (0.17-0.86)	2.62 (1.10-5.23)	na
pH	-	7.15 (6.25 – 8.57)	na	na
Mg/Ca	0.66 (0.18-2.53)	3.67 (1.43-11.05)	0.21 (0.04-0.56)	na

data from Bidwell (2000).

na = not available

Table 6-3 Mean foliar macro elements for eucalypt woodlands/flora of south-western Australia

Foliar elements	(mg g^{-1})
N	9.8
P	0.4
K	6.9

Derived from (Foulds 1993).

Table 6-4 Stepwise multiple linear regression analysis results for *Neoroepera buxifolia* elements as the dependent variables and the soil elements as the independent variables. ([a] = partial R^2, [b] = adjusted R^2).

Dependent variable Foliage	Independent variable soil	B	SE	*β*	P value	R^2
Log. Foliage C	(constant)	12.793	0.065		0.000	
	Log. Soil Al	0.037	0.012	0.548	0.005	0.300
Log. Foliage Ca	(constant)	12.979	1.708		0.000	
	Log. Soil Mg	-0.559	0.216	-0.475	0.016	0.226
Log. Foliage Co	(constant)	-0.969	0.43		0.035	
	Log. Soil Na	0.245	0.061	0.691	0.001	0.245[a]
	Log. soil conductivity	-0.211	0.065	-0.583	0.004	0.141[a]
	Log. Soil Si	0.196	0.075	0.423	0.015	0.153[a]
	overall model				0.001	0.472[b]
Log. foliage Cu	(constant)	-0.707	0.26		0.013	
	Log. Soil Cu	0.863	0.818	0.582	0.000	0.371[a]
	Log. Soil Mo	99.342	21.944	0.552	0.000	0.303[a]
	overall model					0.645[b]
Log. Foliage Fe	(constant)	-6.553442	3.078956		0.044	
	Log. Soil Si	1.70367	0.507052	0.573791	0.003	0.329
Log. Foliage K	(constant)	6.874	0.975		0.000	
	Log. Soil K	0.379	0.108	0.48	0.002	0.280
	Log. Soil Co	0.624	0.184	0.466	0.003	0.231
	Log. Soil Si	-0.28	0.123	-0.312	0.033	0.097
	overall model				0.000	0.552
Log. Foliage Mn	(constant)	10.32059	2.602387		0.001	
	Log. Soil Mg	-1.327643	0.316273	-0.67858	0.000	0.355[a]
	Log. Soil Na	0.634239	0.214615	0.455266	0.008	0.137[a]

Dependent variable Foliage	Independent variable soil	B	SE	*β*	P value	R^2
	Log. Soil Ni	0.503436	0.171871	0.414771	0.008	0.147[a]
	overall model				0.008	0.588[b]
Log. Foliage Mo	(constant)	-0.088	0.066		0.202	
	Log. Zn	0.058	0.015	0.62	0.001	0.415[a]
	Log. Ni	0.033	0.015	0.356	0.039	0.126[a]
	overall model				0.001	0.490[b]
Log. Foliage N	(constant)	8.378	0.243		0.000	
	Log. Cu	0.314	0.097	0.519	0.004	0.294[a]
	Log. Co	0.24	0.102	0.377	0.028	0.141[a]
	overall model				0.002	0.384[b]
Log. Foliage Ni	(constant)	2.769	2.019	0.496	0.184	
	Log. Ni	0.71	0.239	0.496	0.007	0.246[a]
	Log. Ca	-0.568	0.256	-0.372	0.037	0.138[a]
	overall model				0.005	0.328[b]
Log. Foliage P	(constant)	5.73	0.109		0.000	
	Log. Cu	0.369	0.12	0.541	0.005	0.292
Log. Foliage S	(constant)	6.1	0.233		0.000	
	Log. Co	0.341	0.096	0.577	0.002	0.274[a]
	Log. P	0.102	0.043	0.391	0.026	0.150[a]
	overall model				0.002	0.371[b]
Log. Foliage Si	(constant)	6.602	0.156		0.000	
	Log. Mg/Ca	0.297	0.123	0.45	0.024	0.203
Log. Foliage Mg	(constant)	8.58	0.804		0.000	
	pH	0.295	0.93	0.503	0.000	0.344[a]
	Log. Mn	-0.762	0.191	-0.496	0.005	0.276[a]
	Log. Zn	-0.495	0.121	-0.627	0.001	0.073[a]
	Log. Na	0.406	0.13	0.482	0.005	0.101[a]

Dependent variable Foliage	Independent variable soil	B	SE	*β*	P value	R^2
	overall model				0.000	0.752[b]
Log. Foliage Mg/Ca	(constant)	-1.845	1.023		0.085	
	Log. Mg	0.403	0.113	0.544	0.002	0.375[a]
	Log. Co	-0.383	0.158	-0.37	0.024	0.132[a]
	overall model				0.000	0.463[b]
Log. Foliage Na	(constant)	5.016	0.225		0.000	
	Log. P	0.379	0.172	0.417	0.038	0.174
Log. Foliage Zn	(constant)	0.372	0.619		0.553	
	Log. Co	1.043	0.271	0.625	0.001	0.391

6.5 Discussion

Neoroepera buxifolia did not accumulate trace elements and the BF values for the elements Co and Ni did not exceed 1. Plants that are used for phytostabilisation should be native, tolerant of the metals in question, and limit the shoot metal accumulation (Mendez and Maier 2008b). Thus *N. buxifolia* is useful for phytostabilisation following mining within the central Queensland serpentine landscape. The purpose of phytostabilisation is not to eliminate the contaminants but rather to stabilize them to reduce the risk of translocating the metals through the food chain or into the environment (King *et al.* 2008; Mendez and Maier 2008b).

The nutrient concentrations of the soils were typical for serpentine soils, with low concentrations of macronutrients, high Mg/Ca quotients and high concentrations of trace elements, particularly for Ni (*ie*. Brooks 1987). The mean concentrations of *N. buxifolia* foliage macronutrients N, P and K were within the normal range for Australian flora (Table 6-3), supporting the theory that plants species adapted to infertile soils absorb sufficient macronutrients from deficient soils (Chapin 1980; Grime 1979; Kazakou *et al.* 2008; Nagy and Proctor 1997). One of the principal reason for serpentine soil infertility is high Mg/Ca quotients and tolerance of this is an important evolutionary adaptation (Kazakou *et al.* 2008). The foliage Mg/Ca quotients for *Neoroepera buxifolia* were less than that of the soil and there were negative correlations between foliage Ca and soil Mg. Selective Ca transport and Mg retention at the root-to-shoot level is a widespread occurrence on serpentine soils and may be an important adaptation for survival (O'Dell *et al.* 2006). The concentration of foliage Ca of *N. buxifolia* was greater than the foliage Mg. High Ca concentrations in the foliage of serpentine adapted plants may be able to alleviate the toxicity of trace elements (Lyon *et al*. 1971). Calcium has been found to depress the Ni concentrations in roots and reduces the transportation of Ni to the aerial parts of Ni excluder plants (Gabbrielli *et al*. 1990). Whilst the root-to-shoot translocation or root exclusion/accumulation of metals was not determined in this current study, Ni uptake was dependent on the soil Ca availability and the soil extractable Ni. The concentrations of elements Co, Ni, Fe, Mn and Mg were lower compared to the mean foliage elements of the central Queensland serpentine vegetation (Table 6-1).

Neoroepera buxifolia has narrow habitat requirements and is restricted to areas of high soil moisture such as ephemeral and perennial creeks. However, where is does occur it becomes locally abundant and forms dense stands (Hendry *et al.* 2015 in press). It is readily propagated from seed and responds well to marcotting and ground layering propagation techniques. As such *N. buxifolia* may be useful for the phytostabilisation of areas with high soil moisture such as tailing dams, drains and creeks within the central Queensland serpentine landscape.

Chapter 7 An Ecological Risk Characterisation of the serpentine endemic shrub Neoroepera buxifolia of central Queensland.

7.1 Abstract

Ecological Risk Assessment (ERA) provides a systematic method to determine the risk posed by threats to the survival of an ecosystem or species. A five step risk analysis process (AS/NZS ISO 3100:2009) was applied to determine the level of risk to the serpentine endemic shrub to small tree *Neoroepera buxifolia*. This involved establishing the context and identification of the threatening processes. A systematic bibliographic search for threatening processes to serpentine vegetation globally was undertaken, the likelihood and consequences of the threats was assessed and a Risk Matrix was applied. The ERA indicated that *N. buxifolia* populations are at greatest risk from climate change, fire and mining and to a lesser degree from exotic species, grazing and habitat fragmentation. Management options were applied to each of the threats to reduce or mitigate the risk outcomes.

Plate 7-1 Remnant *Neoroepera buxifolia* shrubs in a cleared paddock.

7.2 Introduction

An Ecological Risk Assessment (ERA) is a method to determine the risk posed by a threatening process or stressor to the survival of an ecosystem or species and provides a systematic framework for the evaluation of the potential implications of management decisions when information is sparse, incomplete or uncertain (Burgman *et al.* 1993). The aim of performing an ERA is to estimate the probability and significance of the threatening process or stressor occurring. An ERA can be applied to assess the relative impact potential of multiple threats against a value (Department of Environment 2014b). There are five key steps in the risk analysis process: (i) establish the context; (ii) identify the risks (what are the hazards); (iii) analyse the risks; (iv) evaluate the risks; and (v) manage the risks (AS/NZS ISO 3100:2009).

Vegetation communities on unusual geology and soil types such as serpentine can support a high number of endemic species (Damschen *et al.* 2011). The serpentine landscape of central Queensland on the east coast of Australia is ecologically significant and supports about 17 endemic or rare and endangered flora species (Aola 2014; Batianoff *et al.* 2000; Batianoff *et al.* 1997, Jackes 2005). *Neoroepera buxifolia* Muell.Arg. & F.Muell. (Picrodendraceae) is one such endemic shrub to small tree that occurs in the creeks, riverbanks, terraces and drainage lines of the central Queensland serpentine landscape and is associated with high concentrations of magnesium in the soil (Batianoff *et al.* 2000). It has a restricted distribution and aspects of its ecology and propagation are described in detail earlier in this thesis (Chapters 3, 4, 5 & 6). Only a few studies have dealt directly with threats to Australian or specifically central Queensland serpentine flora (*ie*. Batianoff *et al.* 2000; Bhatia *et al.* 2002). While the conservation advice for *N. buxifolia* has identified a number of threats including vegetation clearing, exotic weeds invasion, mining, grazing and timber harvesting (Department of Environment 2014a), there is no evidence that an Ecological Risk Assessment has been applied to central Queensland serpentine flora to date.

Although threats to *N. buxifolia* have been previously identified (Department of Environment 2014a), it was decided to comprehensively evaluate the likely threatening processes to serpentine flora globally to identify any previously unconsidered threats. The risk for each of these threats was then assessed against a standard risk matrix and the risks were characterised using a semi-qualitative approach (Department of Environment 2014b).

The aim of this chapter was to develop an Ecological Risk Assessment for *N. buxifolia* using the Australian and New Zealand risk analysis framework (AS/NZS ISO 31000:2009). The risk assessment was achieved by developing a likelihood and consequence of threat look-up table and by scoring each of the identified threats.

7.3 Methods

The processes for developing an ERA are illustrated in Figure 7-1 and involve the identification, assessment and evaluation of the risks and the application of management options to reduce the risk from the threats.

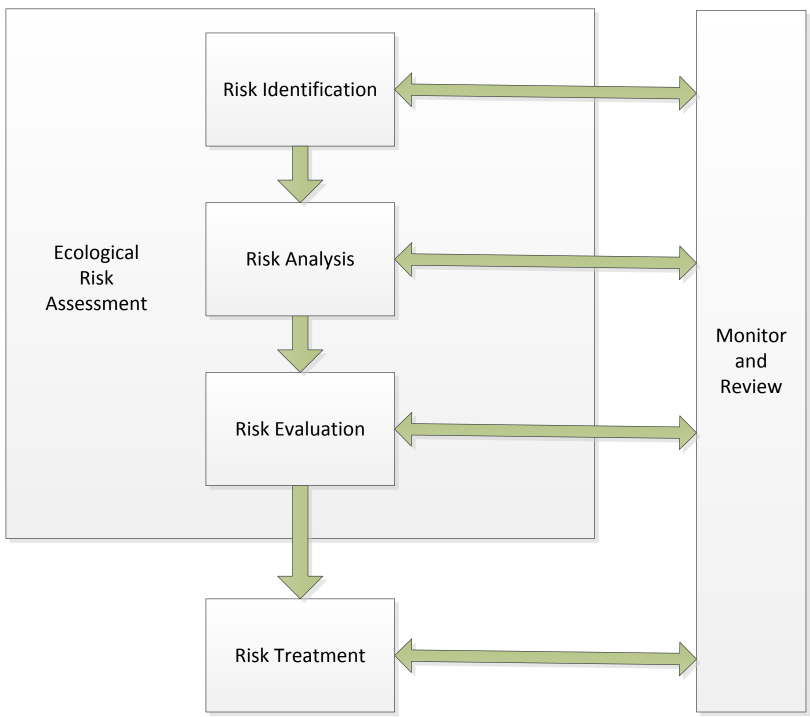

Figure 7-1 Systematic steps for performing Ecological Risk Assessment. Emphasis is on the iterative nature of risk assessments and the need to update based on new data and information (adapted from Standards Australia (2009) AS/NZS ISO 31000:2009).

7.3.1 Risk Identification

In order to identify the threatening process to serpentine flora, a systematic bibliographic search was conducted with Scopus search engines using keywords in the title and abstracts for published papers and reviews (Table 7-1). The initial search parameters were based on the current and future threat syndromes to Australian flora as identified by Burgman *et al.* (2007). A paper was included in the analysis if the threat actually impacted on the serpentine flora. The threatening processes were identified from each study and summarised and their percentage contribution from the literature was then determined.

7.3.2 Assessment of the risk from the threatening processes to *Neoroepera buxifolia* populations.

An assessment of the threats to *N. buxifolia* populations was developed using ecological data and observations presented in this thesis and the EPBC SPRAT database (Department of Environment 2014a). The likelihood of a threat occurring was determined using derived likelihood measures (Table 7-2). The likelihood measures are qualitative descriptions which are quantified into probabilities of the event occurring. A qualitative approach was used to rank the consequences to determine the level of impact from a threat. These rankings provide descriptions of the consequences to act as a guide and were ranked as insignificant to extremely significant (Table 7-3).

Table 7-1 Boolean search terms used for the Scopus search of the threatening processes to serpentine flora globally.

Search terms for keywords in title and abstract

"serpentine" or "serpentinite" or "ultramafic" and	
threats	disease
disturbance	pathogens
agriculture	exotic
farming	introduced
grazing	non native
cattle	fire
livestock	forestry
crops	land clearing
trampling	clearing
climate change	harvesting
climate	logging
CO2	fragmentation
carbon dioxide	afforestation
drought	habitat loss
precipitation	pollution
storms	mining
flooding	extraction
tourism	recreation

Table 7-2 Likelihood measures derived for ecological risk analysis. A threat is defined as an activity that may lead to an undesirable outcome (adapted from Standards Australia (2009) AS/NZS ISO 31000:2009).

Descriptor	Description	Probability of event occurring
Negligible	The threat is unlikely to occur.	<1%
Extremely Low	The threat will only occur in exceptional circumstances	1-5%
Very Low	The threat could occur but not expected	6-25%
Low	The threat could occur	26-50%
Moderate	The threat will probably occur in most circumstances	51-75%
High	The threat is expected to occur in most circumstances	76-100%

Table 7-3 Consequence rankings of threatening processes to *Neoroepera buxifolia* (adapted from Standards Australia (2009) AS/NZS ISO 31000:2009).

Descriptor	Impacts on *N. buxifolia* from threatening process
Insignificant	Population reduction is minimal (<10%) compared to natural processes Reductions in population is not readily detectable (<10% variation).

Descriptor	Impacts on *N. buxifolia* from threatening process
	If the threatening process was removed, recovery is expected; no discernible change in the environment.
Minor	Population reduction is 10-20% compared to loss from other natural processes. Reductions in environment sub-components are 10-20% Population reduction is small compared to known areas of distribution (10-20%) If the threatening process was removed, recovery is expected in months; no loss of keystone species populations, no discernible change in population form and function; no local extinctions.
Moderate	Population reduction is (>20-30% compared to loss from natural processes. Reductions in environment sub-components are >20-30%. Population reduction and threat impact is moderate compared to known area of distribution (>20-30%). If the threatening process was removed, recovery is expected in less than a year ; loss of at least one keystone species or populations, loss of geological form and function, no loss of primary producers; local extinction events.
Major	Population reduction is >30-70% compared to loss from natural processes. Reductions in environment sub-components are >30-70%.

Descriptor	Impacts on *N. buxifolia* from threatening process
	Population reduction and threatening process impact is moderate compared to known area of distribution (>30-70%); likely to cause local extinction. If the threatening process was removed, recovery is expected in less than a decade; loss several keystone species or populations, changes in trophic levels, loss of primary producer populations, loss of ecological form and function; multiple local extinction events; one regional extinction.
Significant	Population reduction is >70% compared to loss from other human-mediated activities; Reductions in environment sub-components are >70%. Environment reduction and area of introduced species impact is small compared to known area of distribution (>70%); likely to cause local extinction. Recovery is not expected; loss of multiple populations causing significant local extinctions and loss of trophic levels, potential trophic cascades resulting in significant changes to ecosystem structure, alteration to biodiversity patterns and changes to ecosystem function, loss of ecological form and function; global extinction

A standard Risk Matrix for *N. buxifolia* was created using a combination of likelihood measures and consequence rankings. The standard Risk Matrix qualitatively listed the risk in terms of low to very high. The Risk Matrix uses a colour key of blue, green, yellow, orange and red to provide an easy visual identification of the level of risk posed. Blue indicates an acceptable level of risk, whereas red indicates extreme risk (Table 7-4). The Risk Matrix was applied to each of the threatening processes to characterise the risk.

Table 7-4 Risk Matrix of consequences vs likelihood (adapted from Standards Australia (2009) AS/NZS ISO 31000:2009)

Likelihood	Consequences				
	Insignificant 1	Minor 2	Moderate 3	Major 4	Significant 5
A (Almost certain)	H	H	VH	VH	E
B (Likely)	M	H	VH	VH	E
C (Possible)	L	M	H	VH	E
D (Unlikely)	L	L	M	H	VH
U (Unknown)	U(L)	U(L)	U(M)	U(H)	UH

(E = extreme; VH = very high, H = high, M = medium, L = low, U = unknown)

7.3.3 Risk Management Matrix

A risk management matrix was developed to consider a range of risk management options to mitigate or minimise the risk of threats and reduce the risk outcomes. Management options were derived from the Threatened Species Scientific Committee (2008) conservation advice for *N. buxifolia* and the Department of Environment and Heritage Protection (2014).

7.4 Results

7.4.1 Identification of threatening processes to serpentine flora

The evaluation of the bibliographic search summarised the threats into 14 key threats. A total of 108 relevant articles were identified. The majority of the studies (n=103) addressed multiple threatening processes, whilst the remaining five studies addressed only single threatening processes. The most common threat to serpentine ecosystems globally was determined to be exotic species invasions (22%), followed by climate change issues (15%), fire (15%), grazing (14%), mining (9%), habitat fragmentation (8%) and pathogens (6%) (Figure 7-2). Processes such as nitrogen pollution, development, landslides, over-collecting, hybridization, quarrying and tourism each contributed to less than 5% of the literature, (Figure 7-2). Although these threats have not been widely considered in the literature it is possible they will be recognised as greater threats to serpentine systems as the pressures from anthropogenic disturbances increase. Additionally, the key threats identified in the Department of Environment (2014a) conservation advice for *N. buxifolia* are vegetation clearing, exotic weeds, mining, grazing and timber harvesting. The majority of the threats to serpentine vegetation are anthropogenic in origin and will be expanded on further in relation to *N. buxifolia*. The definitions for each of the threats is provided in Table 7-5.

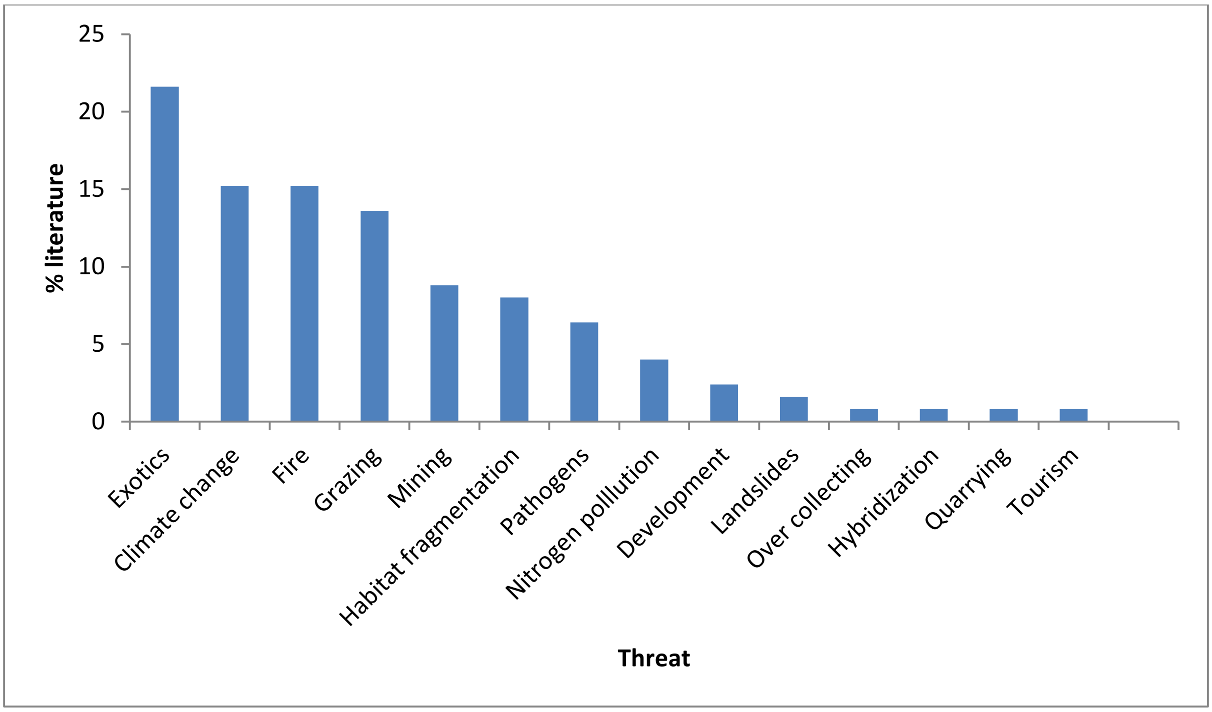

Figure 7-2 Percentage literature addressing each of the threatening processes resulting from the systematic Scopus database search (For years 1981-2014).

Table 7-5 Definitions of the Key threats derived from global literature.

Threat	Definition
Exotics	Non-native or introduced species outside of its native distribution. There are three classes of declared weeds in Queensland that are targeted for control (DAFF 2014). Class 1 – must be eradicated, Class 2 – reasonable steps must be taken to control, Class 3 – steps should be taken to prevent spread.
Climate change	Change in weather patterns such as increased intensity of storms and droughts.
Grazing	Native and domestic livestock

Threat	Definition
Mining	Includes mining and mineral exploration activities
Pathogens	Pathogens such as viruses, bacteria and fungi which cause disease
Habitat fragmentation	Habitat loss caused by afforestation, logging and clearing
Nitrogen pollution	Excess nitrogen may be from fertiliser run-off or atmospheric pollution.
Development	Includes urban development, infrastructure, roads and dams
Landslides	Movement of rock or soil down a slope
Over collecting	Over exploitation of a species
Hybridization	Combining of different varieties of a species to create a new hybrid
Quarrying	Use of rock material for roads or infrastructure development
Tourism	Includes recreation activities such as 4wd, camping, walking and visiting sites

7.4.2 Threats to *Neoroepera buxifolia*

7.4.2.1 Exotic species invasions

Serpentine habitats are generally considered to be less vulnerable to invasion by exotic flora species in comparison to non-serpentine habitats due to their unique edaphic features such as low soil calcium, low nitrogen and high concentrations of trace elements and magnesium (Williamson and Harrison 2002). However, serpentine systems are heterogeneous with a range of soil nitrogen, soil depth, rockiness, heavy metal and calcium concentrations creating a mosaic of niches promoting both native and exotic species diversity (Davies *et al.* 2007). Further to this, riparian zones have greater disturbance regimes and habitat variability and are more susceptible to exotic species invasions (Stohlgren *et al.* 1999). High levels of natural disturbance and intensive livestock grazing of riparian zones within the central Queensland serpentine landscape could expose these areas to exotic species invasions. The declared weed, rubber vine (*Cryptostegia grandiflora*) has been identified as a potential problem in Marlborough and Spring Creeks (Whereat 2002). The vegetation surveys reported in Chapter 3 of this thesis recorded rubber vine and lantana (*Lantana camara*) in low abundances in some creeks. Rubber vine is a class 2 weed that spreads aggressively from waterways into adjoining woodlands and often dominates the vegetation (DAFF 2014; Department of Environment 2012). Lantana is a class 3 weed (DAFF 2014) that increases with the intensity of disturbance, which correlates to an increase in resource availability (Duggin and Gentle 1998).

The consequences of these exotic species in the natural environment are reduced diversity, increases in nutrient cycling and greater fire intensity (Stohlgren *et al.* 1999). Serpentine endemics are considered to be inferior competitors and are successful in their niche as they are able to tolerate the stress of the serpentine soils (Brooks 1987). However, the serpentine environment does not preclude invasion from exotic species which are able to tolerate a wide range of conditions.

7.4.2.2 Climate change

Industrialisation, the use of fossil fuels and land clearing have changed the composition of the atmosphere by increasing CO_2, particulate and NO in the upper atmosphere which trap the outgoing infrared radiation warming the planet accelerating global climate change (Serrat-Capdevila *et al.* 2007; Trenberth 2008). An increase in atmospheric CO_2 will increase the photosynthetic potential and growth in C3 plants, however these benefits may be outweighed by increases in competition and changes in the community composition (Field *et al*. 1995). Modelling indicates there will be greater hydrological extremes with more intense rainfall in La Niña years and more intense drought and higher evaporation rates in El Niño years (Walsh *et al.* 2004).

Under current climate change scenarios the intensity of storms and tropical cyclones south of 30^{o} latitude will increase and more extreme weather events with heavy rainfall and floods are projected to become more frequent (Trenberth 2008; Walsh, *et al.* 2004). Healthy free-flowing rivers respond to changes in flooding via dynamic movements and flow adjustments which will act to buffer the effects of increased heavy rainfall events (Palmer *et al.* 2008).

Riparian plants have numerous morphological, physiological and reproductive adaptations for life in a constantly disturbed environment. They are adapted to frequent flooding, sediment deposition, abrasion and stem breakage (Naiman *et al.* 1998). Indeed floods are critical to channel recharge by providing a seasonal source of soil and sustaining the life cycle of riparian plants (Serrat-Capdevila *et al.* 2007). *Neoroepera buxifolia* is able to reproduce asexually via re-sprouting from branches and stems that are covered by soil deposition or knocked over during flooding and the phenology of *N. buxifolia* seed fall is timed to coincide with the summer monsoon (Hendry *et al.* 2015 in press).

As the severity of droughts increase with the rising global temperatures, forest biomes would be vulnerable to drought induced mortality (Allen *et al.* 2010; Choat *et al.* 2012). The flora of the central Queensland serpentine landscape evolved in the warmer, fluctuating wet/dry conditions of the Tertiary period (Batianoff *et al.* 1995). Historically, during climatic oscillations it is likely the flora contracted into well watered areas during periods of aridity and expanded into the gaps when conditions improved (Batianoff *et al.* 1995). The drainage lines and gullies which form the habitat for *N. buxifolia* generally have more humid microclimates, provide topographic protection against fires and buffer droughts due to enhanced moisture retention in the soil (Bowman 2000; Mac Nally *et al.* 2000). However, drought can lead to the lowering of the riparian water table, causing tree mortality which results in shifts of dominance in the vegetation (Auble *et al.* 1994).

7.4.2.3 Fire

Fire is one of the most common natural disturbances of Australian plant communities and the flora has characteristics to allow persistence following repeated fire cycles (Specht and Specht 1999). Much of the Australian flora has the ability to regenerate vegetatively following fire (Specht and Specht 1999). Fire regimes in Australia are largely driven by latitude, gradient and the summer monsoon activity. The monsoonal north including central Queensland is subjected to frequent low intensity fires, whereas the southern latitudes experience infrequent, high intensity fires (Murphy *et al.* 2013).

In Australia fire is used to manage fuel loads, maintain grazing, protect infrastructure and for biodiversity management (Spencer and Baxter 2006). Developing generic fire management plans is difficult because the impact on plant assemblages is different and floristic composition is driven by many factors (Spencer and Baxter 2006).

Drainage lines and gullies provide topographic protection against fires (Bowman 2000). The majority of *N. buxifolia* populations grow in perennial and ephemeral drainage lines and gullies and on creek banks derived from serpentinite, and thus leverage topographic protection against fire. Further to this, *N. buxifolia* is fire tolerant as it is able to re-sprout basally following a burn. However, it is unknown what the minimum interval between fires should be (Hendry *et al*. in prep). *Neoroepera buxifolia* stores its seeds in the canopy until ripened and further research is required to determine if the seeds persist in the capsules or soil seed bank after a fire. Too frequent or intense fire regimes could eradicate this species from the landscape.

7.4.2.4 Grazing

Until the introduction of domestic livestock in the 1800's, Australian ecosystems had not been subjected to ungulate grazing since the late Pleistocene when large herbivorous marsupial megafauna existed (Fensham and Skull 1999; Freeland 1990). Grazing by domestic livestock and the associated land management has created major ecological changes to the soils, landscape processes, vegetation and fauna in Australia (Lunt *et al.* 2007). Livestock are documented as causing defoliation, and trampling, with defecation and urination increasing soil nitrate-nitrogen, ammonium-nitrogen and available phosphorus in localised areas (Abensperg-Traun *et al.* 1996; Wilson 1990; Yates *et al*. 2000). Livestock impact the soil structure by removing leaf litter, which results in a reduction of soil organic carbon, loss of nutrients, loss of micro-topography, soil compaction, reduced water infiltration rates, increased soil surface erosion and loss of ecosystem functions (Yates, *et al.* 2000). The predominant land use of the central Queensland serpentine landscape is beef cattle grazing on native pastures (Calvert *et al.* 2000; Forster and Baker 2002). The stocking rates of the unfertile undulating hilly country is low with one beast per 10-20 ha, whereas the more fertile adjacent lowlands have higher stocking rates of 1 beast per 4-5 ha (Forster and Baker 2002). The effects of grazing are more intensified around gathering points such as shade, water holes and within riparian zones (Amy and Robertson 2001). As such, *N. buxifolia* plants and seedlings are at risk from trampling, pugging and increased nutrient loadings caused by livestock gathering at natural watering points and when seeking shade.

Despite the soils being characteristically high in trace elements and the associated vegetation carrying above mean concentrations of trace elements in their foliage, only one study on metal accumulation in animals reared on serpentine soil areas was found in the literature (Miranda *et al.* 2009). This study on cattle raised on serpentine soils and forage in Spain found the accumulation of metals in the tissues (liver, kidney and muscle) correlated to the concentration in the soil and forage. The authors concluded many of the animals in the study had near toxic concentrations of nickel and copper (Miranda *et al.* 2009).

7.4.2.5 Mining

Serpentine substrates typically contain deposits of nickel and the laterite deposits of central Queensland contain a series of discrete nickel enrichments over a 30 – 50 km^2 area (Foster and Eggleton 2002). Nickel mining typically involves the removal of the serpentine topsoil down to the bedrock (O'Dell and Claassen 2011). The processed tailings lose their ecological function, water-holding capacity, cation exchange capacity, organic matter, nutrients, seeds and soil microbial communities, becoming a challenge to revegetate (O'Dell and Claassen 2011). The mined serpentine substrates are unlikely to undergo natural colonisation by most plants due to the high concentrations of trace elements, high Mg/Ca quotients and low fertility and the ecological functions have to be rebuilt (Cooke and Johnson 2002; DeGrood *et al.* 2005; O'Dell and Claassen 2011).

Serpentine substrates are vulnerable to erosion when disturbed and mining activities can result in elevated sedimentation, alter hydrology and transport heavy metals into watersheds

(O'Dell and Claassen 2009; Padmavathiamma and Li 2009). It is difficult to predict the effects of increased sedimentation and metal loadings on the plants and on the other riparian vegetation. However, in New Caledonia nickel mining has caused widespread erosion and pollution of rivers and damaged and destroyed the vegetation (Jaffré and Rigault 1989).

Nickel mining on the central Queensland serpentine landscape is taking place at present and will certainly increase in the future (QNI 2013). The impacts on the ephemeral gullies and drainage lines will be localised, but the sediment loading will be transferred to the higher order perennial creeks.

7.4.2.6 Habitat fragmentation

Habitat fragmentation is a major factor influencing the persistence of species globally (Hobbs and Yates 2003). Fragmentation involves the creation of isolated patches of remnant vegetation of various sizes within areas of urban and agricultural developments (Fahrig 2003; Hobbs and Yates 2003).

Plants on serpentine soils are distinct from adjacent vegetation on non-serpentine soils and are geographically isolated and patchily distributed (Brooks 1987; Kruckeberg 1954). Habitat fragmentation caused by land clearing exacerbates the isolation of serpentine outcrops. The challenge for ecologists is to determine if the pre-adaption to geographical isolation allows serpentine adapted plants to survive and persist in increasingly isolated pockets or if they require continuity of landscape via remnant vegetation for long-term survival (Harrison and Rajakaruna 2011; Harrison *et al.* 2006).

Populations can only exist in islands if long-term reproduction outweighs long-term mortality (Fahrig 2003). *Neoroepera buxifolia* is endangered due to its restricted distribution and overall small population size, and is vulnerable to catastrophic events. There is a risk that if a population dies out within a creek line, limited seed dispersal or low seed bank persistence would impede the regeneration of *N. buxifolia* leading to localised extinction (Hendry *et al*. 2015 in prep.). Further research is required to determine the persistence of the seed bank.

7.4.2.7 Pathogens

It is believed that the edaphic properties of serpentine soils, such as high trace element concentration may provide a refuge from pathogen attack (Kruckeberg 1992; Springer *et al.* 2007). Studies have found that the elemental defences of hyperaccumulator plants supplement the defensive compounds found in most plants (Boyd and Moar 1999; Martens and Boyd 1994). However, elemental defence is not absolute and high concentrations of nickel and metal ions in the soil are not guarantees of protection against introduced pathogens. For example, the Tasmanian serpentine endemic herb *Tetratheca gunnii* is susceptible to the root fungus *Phytophora cinnamoni*. The fungus is spread by off-road vehicle use, wood harvesting and mining activities. Management for *P. cinnamoni* includes the use of protective sprays (Potts and Barker 1999).

The pathogen Myrtle rust *Puccinia psidii sensu lato* has a preference for moist climates with moderate temperatures in the wet tropics and sub-tropics (Kriticos *et al.* 2013). Since its detection in Australia in 2010, Myrtle rust spread rapidly into Queensland and has been detected within the Rockhampton Regional Council management area (DAFF 2014). Myrtle rust spreads rapidly via spores and is extremely difficult to eradicate (DAFF 2014). Australia has a multiplicity of species in the family Myrtaceae which are susceptible to Myrtle rust and it poses a significant threat to the conservation of native plants and ecosystems and the forestry industry (Kriticos *et al.* 2013). As the central Queensland serpentine landscape is dominated by two eucalypts, if one or both are susceptible to myrtle rust it is likely to have a major impact. Although *N. buxifolia* is not from the Myrtaceae family, alterations to the overlying vegetation could have profound effects on the entire ecosystem.

7.4.2.8 Nitrogen pollution

Ecosystems which are nitrogen-limited such as serpentine respond strongly to additions of nitrogen causing significant and rapid changes to productivity and species composition (Weiss 1999). Anthropogenic sources of nitrogen are increasingly being emitted into the atmosphere from the combustion of fossil fuels, fertilizer drift and manure (Bobbink *et al.* 2010; Galloway *et al.* 2004; Neff *et al.* 2002). It is unlikely that fertilizer drift or atmospheric pollution will cause an increase in nitrogen on the central Queensland serpentine landscape; however, there is a risk of increased fertilization caused by livestock grazing.

7.4.2.7 Development

The life histories of riparian plants evolve in direct response to natural flow regimes and alterations to flow can lead to recruitment failure, loss of native biodiversity and invasion of exotic species (Bunn and Arthington 2002). The instalment of weirs and dams raises the water line of the river causing permanent flooding of areas upstream of the structure. Further to this, changes to the flow rates in streams due to dams and weirs can result in channel narrowing because of the deposition of new material adjacent to the channel (Auble *et al.* 1994).

The raising of the Eden Bann weir on the Fitzroy River would impact on the Marlborough and Princhester feeder creeks draining the central Queensland serpentinite area. This will be a two stage development, increasing the current level of the weir from reservoir level (RL) 14.5m to stage 2 RL 18.2m and stage 3 20.2m (GHD 2010). The raising of the weir will directly impact on Marlborough and Princhester creek systems which are essential habitat for

N. buxifolia. *Neoroepera buxifolia* predominantly occurs above the water line indicating they prefer to grow in drained soils. When inundated the initial effects on plants is through the root systems, as the soil become anoxic the plant becomes oxygen stressed and eventually dies (Nilsson and Berggren 2000). It is unknown how *N. buxifolia* would respond to long term inundation, but given that current populations are only found above the water line this indicates they may be susceptible to extended periods of inundation.

7.4.2.8 Landslides, Hybridization, Quarrying, Tourism

These remaining threats are unlikely to impact on *N. buxifolia* populations at this time. The local Livingstone shire council no longer uses quarried serpentinite rock as road base due to the presence of chrysotile (white asbestos) which is a class 1 carcinogen associated with mesothelioma (Hendrickx 2009). Whilst landslides are possible on the steeper slopes, they are unlikely to occur as a search on Australian Government Geoscience Australia database did not reveal any documented landslides within the serpentine landscape (Geoscience Australia 2014). Natural hybridization is indeterminate at this stage as the life history of *N. buxifolia* is relatively undescribed. Tourism is low to negligible as much of the landscape is actively managed for grazing and recreational 4WD and motorbike access is limited. There are no camping reserves within the area.

7.4.2.9 Risk Characterisation and Management

The threatening processes for *N. buxifolia* were characterised by applying the standard Risk Matrix (Table 7-4) to each of the threats. The potential risk from exotics species on *N. buxifolia* populations has been assessed as Very High (Table 7-6). If exotic species spread aggressively throughout Marlborough Creek (Chapter 3 this thesis) and the other perennial creek systems it would cause a significant reduction in *N. buxifolia* populations. If this occurred priority management actions (Table 7-7) through the implementation of a Pest

Management Plan would be required to reduce exotic species incursions. This would reduce the threat to *N. buxifolia* populations to Moderate (Table 7-8 Risk management outcomes from management actions).

The potential risk of climate change on *N. buxifolia* populations has been assessed as Very High (Table 7-6). This is because climate change effects will be widespread throughout *N. buxifolia* habitat. The impacts from extreme droughts or frequent flooding or fire events could be major, causing local extinction of populations. A priority action plan (Table 7-7) to protect and manage populations of *N. buxifolia* within protected areas and to expand the population into other suitable habitats and implementing fire management plans would build resilience in the population and mitigate the threat of climate change. Such actions would reduce the threat to *N. buxifolia* populations to Moderate (Table 7-8 Risk management outcomes from management actions).

The risk from fire has been assessed as Very High (Table 7-6). Fire in the serpentine landscape is certain and the impacts are major. Unmanaged or too frequent fire regimes would have a deleterious effect on *N. buxifolia,* reducing the population and potentially causing local extinctions. Priority fire and pest management plans to reduce the intensity and frequency of fire affecting the ephemeral gullies and creeks planting new populations and the creation of protected areas would reduce the threat of fire on *N. buxifolia* populations to Medium (Table 7-8).

The impact of livestock grazing on *N. buxifolia* has been assessed as High (Table 7-6). This is attributable to the impacts from livestock likely to be confined to areas with permanent water where the cattle gather around access points. *Neoroepera buxifolia* habitat also encompasses ephemeral gullies and creeks and these habitats are unlikely to be affected by gathering cattle. The impacts from livestock could be easily managed by fencing the gathering points and pumping water to shaded watering stations established out of the riparian zone. Focused management of livestock and the creation of protected areas would reduce the threats from grazing to Low (Table 7-8 Risk management outcomes from management actions).

The threat from mining has been assessed as Very High (Table 7-6). Serpentine landscapes are vulnerable to erosion when disturbed and it is difficult to predict if erosion control measures will be suitably effective. The most effective priority management will be to establish rehabilitation protocols before mining commences and expand populations into other suitable habitat and to ensure populations of *N. buxifolia* are protected in unmined protected areas This would reduce the risk of mining to *N. buxifolia* to High (Table 7-8 Risk management outcomes from management actions).

The risk of threat from habitat fragmentation on *N. buxifolia* has been assessed as High (Table 7-6). *Neoroepera buxifolia* has limited seed dispersal and fragmentation of its habitat could have a major impact on the persistence of populations. A focused plan to actively introducing *N. buxifolia* into suitable habitats would reduce the threat of habitat fragmentation to Low (Table 7-8 Risk management outcomes from management actions).

The incidence of reported pathogens on serpentine flora in Australia is low (only 1 case found in literature review on a Tasmanian serpentine herb (Potts and Barker 1999). However, land managers need to remain aware that introduction is possible and the potential impacts from pathogens could be profound on forest ecosystems. Management should focus on preventing the entry of new strains of myrtle rust into Australia (Booth 2011). The vegetation around mining and exploration sites of the central Queensland serpentine landscape should be monitored for introduction or establishment of invasive pathogens, with immediate action if discovered. More research is required to ascertain the infection risk on the central Queensland serpentine flora.

The threat from pathogens on *N. buxifolia* is currently Low (Table 7-6). It is unlikely that the populations will be threatened by pathogens at this time. However, other management actions proposed for the species should include monitoring and protocols for preventing new pathogens from entering the serpentine landscape.

The threat of nitrogen pollution on *N. buxifolia* populations is currently Low (Table 7-6) and managing livestock grazing activities by fencing water sources will assist in keeping the threat from nitrogen pollution as Low.

The threat from developments was assessed as Medium (Table 7-6). If raising the Eden Bann weir does impact on *N. buxifolia* populations, the impacts are likely to be limited to lower lying populations resulting in a moderate reduction in population size. Intermittent monitoring and management options to mitigate the threat would include introducing *N. buxifolia* into new suitable habitat above the water line (Table 7-8 Risk management outcomes from management actions).

Table 7-6 Risk characterisation assessment for *Neoroepera buxifolia*

Threat	Impact on *N. buxifolia* populations
Exotics	VH
Climate change	VH
Fire	VH
Grazing	H
Mining	VH
Habitat fragmentation	H
Pathogens	L
Nitrogen pollution	L
Development	M
Landslides	L
Over collecting	L
Hybridization	L
Quarrying	L
Tourism	L

Table 7-7 Risk management actions identified for *Neoroepera buxifolia* (modified from Campbell and Hewitt (2008)).

Risk	Likely management action(s)
Low	Nil
Moderate	Intermittent monitoring
High	Focused scientific research and applied management actions required
Very high	Priority scientific research and applied management actions required
Extreme	Intensive scientific research and applied management actions required

Table 7-8 Risk management outcomes from management actions

	Management Option					
Threat	Natural regeneration	Fencing off water sources	Pest Management plan	Implement fire management plan	Plant new populations	Create a protected area
Exotics	H	M	M	M	M	M
Climate change	VH			M	M	M
Fire	VH		M	M	L	M
Grazing	H	L				L
Mining	VH				H	H
Habitat fragmentation	H				L	
Pathogens	L	L	L		L	L
Nitrogen pollution	M	L	L	L	L	L

Development	M				L	L
Landslides	L					
Over collecting	L					L
Hybridization	L					
Quarrying	L					L
Tourism	L					L

7.5 Conclusions

The Ecological Risk Assessment process indicates *N. buxifolia* populations are at greatest risk from climate change, fire and mining and to a lesser degree exotic species, grazing and habitat fragmentation. If continued to be exposed to these threats it is likely the overall population will decline. *Neoroepera buxifolia* has a naturally restricted distribution within the central Queensland serpentine habitat and without management intervention the threatening processes have the potential to cause localised extinctions. Applying management actions such as implementing fire management plans, planting new populations, creating protected areas, controlling exotics will build resilience into *N. buxifolia* populations and likely reduce and mitigate the risks due to threatening processes.

Data on the ecological aspects of *N. buxifolia* are limited and using an incomplete knowledge base presents a challenge for undertaking an Ecological Risk Assessment. Whilst semi-quantitative and qualitative assessments are robust techniques and are useful when limited data is available (Fox and Burgman 2008), it was identified in Table 7-7 that scientific research is required for Risk classified as High or above ratings. This presents opportunities for targeted research to better inform management decisions. Priorities for research should be on Very High risks of climate change, fire and mining, followed by the High risks from exotics, grazing and habitat fragmentation.

Threatening processes are synergistic (Burgman *et al*. 2008) and removing or mitigating the very high and high Risks will also have flow-on effects and likely reduce the lower ranked risks. For example managing grazing by controlling stock access to riparian zones by fencing gathering points and establishing watering stations would reduce nitrogen inputs and the spread of exotic species in the riparian zones.

Whilst this ecological risk characterisation did not identify any extreme threats to *N. buxifolia* populations the threats from climate change, fire and mining were assessed as being very high. These threats have the potential to have a major influence on *N. buxifolia* potentially causing local extinctions. Combined with the high risk from exotic species, grazing and habitat fragmentation these threats highlight the need for management actions. Management actions will mitigate and control multiple threats to *N. buxifolia* populations.

Chapter 8 Conclusions and future research

This thesis studied the ecology of the central Queensland serpentine vegetation and determined that the structure and composition of the overlying vegetation can be used as a bio-indicator of the severity of the serpentine soils. In particular the relative basal area of the endemic overstorey tree *Corymbia xanthope* was negatively correlated with soil calcium status. The assemblages of the flora varied with the landform patterns reflecting differences in the soil chemistry. The species assemblages were greater on the mountains where there was more soil calcium and less extractable nickel compared to the other landform types. The overall species diversity decreased with the soil magnesium and there were more endemic and rare species on the hills reflecting high soil extractable nickel and high magnesium, skeletal soils and low soil moisture holding capacity. Species richness increased with soil boron supporting the theory that boron may be a more important influence on serpentine vegetation than previously considered (Sambatti and Rice 2007).

The herbaceous species were negatively influenced by the extractable nickel supporting the findings of Lazarus and Richards (2011), whereas the woody species were influenced by the soil Mg/Ca quotients supporting findings of Alexander (1988), Bauer (2011) and O'Dell (2006). The biomass of the woody shrub to small tree *Neoroepera buxifolia* is correlated most strongly to the soil nickel rather than with the soil Mg/Ca. The standing volume of *N. buxifolia* increased with soil extractable nickel and the height increased with the soil extractable nickel and to a lesser degree the Mg/Ca quotients. The standing volume of *N. buxifolia* was also greatest in the upper reaches of the 1st order stream channels where the concentrations of nickel were highest. *Neoroepera buxifolia* is associated with high concentrations of magnesium in the soil consistent with the observations of Batianoff *et al.* (2000) and tolerates very high Mg/Ca quotients of up to 11. *Neoroepera buxifolia* is able to maintain its foliage Mg/Ca quotients below 1 regardless of the soil Mg/Ca quotient. It is well adapted to the high concentrations of magnesium and within its niche variations in magnesium appear to have little influence on biomass. These results show that it is difficult to make generalisations about the functional groups of serpentine vegetation being influenced by either the soil Mg/Ca quotients or nickel.

Neoroepera buxifolia seeds have a high germination rate and no dormancy relieving techniques were required to improve germination rates. *Neoroepera buxifolia* was readily propagated using marcotting and ground layering techniques. The application of the hormone 3 IBA gel improved the development of the root ball. In comparison, the use of cuttings were not successful with low strike rates. However, this may have been because the mist house did not provide a desirable environment for rooting or the season cutting material was obtained.

Neoroepera buxifolia is recommended for phytostabilisation as it does not accumulate metals in its foliage, is a metallophyte and is readily propagated. The aims of phytostabilisation are to stabilise soils and reduce the risk of translocating metals into the environment or through the food chain. *Neoroepera buxifolia* is efficient at heavy metal regulation as the foliage bioaccumulation factors for the metals nickel and cobalt did not exceed a factor of 1. However, *N. buxifolia* has narrow habitat requirements and is restricted to the high soil moisture habitats such as creeks and channels. Thus it would be most suitable for phytostabilisation at tailing dams, drains and creeks within this serpentine landscape.

Further work is recommended to determine the effects of nickel and Mg/Ca quotients on germination, growth rates and survival of *N. buxifolia* seedlings. Serpentine plants are slow growing and more research is required to determine *N. buxifolia* growth rates and the survival of the marcotted and ground layered branches to completely assess its suitability for phytostabilisation and also improve conservation.

An Ecological Risk Assessment of threatening processes determined *N. buxifolia* populations are at risk from climate change, fire and mining and to a lesser extent exotic species, livestock grazing and habitat fragmentation. *Neoroepera buxifolia* has a naturally restricted distribution and management actions are needed to reduce or mitigate threatening processes. The challenge of undertaking an Ecological Risk Assessment for *N. buxifolia* was using incomplete data on ecological aspects thus highlighting the need for further scientific research.

8.1 Future research

8.1.1 Model system for climate change

Serpentine systems are useful as model systems for ecological studies (Damschen *et al.* 2011) and the central Queensland serpentine landscape would make an interesting model to study the effects of climate change on eucalypt woodlands and the dominance of ironbarks (*Eucalyptus* spp.) over the sub-dominant bloodwoods (*Corymbia* spp.) within the realized and fundamental niches of the species. Bloodwoods are more drought resistant as they have a deeper root systems and greater xylem pressure relative to ironbarks. Ironbarks allocate a greater biomass to above ground parts at the expense of an expansive root system compared to bloodwoods. Generally the faster growing ironbarks dominate, but if droughts become more frequent or more severe the slower growing bloodwoods could shift to dominate over time (Rice *et al*. 2004; Fensham and Fairfax 2007). Serpentine species do not have the potential for migration with changing climate. However, eucalypts have the potential for rapid evolutionary responses as hybridization is common in eucalypts and *Corymbia* (McKinnon *et al* 2004). A long-term study on the dominance of the eucalypt species using the basal area data from this thesis as the basis for measuring changes over time could be undertaken to assess the impacts of climate change on eucalypt woodlands.

8.1.2 Root-to-shoot exclusion mechanisms of *Neoroepera buxifolia*

Investigations into the root-to-shoot exclusion of metals in *N. buxifolia* could provide a useful insight into tolerance mechanisms of metallophytes. The mechanisms of metal exclusion in serpentine tolerant plants tend to be underplayed and research is often focussed on accumulation (Baker and Walker 1990). The mechanisms for metal tolerance vary between species and between metals (Baker and Walker 1990). The avoidance or restriction of uptake of trace elements is not a common feature in vascular plants and there is a tendency for plants to immobilise trace elements in the root tissue (Baker and Walker 1990). Metal tolerance may be associated with enhanced root accumulation and restriction of internal metal transport (Baker and Walker 1990). Plants may also have external mechanisms for metal tolerance by releasing exudates into the soil to chelate the metals (Pushenreiter *et al*. 2003; Martinez-Alcala *et al.* 2009). Mycorrhizal relationships with ericoid, vesicular (VAM) and ectomycorrhizal fungi also have functional roles in the exclusion of metals and uptake of nutrients in some species (Baker and Walker 1990; Schechter and Bruns 2008; Kidd *et al.* 2009). Given its extreme tolerance of high soil Mg/Ca quotients and high soil extractable nickel, *N. buxifolia* would be a useful candidate for further research into the mechanisms of metal tolerance.

References

Abensperg-Traun M G T, Smith G W, Arnold E,Stevens D (1996) The effects of habitat fragmentation and livestock-grazing on animal communities in remnants of gimlet Eucalyptus salubris woodland in the Western Australian wheatbelt. I. Arthropods. *Journal of Applied Ecology*, 1281-1301.

Abu-Zahra T R, Hasan M, Al-Shadaideh A N & Abubaker S (2011). Effect of different auxin concentrations on umbrella tree (*Schefflera arboricola*) rooting. *Agricultural Science Digest*, *31*(4), 312-315.

Agulló-Antón, M Á, Sánchez-Bravo J, Acosta M, & Druege U (2011). Auxins or sugars: what makes the difference in the adventitious rooting of stored carnation cuttings?. *Journal of Plant Growth Regulation*, *30*(1), 100-113.

Airy Shaw H (1980) A partial synopsis of the Euphorbiaceae-Platylobeae of Australia (excluding Phyllanthus, Euphorbia and Calycopeplus). *Kew Bulletin* **35**(3), 577-700.

Allen C D, Macalady A K, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Cobb N (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, ***259***(4), 660-684.

Alexander E B (1988) Morphology, fertility and classification of productive soils on serpentinized peridotite in California (USA). *Geoderma* **41**, 337–351. doi:10.1016/0016-7061(88)90069-9

Alloway B J (1995) Soil processes and the behaviour of trace elements. In 'Heavy metals in soils. (Ed. BJ Alloway) pp. 11-35. (Blackie Academic and Professional: Glasgow).

Amy J, Robertson A I (2001) Relationships between livestock management and the ecological condition of riparian habitats along an Australian floodplain river. *Journal of Applied Ecology* **38**(1), 63-75.

Aola (2014) Atlas of Living Australia, Occurrence records map for *Olearia macdonnellensis* D. A. Cooke, http://www.bie.ala.org.au/species/oleria+macdonnellensis#. accessed Dec. 2014.

Aristizábal E, Roser B, Yokota S (2005) Tropical chemical weathering of hillslope deposits and bedrock source in the Aburrá Valley, northern Colombian Andes. *Engineering Geology* **81**(4), 389-406.

Ashwath N (2002). Rare and threatened species of Marlborough nickel project, Marlborough. Marlborough Serpentine, landscape management and conservation, Rockhampton, Centre for Environmental Management, Central Queensland University.

Asemaneh T, Ghaderian S M, Baker A J M (2007) Responses to Mg/Ca balance in an Iranian serpentine endemic plant, *Cleome heratensis* (Capparaceae) and a related non-serpentine species, *C. foliolosa*. *Plant Soil* **293**, 49-59.

Auble G T, Friedman J M, Scott M L (1994) Relating riparian vegetation to present and future streamflows. *Ecological Applications* **4**(3), 544-554.

Batianoff G N, Singh S (2001) Central Queensland serpentine landforms, plant ecology and endemism. *South African Journal of Science* **97**, 495.

Batianoff G N, Neldner V J, Singh S (2000) Vascular plant census and floristic analysis of serpentine landscapes in Central Queensland. *Proceedings of the Royal Society of Queensland* **109**, 1-30.

Batianoff G N, Reeves R D, Specht R L (1997) The effects of serpentine on vegetation structure, species diversity and endemism in Central Queensland. In 'The Ecology of Ultramafic and Metalliferous Areas. (Eds T Jaffré, R D Reeves and T Becquer) pp. 147-154. (Centre ORSTOM: Noumea, New Caledonia).

Baker A J M (1981) Accumulators and excluders - strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**(1/4), 643-654.

Baker A J M, Walker P L (1990) Ecophysiology of metal uptake by tolerant plants. In 'Heavy metal tolerance in plants: evolutionary aspects. (Ed. A J Shaw). (CRC Press Inc.: Boca Raton, Florida; USA).

Balabushka V K (1985). Stimulation of vegetative propagation of introduced woody species with growth powder and honey. *Lesnoi Zhurnal* **6**: 118-119.

Bansal R, Bhati P, Sen D (1980) Differential specificity in water imbibition of Indian arid zone seeds. *Biologia Plantarum* **22**(5), 327-331.

Basic N, Keller C, Fontanillas P, Vittoz P, Besnard G, Galland N (2006) Cadmium hyperaccumulation and reproductive traits in natural *Thlaspi caerulescens* populations. *Plant Biology (Stuttgart)* **8**(1), 64-72.

Baskin C C, Baskin J M (1998a) Chapter 2 - Ecologically Meaningful Germination Studies. In 'Seeds. (Eds CC Baskin and JM Baskin) pp. 5-26. (Academic Press: San Diego).

Baskin C C, Baskin J M (1998b) 'Seeds: ecology, biogeography, and evolution of dormancy and germination.' (Academic Press San Diego).

Batianoff G N, Neldner V J, Singh S (2000) Vascular plant census and floristic analysis of serpentine landscapes in Central Queensland. *proceedings of the Royal Society of Queensland* **109**, 1-30.

Batianoff G N, Reeves R D, Specht R L (1990) *Stackhousia tryonii* Bailey: a nickel-accumulating serpentinite-endemic species of central Queensland. *Australian Journal of Botany* **38**, 121-130.

Batianoff G N, Singh S (2001) Central Queensland serpentine landforms, plant ecology and endemism. *South African Journal of Science* **97**(11/12), 495.

Batianoff G N, Specht R L (1991) Queensland (Australia) serpentine vegetation. In 'The Vegetation of Ultramafic (Serpentine) Soils, Proceedings from the First International conference on Serpentine Ecology. (Eds A J M Baker, J Proctor and R D Reeves) pp. 109 - 128. (Intercept Ltd: Andover, Hampshire, United Kingdom).

Bauer P, Elbaum R, Weiss I M (2011) Calcium and silicon mineralization in land plants: transport, structure and function. *Plant Science* **180**, 746–756. doi:10.1016/j.plantsci.2011.01.019

Bell D, Plummer J, Taylor S (1993a) Seed germination ecology in southwestern Western Australia. *Botanical Review* **59**(1), 24-73.

Bell L C. (2001). Establishment of native ecosystems after mining — Australian experience across diverse biogeographic zones. *Ecological Engineering* **17**(2–3): 179-186.

Bever J (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil* **244**(1-2), 281-290.

Bhatia N P (2003) Ecophysiology of nickel hyperaccumulation in *Stackhousia tryonii* Bailey. PhD Thesis, Central Queensland University, Rockhampton

Bhatia N P, Baker A J M, Walsh K B, Midmore D J (2005) A role for nickel in osmotic adjustment in drought-stressed plants of the nickel hyperaccumulator *Stackhousia tryonii* Bailey *Planta* **223**(1), 1432-2048.

Bhatia N P, Bhatia P, Ashwath N (2002) Asexual propagation of *Stackhousia tryonii*: a step towards restoration of a rare metallophyte. *Australian Journal of Botany* **50**, 577-582.

Bidwell S D (2000). Hyperaccumulation of metals in Australian native plants. PhD thesis, The University of Melbourne.

Blakesley D, Weston G, Elliott M (1991) Endogeneous levels of indole-3-acetic acid and abscisic acid during the rooting of Cotinus coggygria cuttings taken at different times of the year. *Plant growth regulation* **10**(1), 1-12.

BOM (2014) Bureau of Meterology Climate data for Rockhampton. http://www.bom.gov.au/climate. Date accessed 2014.

BOM (2009) Bureau of Meterology Climate data for Rockhampton. http://www.bom.gov.au/climate. Date accessed 2009.

Borhidi A (1992) The serpentine flora and vegetation of Cuba. In 'The vegetation of ultramafic (serpentine) soils. (Eds AJM Baker, J Proctor and RD Reeves) pp. 83-96. (Intercept: Hampshire).

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore, De Vries W (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications*, **20**(1), 30-59.

Booth C (2011) Myrtle Rust: how big a threat to native plants? *ECOS* **2011**(162).

Boulet F (2003) Mycorrhizal symbiosis as a strategy for survival in ultramafic soils. James Cook University.

Bowman D M J S (2000) 'Australian Rainforests, islands of green in a land of fire.' Cambridge University Press, UK. .

Boyd R S (2007) The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. *Plant Soil* **7**, 153–176.

Boyd R S, Moar W J (1999) The defensive function of Ni in plants: response of the polyphagous herbivore *Spodoptera exigua* (Lepidoptera: Noctuidae) to hyperaccumulator and accumulator species of *Streptanthus* (Brassicaceae). *Oecologia* **118**(2), 218-224.

Bradshaw AD,Chadwick MJ (1980). The Restoration of Land, Blackwell Scientific Publication.

Bradshaw A (1997) Restoration of mined lands – using natural processes. *Ecological Engineering* **8**, 255–269. doi:10.1016/S0925-8574(97)00022-0

Brady K U, Kruckeberg A R, Bradshaw Jr H D (2005) Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics* **36**, 243-266.

Braithwaite L W, Turner J, Kelly J (1984) Studies on the arboreal marsupial fauna of eucalypt forests being harvested for woodpulp at Eden, N.S.W. III. Relationships between faunal densities, eucalypt occurrence and foliage nutrients and soil parent materials. *Australian. Wildlife Research.* **11**, 41-48.

Brooks R R (1987) 'Serpentine and its Vegetation.' (Dioscorides Press: Portland, Oregon).

Brooks R R, Lee J, Jaffré T (1974) Some New Zealand and New Caledonian plant accumulators of nickel. *Journal of Ecology* **62**, 493–499. doi:10.2307/2258995

Bruce M C, Niu Y (2000) Evidence for Palaeozoic magmatism recorded in the Late Neoproterozoic Marlborough ophiolite, New England Fold Belt, central Queensland. *Australian Journal of Earth Sciences* **47**, 1065–1076. doi:10.1046/j.1440-0952.2000.00834.x

Bruce M, Niu Y, Harbort T, Holcombe R (1998) Geochemistry and geochronology of the Marlborough ophiolite: implications for the tectonic history of the northern New England fold belt. Geological Society of Australia Abstracts **49**, 58.

Bruce M C, Niu Y, Harbort T A, Holcombe R J (2000) Petrological, geochemical and geochronological evidence for a Neoproterozoic ocean basin recorded in the Marlborough terrane of the northern New England Fold Belt. *Australian Journal of Earth Sciences* **47**(6), 1053 - 1064.

Bunn S E, Arthington A H (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* **30**(4), 492-507.

Burgman M, Keith D, Hopper S, Widyatmoko D, Drill C (2007) Threat syndromes and conservation of the Australian flora. *Biological Conservation* **134**(1), 73-82.

Callahan D L (2007) The coordination of nickel in hyperaccumulating plants. PhD Thesis, University of Melbourne, Melbourne

Calvert M, Simpson J, Adsett K (2000) Land use mapping of the Fitzroy Catchment. *The State of Queensland, Department of Natural Resources, The State of Queensland, Department of Natural Resources, Coorparoo, Qlnd*.

Campbell M L, Hewitt C L (2008) Introduced marine species risk assessment–aquaculture. *Understanding and applying risk analysis in aquaculture. FAO Fisheries and Aquaculture Technical Paper* **519**, 121-133.

Casper B B, Bentivenga S P, Ji B, Doherty J H, Edenborn H M, Gustafson D J (2008) Plant-soil feedback: Testing the generality with the same grasses in serpentine and prairie soils. *Ecology* **89**(8), 2154-2164.

Casper B B, Castelli J P (2007) Evaluating plant–soil feedback together with competition in a serpentine grassland. *Ecology Letters* **10**(5), 394-400.

Chapin S F (1980) The mineral nutrition of wild plants. *Annual Review of Ecology, Evolution, and Systematics* (11), 233-260.

Chiarucci A, Robinson B H, Bonini I, Petit D, Brooks R R, De Dominicis V (1998) Vegetation of Tuscan ultramafic soils in relation to edaphic and physical factors. *Folia Geobotanica* **33**, 113–131. doi:10.1007/BF02913340

Choat B, Jansen S, Brodribb T J, Cochard H, Delzon S, Bhaskar Zanne A E (2012). Global convergence in the vulnerability of forests to drought. *Nature*, ***491***(7426), 752-755.

Chen C, Huang D, Liu J (2009) Functions and toxicity of nickel in plants: Recent advances and future prospects. *Clean* **37**(4-5), 304-313.

Clarke K R, Gorely R N (2006) 'PRIMER v6: user manual/tutorial.' (PRIMER-E: Plymouth, UK)

Clarke K R, Warwick R M (2001) 'Change in marine communities: an approach to statistical analysis and interpretation.' 2nd edn. (PRIMER-E: Plymouth, UK)

Cochrane A, Kelly A, Brown K, Cunneen S (2002) Relationships between seed germination requirements and ecophysiological characteristics aid the recovery of threatened native plant species in Western Australia. *Ecological Management and Restoration* **3**(1), 47-60.

Coleman R G, Jove C (1992) Geological origin of serpentines. In 'The vegetation of ultramafic (serpentine) soils. (Eds AJM Baker, J.Proctor and R.D.Reeves) pp. 1-17. (Intercept: Hampshire).

Coolbear P, Francis A, Grierson D (1984) The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany* **35**(11), 1609-1617.

Cooke J, Leishman M R (2011) Is plant ecology more siliceous than we realise? *Trends in Plant Science* **16**, 61–68. doi:10.1016/j.tplants.2010.10.003

Cooke J, Johnson M (2002) Ecological restoration of land with particular reference to the mining of metals and industrial minerals: A review of theory and practice. *Environmental Reviews* **10**(1), 41-71.

Cornara L, Roccotiello E, Minganti V, Drava G, Pellegrine R D, Mariotti M G (2007) Level of trace elements in Pteridophytes growing on serpentine and metalliferous soils. *Journal of Plant Nutrition and Soil Science* **170**, 781–787. doi:10.1002/jpln.200720099

Crooke W M (1956) Effect of soil reaction on uptake of nickel from a serpentine soil. *Soil Science* **81**, 269–276. doi:10.1097/00010694-195604000-00003

da Costa C T, de Almeida M R, Ruedell C M, Schwambach J, Maraschin F S,Fett-Neto A G (2013). When stress and development go hand in hand: main hormonal controls of adventitious rooting in cuttings. *Frontiers in Plant Science*, **4,** 144.

DAFF (2014) Declared plants of Queensland, Department of Agriculture, Fisheries and Forestry, 2010-2014, https://www.daff.qld.gov.au/plants/weeds-pest-animals-ants/weeds/declared-plants. Accessed Dec 2014.

DAFF (2014) Department of Agriculture, Fisheries and Forestry, Queensland Government. Myrtle rust, https://www.daff.qld.gov.au/plants/health-pests-diseases/a-z-significant/myrtle-rust. Accessed Dec. 2014.

Damschen E I, Harrison S, Going B M, Anacker B L (2011) Climate change and plant communities on unusual soils. In: *Serpentine: The Evolution and Ecology of a Model System,* 359-381. Eds, Harrison, S. & Rajakaruna, N. University of California Press

Danehloueipour N, Yan G, Clarke H J, Siddique K H M (2006) Successful stem cutting propagation of chickpea, its wild relatives and their interspecific hybrids. *Animal Production Science*, **46**(10), 1349-1354.

Davies K F, Harrison S, Safford H D, Viers JH (2007) Productivity alters the scale dependence of the diversity-invasibility relationship. *Ecology* **88**(8), 1940-1947.

Day J S Loveys B R (1998) Propagation from cuttings of two woody ornamental Australian shrubs, *Boronia megastigma* Nees (brown boronia) and *Hypocalymma* *angustifolium* Endl. (white myrtle). *Australian Journal of Experimental Agriculture* **38**: 201-206.

de Almeida M R, Ruedell C M (2010). Reference gene selection for quantitative reverse transcription-polymerase chain reaction normalization during in vitro adventitious rooting in *Eucalyptus globulus* Labill. *BMC Molecular Biology* **11**: 73-73

DeGrood S H, Claassen V P, Scow K M (2005) Microbial community composition on native and drastically disturbed serpentine soils. *Soil biology and Biochemistry* **37**, 1427-1435.

De Klerk G J, Ter Brugge J, Marinova S (1997). Effectiveness of indoleacetic acid, indolebutyric acid and naphthaleneacetic acid during adventitious root formation in vitro in *Malus* 'Jork 9'. *Plant Cell, Tissue and Organ Culture*, **49**(1), 39-44.

De Klerk G J, Keppel M, Ter Brugge J, Meekes H (1995). Timing of the phases in adventitous root formation in apple microcuttings. *Journal of Experimental Botany*, **46**(8), 965-972.

Demchenko N P, Kalimova I B, Demchenko K N (2005) Effect of nickel on growth, proliferation, and differentiation of root cells in *Triticum aestivum* seedlings. *Russian Journal of Plant Physiology* **52**(2), 220-228.

Denaxa N-K, Vemmos S N, Roussos P A (2012) The role of endogenous carbohydrates and seasonal variation in rooting ability of cuttings of an easy and a hard to root olive cultivars (*Olea europaea* L.). *Scientia Horticulturae* **143**, 19-28.

Department of Environment (2012) Weeds of National Signficance In. Vol. 2014. Ed. AF Government))

Department of Environment and Heritage Protection (2014) Management Plan for protected plants in Queensland 2014-19, Nature conservation act 1992. *Queensland Government,* http://environment.gov.au/system/files/resources.Accessed,1 December 2014.

Department of Environment (2014a) *Neoroepera buxifolia* in species profiles and threats database. *Department of Environment, Canberra,* available from*:* http://www.environment.gov.au/sprat. Accessed Mon, 1 Dec 2014.

Department of Environment (2014b). Ecological Risk Assessment. *Australian Government*, http://www.environment.gov.au/science/supervising-scientist/research/ecological-risk. Accessed Dec. 2014.

Doncheva S, Poschenrieder C, Stoyanova Z, Georgieva K, Velichkova M, Barceló J (2009) Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties. *Environmental and Experimental Botany* **65**(2–3), 189-197.

DNRM (2014) Department of Natural Resources and Mines Queensland State Government Mapping and Data https://www.dnrm.qld.gov.au/mapping. Accessed Aug. 2014.

Duggin J, Gentle C (1998) Experimental evidence on the importance of disturbance intensity for invasion of *Lantana camara* L. in dry rainforest–open forest ecotones in north-eastern NSW, Australia. *Forest Ecology and Management* **109**(1), 279-292.

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant-soil system. *Annual Review of Environment and Resources.* **30**, 75-115.

EPA (2007) 'Regional ecosystem description database (REDD). Version 5.2.' (Ed. DmbQ Herbarium) (Environmental Protection Agency: Brisbane.)

EPA (2009) 'Copy of the certified regional ecosystem map for the purpose of Vegetation Management Act 1999.' Online REDATA. (Environmental Protection Agency: Brisbane) Available at http://www.epa.qld.gov.au/REDATA. Accessed July 2009

EPA (2010) 'Wildlife online defined area search for latitude 22.63–23.13 and longitude 149.77–150.44.' (Environmental Protection Agency: Brisbane) Available at http://www.epa.qld.gov.au/wildlife/wildlife-online. Accessed August 2010

Epstein E (1999) SILICON. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**(1), 641-664.

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, **34** 487-515.

Farooq M, Basra S, Ahmad N, Hafeez K (2005) Thermal hardening: a new seed vigor enhancement tool in rice. *Journal of Integrative Plant Biology* **47**(2), 187-193.

Fensham, R. Fairfax R. (2007). Drought-related tree death of savanna eucalypts: Species susceptibility, soil conditions and root architecture. *Journal of Vegetation Science* **18**(1): 71-80.

Fensham R, Skull S (1999) Before Cattle: A Comparative Floristic Study of *Eucalyptus* Savanna Grazed by Macropods and Cattle in North Queensland, Australia. *Biotropica* **31**(1), 37-47.

Forster B A, Baker D E (2002) Soils of the Marlborough serpentinite landscape of Central Queensland In 'Marlborough serpentine landscape management and conservation', 2002, Rockhampton. Eds A Exelby, K Wormington and A Melzer).

Forster B A, Baker D E (1995) Characterisation of the serpentinite soils of central Queensland, Australia. In 'Second international conference of serpentine ecology', Nouméa, New Caledonia.

Foster L, Eggleton R (2002) The Marlborough Nickel Laterite Deposits. In 'Regolith and Landscapes in Eastern Australia. (Ed. IC Roach) pp. 33-36. (CRC LEME).

Foulds W (1993) Nutrient concentrations of foliage and soil in South-western Australia. *New Phytologist* **125**, 529-546.

Fox D R,Burgman M (2008). Ecological risk assessment.In Encyclopedia of Quantitative Risk Analysis and Assessment.

Freeland W J (1990) Large herbivorous mammals: exotic species in northern Australia. *Journal of Biogeography* **17**(445-449).

Friedland A J (1989) The movement of metals through soils and ecosystems. In Heavy metal tolerance in plants: Evolutionary aspects. (Ed. AJ Shaw). (CRC: Boca Raton).

Field C B, Jackson R B, Mooney H A (1995) Stomatal responses to increased CO2: implications from the plant to the global scale. *Plant, Cell and Environment* **18**, 1214-1225.

Gabbrielli P, Pandolfini T, Vergnano O, Palandri M R (1990). Comparison of two serpentine species with different nickel tolerance strategies. *Plant and Soil*, **122**(2), 271-277.

Galloway, J N, Dentener, F J, Capone, D G, Boyer, E W, Howarth, R W, Seitzinger, S P, Asner G P. Cleveland C C, Green P A, Holland E A, Karl D M, Michaels A F, Porter J H, Townsend A R, Vöosmarty C J (2004)Nitrogen cycles: Past, present, and future *Biogeochemistry* **70**(2) 153-226.

Geoscience Australia (2014) Landslide search, http://www.ga.gov.au/landslides-web/landslips.htm; area of interest lat. -22.8255 long.149.8342 and lat. -23.4821 and long. 150.8065; Accessed 1/12/14.

GHD (2010) Lower Fitzroy River Infrastructure Project, initial advice statement

Ghasemi R, Ghaderian S M (2009) Responses of two populations of an Iranian nickel-hyperaccumulating serpentine plant, *Alyssum inflatum* Nyar., to substrate Ca/Mg quotient and nickel. *Environmental and Experimental Botany* **67**(1), 260-268.

Grace J B, SaVord H D, Harrison S (2007) Large-scale causes of variation in the serpentine vegetation of California. *Plant Soil* **7**, 121-132.

Grime P J (1979) Plant Strategies and Vegetation Processes. *Wiley*, *New York*

Gutterman Y, Shem-Tov S (1997) The efficiency of the strategy of mucilaginous seeds of some common annuals of the Negev adhering to the soil crust to delay collection by ants. *Israel Journal of Plant Sciences* **45**(4), 317-327.

Goodwin-Bailey C I, Woodell S R J, Loughman B C (1992) The reponse of serpentine, mine spoil and saltmarsh races of *Armeria maritima* (Mill.) Willd. to each others' soil. In 'The vegetation of ultramafic (serpentine) soils. (Eds AJM Baker, J Proctor and RD Reeves) pp. 375-390. (Intercept Limited: Andover, UK).

Grice T (2004) Weed ecology and dynamics. *Healthy Rangelands: Principles for Sustainable Systems,* Tropical Savannas CRC, Darwin, 115-45.

Haissig B E (1989). Removal of the stem terminal and application of auxin change carbohydrates in Pinus banksiana cuttings during propagation. *Physiologia Plantarum* **77**(2): 179-184

Hansen E M (1999) Disease and diversity in forest ecosystems. *Australasian Plant Pathology* **28**(4), 313-319.

Hartmann H T (2014). Hartmann & Kester's Plant Propagation Principles and Practices, Pearson.

Harrison S (1999) Local and regional diversity in a patchy landscape: native, alien, and endemic herbs on serpentine. *Ecology* **80**, 70–80. doi:10.1890/0012-9658(1999)080[0070:LARDIA]2.0.CO;2

Harrison S, Rajakaruna N (2011) 'Serpentine: the evolution and ecology of a model system.', Univ of California Press.

Harrison S, Safford H D, Grace J B, Viers J H, Davies K F (2006) Regional and local species richness in an insular environment: Serpentine plants in California. *Ecological Monographs* **76**(1), 41-56.

Henderson R J F (1992) Studies in Euphorbiaceae A.L.Juss., Sens. Lat.2*. A revision of *Neoroepera* Muell. Arg. & F. Muell. (Oldfieldioideae Kohler & Webster, Caletiteae Muell. Arc.) *Austrobaileya* **3**(4), 615-625.

Hendry R A, Wormington K R (2014) Eucalypt forests as indicators of the gradients within the central Queensland serpentine landscape of Australia. *Australian Journal of Botany* **61**(7), 544-551.

Hendrickx M (2009) Naturally occurring asbestos in eastern Australia: a review of geological occurrence, disturbance and mesothelioma risk. *Environmental Geology* **57**(4), 909-926.

Hobbs R J, Yates C J (2003) Turner Review No. 7. Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. *Australian Journal of Botany* **51**(5), 471-488.

Hobbie S E, Gough L (2002) Foliar and soil nutrients in tundra on glacial landscapes of contrasting ages in northern AlaskaO. *Oecologia* **131**, 453-462.

Hutchinson T C (1981) Nickel. In 'Effect of heavy metal pollution on plants. (Ed. NW Lepp) pp. 171-211. (Applied Science Publishers Ltd: Liverpool).

IBM Corp. Released (2010) IBM SPSS Statistics for Windows, Version 19.0. Armonk NY: IBM Corp

Iturralde R B (2001) The influence of ultramafic soils on plants in Cuba. *South African Journal of Science* **97**, 510-512.

Jackes B R (2005) Revision of Myrsine (Myrsinaceae) in Australia. *Australian Systematic Botany* **18**(5), 399-438.

Jaffré T, Rigault F (1989) Recherches sur les possibilités d'implantation végétale sur sites miniers. In 'Rapport final de la convention SLN-ORSTOM'

Jouquet P, Dauber J, Lagerlöf J, Lavelle P, Lepage M (2006) Soil invertebrates as ecosystem engineers: intended and accidental effects on soil and feedback loops. *Applied Soil Ecology* **32**(2), 153-164.

Jones J B (2001) ‘Laboratory guide for conducting soil tests and plant analysis.’ (CRC Press LLC: New York)

Kabata-Pendias A (2004). Soil–plant transfer of trace elements—an environmental issue. *Geoderma* **122**(2–4): 143-149.

Kazakou E, Dimitrakopoulos P G, Baker A JM, Reeves R D, Troumbis A Y (2008) Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. *Biological Reviews* **83**(4), 495-508.

Khan M, Niazi Z, Khan D (1988) Rooting of different types of guava stem cuttings using growth regulator. *Pakistan Journal of Agricultural Research* **9**(3), 363-365.

Kidd, P, Barceló, J, Bernal, M P, Navari-Izzo, F, Poschenrieder, C, Shilev, S, Monterroso, C. (2009). Trace element behaviour at the root–soil interface: implications in phytoremediation. *Environmental and Experimental Botany*, **67**(1), 243-259.

King D J, Doronila A I, Feenstra C, Baker A J, Woodrow I E (2008) Phytostabilisation of arsenical gold mine tailings using four *Eucalyptus* species: Growth, arsenic uptake and availability after five years. *Science of the total environment* **406**(1), 35-42

Kinraide T B (1994) Use of a Gouy-Chapman-Stern model for membrane-surface electrical potential to interpret some features of mineral rhizotoxicity *Plant Physiology* **106**, 1538-1592.

Kriticos D J, Morin L, Leriche A, Anderson R C, Caley P (2013) Combining a climatic niche model of an invasive fungus with its host species distributions to identify risks to natural assets: Puccinia psidii sensu lato in Australia. *PLoS ONE* **8**(5), e64479.

Kruckeberg A R (1954) The ecology of serpentine soils: A symposium. III. Plant species in relation to serpentine soils. . *Ecology* **35**, 267-274.

Kruckeberg A R (1992) Serpentine biota of Western North America. In 'The vegetation of ultramafic (Serpentine) soils. (Eds AJM Baker, J Proctor and RD Reeves) pp. 19-33. (Intercept: Hampshire).

Kulmatiski A, Beard K H, Stevens J R, Cobbold S M (2008) Plant–soil feedbacks: a meta-analytical review. *Ecology Letters* **11**(9), 980-992.

Lambert M J, Turner J (1983) Soil nutrient-vegetation relationships in the Eden area, N.S.W. III. Foliage nutrient relationships with particular reference to *Eucalyptus* subgenera. . *Austalian Forestry* **46**, 200-209.

Lazarus B, Richards J, Claassen V, O'Dell R, Ferrell M (2011) Species specific plant-soil interactions influence plant distribution on serpentine soils. *Plant & Soil* **342**(1/2), 327-344.

Leakey R R B., Mesén J F T, Tchoundjeu Z, Longman, K A, Newton A. Dick J McP, Matin A, Grace J, Munro R C, and Muthoka P N. (1990) Low-technology techniques for the vegetative propagation of tropical trees. ***Commonwealth*** *Forestry Institute* **69**, no. 3: 247-257.

Luo Y, Chen H Y H (2011) Competition, species interaction and ageing control tree mortality in boreal forests. *Journal of Ecology* **99**, 1470–1480. doi:10.1111/j.1365-2745.2011.01882.x

Lunt I D, Eldridge D J, Morgan J W, Witt G B (2007) Turner Review No. 13. A framework to predict the effects of livestock grazing and grazing exclusion on conservation values in natural ecosystems in Australia. *Australian Journal of Botany* **55**(4), 401-415.

Lyon G L, Peterson P J, Brooks R R, Butler G W (1971*a*) Calcium, magnesium and trace elements in a New Zealand serpentine. *Journal of Ecology* **59**, 421–429. doi:10.2307/2258322

Lyon G L, Peterson P J, Brooks R R, Butler G W (1971*b*) Calcium, magnesium and trace elements in a New Zealand serpentine flora. *Journal of Ecology* **59**, 421–429. doi:10.2307/2258322

Mac Nally R, Soderquist T R, Tzaros C (2000) The conservation value of mesic gullies in dry forest landscapes: avian assemblages in the box–ironbark ecosystem of southern Australia. *Biological Conservation* **93**(3), 293-302.

Martens S N, Boyd R S (1994) The ecological significance of nickel hyperaccumulation: a plant chemical defense. *Oecologia* **98**(3-4), 379-384.

Martínez-Alcalá I, Clemente R, Bernal M P (2009). Metal availability and chemical properties in the rhizosphere of *Lupinus albus* L. growing in a high-metal calcareous soil. *Water, Air, and Soil Pollution*, **201**(1-4), 283-293.

McCarten N F (1986) Ecology of the Serpentine vegetation in the San Francisco Bay region. In 'Conservation and management of rare and endangered plants', 1986, California. (Ed. TS Elias), pp. 335-340

McColl J G, Humphreys F R (1967) Relationships between some nutritional factors and the distributions of *Eucalyptus gummifera* and *Eucalyptus maculata*. *Ecological Society of America* **48**(5), 766-771.

Melzer R (2004) ‘A system for the collection of site data.’ (Environmental Protection Agency: Brisbane)

Melo J, D’souza S (2004) Removal of chromium by mucilaginous seeds of *Ocimum basilicum*. *Bioresource technology* **92**(2), 151-155.

Mendez M, Maier R (2008a) Phytoremediation of mine tailings in temperate and arid environments. *Reviews in Environmental Science and Bio/Technology* **7**(1), 47-59.

Mendez M O, Maier R M (2008b) Phytostabilization of mine tailings in arid and semiarid environments-an emerging remediation technology. *Environmental health perspectives* **116**(3), 278.

Mertens J, Vervaeke P, Schrijver A D, Luyssaert S (2004). Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation. *Science of the Total Environment*, **326**(1), 209-215.

McGrath S P, Zhao F J (2003). Phytoextraction of metals and metalloids from contaminated soils *Current Opinion in Biotechnology* **14**: 277-282.

Miranda M, Benedito J, Blanco-Penedo I, López-Lamas C, Merino A, Lopez-Alonso M (2009) Metal accumulation in cattle raised in a serpentine-soil area: Relationship between metal concentrations in soil, forage and animal tissues. *Journal of Trace Elements in Medicine and Biology* **23**(3), 231-238.

Morris C, Grossl P R, Call C A (2009) Elemental allelopathy: processes, progress, and pitfalls. *Plant Ecology* **202**, 1-11.

Murphy, B P, Bradstock, R A, Boer M M, Carter, J, Cary, G J, Cochrane, M A, Fensham, R J, Russell-Smith, J, Williamson, G J, Bowman D M J S (2013) Fire regimes of Australia: a pyrogeographic model system. *Journal of Biogeography* **40**(6): 1048-1058.

Murray C G (1969) The petrology of the ultramafic rocks of the Rockhampton District, Queensland. *Geological Survey of Queensland* **343**.

Murray C G (2007) Devonian supra-subduction zone setting for the Princhester and Northumberland serpentinites: implications for the tectonic evolution of the northern New England Orogen. *Australian Journal of Earth Sciences* **54**, 899–925. doi:10.1080/08120090701392747

Murray C G, Blake P R (2005) Geochemical discrimination of tectonic setting for Devonian basalts of the Yarrol Province of the New England Orogen, central coastal Queensland: An empirical approach. *Australian Journal of Earth Sciences* **52**(6), 993 - 1034.

Naiman R J, Fetherston K L, McKay S J, Chen J (1998) Riparian forests. River ecology and management: lessons from the Pacific coastal ecoregion. Springer-Verlag, New York, 289-323.

Nagy L, Proctor J (1997) Plant growth and reproduction on a toxic alpine ultramafic soil: adaptation to nutrient limitation. *New Phytologist* **37**(267-274).

Neave I A, Davey S M, Russell-Smith J J, Florence R G (1995) The relationship between vegetation patterns and environment on the south coast of New South Wales. *Forest ecology and management* **72**, 71-80.

Neff J C, Holland E A, Dentener F J, McDowell W H, Russell K M (2002) The origin, composition and rates of organic nitrogen deposition: a missing piece of the nitrogen cycle? *Biogeochemistry* **57**(1), 99-136.

Neldner V J, Wilson B A, Thompson E J, Dillewaard H A (2005) 'Methodolgy for survey and mapping of regional ecosystems and vegetation communities version 3.1.' (Queensland Herbarium, Enviornmental Protection Agency: Brisbane).

Nilsson C, Berggren K (2000) Alterations of Riparian Ecosystems Caused by River Regulation. *BioScience* **50**(9), 783.

Nkoane B B M, Sawula G M, Wibetoe G, Lund W (2005) Identification of Cu and Ni indicator plants from mineralised locations in Botswana. *Journal of Geochemical Exploration* **86**(3), 130-142

O'Dell R E, Claassen V P (2006) Relative performance of native and exotic grass species in response to amendment of drastically disturbed serpentine substrates. *Journal of Applied Ecology* **43**(5), 898-908.

O'Dell R E, Claassen V P (2009) Serpentine Revegetation: A review. *Northeastern Naturalist* **16**(Special volume 5: Soil and Biota of Serpentine: A world view), 253-271.

O'Dell R E, Claassen V P (2011) Restoration and revegetation of harsh soils. *Serpentine: The Evolution and Ecology of a Model System*, 383-413.

O'Dell R E, James J J, Richards J H (2006) Congeneric serpentine and non-serpentine shrubs differ more in leaf Ca : Mg than in tolerance of low N, low P, or heavy metals. *Plant and Soil* **280**, 49–64. doi:10.1007/s11104-005-3502-y

Ofori, D, Newton, A C, Leakey, R R B Grace J. (1997). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of maturation, coppicing, cutting length and position on rooting ability. *Journal of Tropical Forest Science*, **10**(1), 115-129.

Padmavathiamma P K, Li LY (2009) Phytoremediation of metal contaminated soil in temperate humid regions of British Columbia, Canada. *International Journal of Phytoremediation* **11**(6), 575-590.

Palmer M A, Reidy Liermann C A, Nilsson C, Flörke M, Alcamo J, Lake P S, Bond N (2008) Climate change and the world's river basins: anticipating management options. *Frontiers in Ecology and the Environment* **6**(2), 81-89.

Palomino M, Kennedy P G, Simms E L (2007) Nickel hyperaccululation as an anti-herbivore trait: considering the role of tolerance to damage. *Plant soil* **293**, 189-195.

Peters J (2000) Tetrazolium testing handbook. *Association of Official Seed Analysts* (29).

Pernilla Brinkman E, Van der Putten W H, Bakker E J, Verhoeven K J (2010) Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology* **98**(5), 1063-1073.

Phipps D A (1981) Chemistry and biochemistry of trace metals in biological systems. In *Effects of heavy metal pollution on plants* (1-54). Springer Netherlands.

Phipps D A (1976) Metals and Metabolism. Oxford University Press, Oxford.

Pop, TIPamfil D (2011). Auxin Control in the Formation of Adventitious Roots. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **39**(1): 307-316.

Potts W, Barker P (1999) *Tetratheca gunnii* Recovery Plan 2001-2005. Department of Primary Industries, Water and Environment, Hobart.

Prasad, M N V, Freitas H M D O (1999). Feasible biotechnological and bioremediation strategies for serpentine soils and mine spoils. *Electronic Journal of Biotechnology* **2**(1): 20-34.

Proctor J, McGowan I (1976) Influence of magnesium on nickel toxicity. *Nature* **260** 176:234.

Proctor J, Nagy L (Eds) (1992) 'Ultramafic rocks and their vegetation: an overview.' The vegetation of ultramafic (serpentine soils).Intercept Limited, Andover.

Price J N, Morgan J W (2010) Small-scale patterns of species richness and floristic composition in relation to microsite variation in herb-rich woodlands. *Australian Journal of Botany* **58**, 271–279.

Puschenreiter, M, Wieczorek, S, Horak, O, Wenzel, W W. (2003). Chemical changes in the rhizosphere of metal hyperaccumulator and excluder *Thlaspi* species. *Journal of Plant Nutrition and Soil Science*, **166**(5), 579-584.

Pulford I D, Watson C (2003). Phytoremediation of heavy metal-contaminated land by trees—a review. *Environment International* **29**(4): 529-540.

Rayment G E, Lyons D J (2010) ‘Soil chemical methods – Australasia’,CSIRO Publishing: Melbourne.

Reeves R D (2003) Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant and Soil* **249**, 57–65. doi:10.1023/A:1022572517197

Reeves R D (2002) Metal uptake by the serpentine plants of Queensland. In 'Marlborough serpentine, landscape managment and conservation. ' (Eds A Exelby, K Wormington and A Melzer) pp. 92-101. (Central Queensland University Unit: Rockhampton)

Russell J S (1970) Plant growth on a low calcium status solodic soil in a subtropical environment. *Australian Journal of Agricultural Research* **21**, 407–425. doi:10.1071/AR9700407

Robinson B H, Brooks R R, Kirkman J H, Gregg P E, Gremigni P (1996) Plant-available elements in soils and their influence on the vegetation over ultramafic ("serpentine") rocks in New Zealand. *Journal of the Royal Society of New Zealand* **26**(4), 457-468.

Sambatti J B, Rice K J (2007) Functional ecology of ecotypic differentiation in the Californian serpentine sunflower (*Helianthus exilis*). *New Phytologist* **175**, 107–119. doi:10.1111/j.1469-8137.2007.02068.x

Samecka-Cymerman, A, Garbiec, K, Kolon, K, Kempers, A J. (2009). Factor analysis of the elemental composition of *Pteridium aquilinum* from serpentine and granite soils as a tool in the classification of relations between this composition and the type of parent rock in the Ślęża Massif in Lower Silesia, Poland. *Environmental geology*, **58**(3), 509-514.

Seregin I V, Kozhevnikova A D (2006) Physiological role of nickel and its toxic effects on higher plants. *Russian Journal of Plant Physiology* **53**(2), 257-277.

Schutz W, Milberg P, Lamont B B (2002) Germination requirements and seedling responses to water availability and soil type in four eucalypt species. *Acta Oecologica* **23**, 23–30. doi:10.1016/S1146-609X(01)01130-4

Serrat-Capdevila A, Valdés J B, Pérez J G, Baird K, Mata L J, Maddock III T (2007) Modeling climate change impacts–and uncertainty–on the hydrology of a riparian system: The San Pedro Basin (Arizona/Sonora). *Journal of Hydrology* **347**(1), 48-66.

Schechter S P.Bruns T D (2008). Serpentine and non-serpentine ecotypes of Collinsia sparsiflora associate with distinct arbuscular mycorrhizal fungal assemblages. *Molecular Ecology* **17**(13): 3198-3210.

Smulders M J M, Van de Ven E T W M, Croes A F,Wullems G J (1990). Metabolism of 1-naphthaleneacetic acid in explants of tobacco: evidence for release of free hormone from conjugates. *Journal of Plant Growth Regulation*, **9**(1-4), 27-34.

Specht R L, Batianoff G N, Reeves R D (2002) The vegetation continuum on the Serpentine soil catena in the Rockhampton-Marlborough area. In ‘Marlborough serpentine, landscape management and conservation, 2002, Rockhampton’. (Eds A Exelby, K Wormington, A Melzer) pp. 23–40.

Specht R, Batianoff G, Reeves R (2006) Vegetation structure and biodiversity along the eucalypt forest to rainforest continuum on the serpentinite soil catena in a subhumid area of central Queensland, Australia. *Austral Ecology* **31**, 394–407. doi:10.1111/j.1442-9993.2006.01628.x

Specht R L, Specht A (1999) Australian communities: dynamics of structure, growth and biodiversity. Oxford University Press. Melbourne.

Spencer R J, Baxter G S (2006) Effects of fire on the structure and composition of open eucalypt forests. *Austral Ecology* **31**(5), 638-646.

Springer Y P, Hardcastle B A, Gilbert G S (2007) Soil calcium and plant disease in serpentine ecosystems: a test of the pathogen refuge hypothesis. *Oecologia* **151**(1), 10-21.

Standards Australia (2009) AS/NZS ISO 31000:2009 Risk management - Principles and guidelines, Standards Australia/Standards new Zealand

Stohlgren T J, Binkley D, Chong G W, Kalkhan M A, Schell L D, Bull K A, Son Y (1999). Exotic plant species invade hot spots of native plant diversity. *Ecological Monographs*, **69**(1), 25-46.

Tchinda N D, Messi H J C M, Nzweundji G, Tsabang N, Dongmo B, Oumar D, Tarkang P A, Caver A, Ndoumou D O (2013). Improving propagation methods of *Ricinodendron heudelotti* Baill. from cuttings *South African Journal of Botany* **88**: 3-9.

Threatened Species Scientific Committee (2008) Commonwealth Conseration Advice on *Neoroepera buxifoli*a. In. ' Ed. W Department of the Environment, Heritage and the Arts).

Trenberth K E (2008) The impact of climate change and variability on heavy precipitation, floods, and droughts. *Encyclopedia of hydrological sciences*.

Van Der Stoel C D, Van Der Putten W H, Duyts H (2002) Development of a negative plant–soil feedback in the expansion zone of the clonal grass *Ammophila arenaria* following root formation and nematode colonization. *Journal of Ecology* **90**(6), 978-988.

Vöosmarty C J (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry*, **70**(2), 153-226.

Walsh K, Nguyen K-C, McGregor J (2004) Fine-resolution regional climate model simulations of the impact of climate change on tropical cyclones near Australia. *Climate Dynamics* **22**(1), 47-56.

Wang G G (2000) Use of understory vegetation in classifying soil moisture and nutrient regimes. *Forest ecology and management* **129**(1), 93-100.

Weiss S B (1999) Cars, Cows, and Checkerspot Butterflies: Nitrogen Deposition and Management of Nutrient-Poor Grasslands for a Threatened Species. *Conservation Biology* **13**(6), 1476-1486.

Whereat C (2002) Marlborough Nickel Project environmental management pre-development *Marlborough serpentine, Landscape Management and Conservation,* eds Exelby A., Wormington k., Melzer A., Central Queensland University, Rockampton, Australia

Wilcock S (1998) Sediment-hosted magnesite deposits. *AGSO Journal of Australian Geology & Geophysics* **17**(4), 247-251.

Williamson J, Harrison S (2002) Biotic and abiotic limits to the spread of exotic revegetation species. *Ecological applications* **12**(1), 40-51.

Williamson A, Johnson M S (1981) Reclamation of metalliferous mine wastes. In 'Effect of heavy metal pollution on plants. Vol. 2'. (Ed. NW Lepp) pp. 185–212. Springer: The Netherlands.

Wilson A D (1990) The effect of grazing on Australian ecosystems. *Proceedings of the Royal Society of Australia* **16**, 235-244.

Wolf A (2001) Conservation of endemic plants in serpentine landscapes. *Biological Conservation* **100**(1), 35.

Wormington K R, Lamb D, McCallum H I, Moloney D J (2007) Leaf nutrient concentrations and timber productivity in the dry sclerophyll forests of south-east Queensland, Australia: implications for arboreal marsupials. *Forest Science* **53**, 627–634

Wormington K R, Houston W (2008) Management for biodiversity in grazing areas of Central Queensland, report to the Fitzroy Basin Association. Centre for Environmental Managment, Central Queensland University, Rockhampton.

Yates C J, Norton D A, Hobbs R J (2000) Grazing effects on plant cover, soil and microclimate in fragmented woodlands in south-western Australia: implications for restoration. *Austral Ecology* **25**(1), 36-47.

Yang X, Baskin J M, Baskin C C, Huang Z (2012) More than just a coating: Ecological importance, taxonomic occurence and phylogenetic relationships of seed coat mucilage. *Perspectives in Plant Ecology, Evolution and Systematics* **14**, 434-442.

QNI (2013) QNI Resources Pty., Ltd. Brolga Project - stage 2, Environmental Management Plan

Zare A R, Solouki M, Omidi M, Irvani N, Abasabadi A O, Nezad NM (2011) Effect of various treatments on seed germination and dormancy breaking in *Ferula* assa *foetida* L. (Asafetida) a threatened medicinal herb *Trakia Journal of Sciences* **9**(2), 57-61.

Appendix 1. Plant species list for combined sites. Q indicates Queensland conservation status under the *Nature Conservation Act 1992* , C indicates Australian conservation status under the *Environmental Protection and Biodiversity Conservation Act 1999* , Codes are + indicates the taxon is present at site, * introduced taxon and has naturalised, Endangered (E), Vulnerable (V), Near Threatened (NT), Site numbers: S1 - S30 Chapter 2, NB1 - NB25 Chapter 3 *N. buxifolia* sites, T1 - T10 Chapter 3 non- *N. buxifolia* sites.

Site number (S1–S30)

Q, C	***Plant name***	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
	Acanthaceae																														
	Brunoniella australis		+					+	+	+													+				+		+	+	+
	Pseuderanthemum variabile							+			+																				
	Rostellularia adscendens						+						+	+	+		+	+						+			+			+	+
	Adiantaceae																														
	Cheilanthes sieberi subsp. *sieberi*	+				+																									
	Amaranthaceae																														
	Alternanthera denticulata var. *micrantha*		+																										+		
	Anacardiaceae																														
	Euroschinus falcatus																														
	Annonaceae																														
	Uvaria leichhardtii																														
	Apocynaceae																														
	Alstonia constricta																														
	Alyxia ruscifolia																														
	Asclepias curassavica																														
	Carissa spinarum																														
	Cryptostegia grandiflora																														
	Gomphocarpus physocarpus																														
V	*Marsdenia brevifolia*				+	+					+																				
	Araliaceae																														
	Astrotricha intermedia			+		+																	+	+				+			+
	Araucariaceae																														
	Araucaria cunninghamii																														
	Arecaceae																														
	Livistona decipiens																														
	Asteraceae																														
	Acmella grandiflora var. *brachyglossa*		+																												
*	*Ageratum houstonianum*	+	+						+																						
	Camptacra gracilis		+																							+		+			
	Cyanthillium cinereum	+							+					+					+		+	+		+			+		+	+	+
*	*Emelia sonchifolia* var. *sonchifolia*								+														+								+
	Glossocardia bidens							+																							
E	*Olearia macdonnellensis*					+																									
	Peripleura bicolor													+																	
	Peripleura hispidula																														+
	Peripleura hispidula var. *setosa*										+																				
	Pterocaulon redolens							+	+		+					+							+								
	Boraginaceae																														
	Trichodesma zeylanicum var. zeylanicum										+																				
	Capparaceae																														
	Capparis canescens		+			+				+			+	+									+								
V	*Capparis thozetiana*										+				+	+	+									+		+			
	Commelina diffusa		+				+																	+							
	Commelinaceae																														
	Casuarinaceae																														
	Casuarina cunninghamiana																														
	Convolvulaceae																														
	Evolvulus alsinoides								+		+					+															
	Jacquemontia paniculata var. *paniculata*													+							+										
	Crassulaceae																														
	Bryophyllum sp.																														
	Cycadaceae																														
E	*Cycas ophiolitica*																												+		
	Cyperaceae																														
	Abildgaardia ovata																										+	+			+
	Cyperus sp.																														
	Gahnia aspera			+				+	+			+	+			+	+	+	+		+	+	+		+			+	+	+	+
	Gahnia sieberiana			+																											
	Scleria brownii			+	+	+	+	+				+	+									+		+	+	+	+		+		+

Site number (NB1–NB25, T1–T10)

Q, C	***Plant name***	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10	NB11	NB12	NB13	NB14	NB15	NB16	NB17	NB18	NB19	NB20	NB21	NB22	NB23	NB24	NB25	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
	Acanthaceae																																			
	Brunoniella australis																																			
	Pseuderanthemum variabile																																			
	Rostellularia adscendens																									+										
	Adiantaceae																																			
	Cheilanthes sieberi subsp. *sieberi*				+																															
	Amaranthaceae																																			
	Alternanthera denticulata var. *micrantha*																																			
	Anacardiaceae																																			
	Euroschinus falcatus																						+													
	Annonaceae																																			
	Uvaria leichhardtii																												+							+
	Apocynaceae																																			
	Alstonia constricta								+	+																										
	Alyxia ruscifolia		+						+																											
	Asclepias curassavica														+																					
	Carissa spinarum		+						+	+																	+									
	Cryptostegia grandiflora														+	+																				
	Gomphocarpus physocarpus																															+				
V	*Marsdenia brevifolia*			+			+						+																+							
	Araliaceae																																			
	Astrotricha intermedia					+	+																													
	Araucariaceae																																			
	Araucaria cunninghamii																						+													
	Arecaceae																																			
	Livistona decipiens										+	+	+			+		+										+								+
	Asteraceae																																			
	Acmella grandiflora var. *brachyglossa*																																			
*	*Ageratum houstonianum*														+																					
	Camptacra gracilis																																			
	Cyanthillium cinereum	+		+		+							+	+							+					+										
*	*Emelia sonchifolia* var. *sonchifolia*																				+															
	Glossocardia bidens																																			
E	*Olearia macdonnellensis*																																			
	Peripleura bicolor																																			
	Peripleura hispidula																																			
	Peripleura hispidula var. *setosa*																																			
	Pterocaulon redolens																+		+	+	+				+			+				+				+
	Boraginaceae																																			
	Trichodesma zeylanicum var. zeylanicum																				+															
	Capparaceae																																			
	Capparis canescens																																			
V	*Capparis thozetiana*					+	+																													
	Commelina diffusa																																			
	Commelinaceae																																			
	Casuarinaceae																																			
	Casuarina cunninghamiana										+	+			+	+	+	+		+		+	+	+		+		+								+
	Convolvulaceae																																			
	Evolvulus alsinoides																																			
	Jacquemontia paniculata var. *paniculata*																													+						
	Crassulaceae																																			
	Bryophyllum sp.														+																					
	Cycadaceae																																			
E	*Cycas ophiolitica*																																			
	Cyperaceae																																			
	Abildgaardia ovata																																			
	Cyperus sp.											+				+		+				+														
	Gahnia aspera	+	+	+	+	+	+		+	+	+	+			+	+	+	+		+	+	+	+	+		+	+	+	+	+						+
	Gahnia sieberiana																																			
	Scleria brownii				+	+	+							+	+			+																		

Q, C	Plant name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10	NB11	NB12	NB13	NB14	NB15	NB16	NB17	NB18	NB19	NB20	NB21	NB22	NB23	NB24	NB25	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
	Scleria mackaviensis							+																																																										
	Ebenaceae																																																																		
	Diospyros australis																										+						+							+	+					+				+							+	+									
	Diospyros geminata																																						+			+																+									
	Ericaceae																																																																	
V	*Leucopogon cuspidatus*												+					+	+	+				+	+	+		+	+	+	+		+		+	+	+	+													+															
	Leucopogon mitchellii			+	+																+	+																						+															+							
	Fabaceae																																																																	
*	*Aeschynomene micranthos*																										+																																							
	Austrosteenisia blackii																																							+																										
	Cajanus confertiflorus														+	+		+					+	+																																										
	Cajanus reticulatus var. *reticulatus*	+		+		+														+									+			+	+	+		+	+											+			+				+			+		+	+	+			+	+
	Chamaecrista concina													+																																																				
	Crotalaria montana	+	+	+	+	+	+						+	+	+	+	+		+	+			+				+	+		+	+	+		+		+	+							+				+																		
	Daviesia filipes												+																	+																																		
	Desmodium brachypodum										+																		+																																			
	Desmodium rhytidophyllum						+		+				+			+		+									+	+	+	+	+																																			
	Flemingia parviflora																										+																																						
	Galactia tenufolia		+			+																																																											
	Glycine sp.	+								+														+	+	+				+																																			
	Glycine tabacina													+																																																			
	Hardenbergia violacea														+																																																		
	Hovea longipes																+														+	+	+		+		+					+					+		+	+								+							
	Hovea tholiformis												+		+				+	+							+																										+												
	Indigofera australis																									+																													+											
	Indigofera linifolia								+																																																								
	Indigofera sp.	+			+																							+																																					
	Neptunia gracilis		+																																																														
V	*Pultenaea setulosa*											+	+		+				+		+												+											+										+												
	Rhynchosia minima var. *minima*													+																																																			
	Senna gaudichaudii																														+																																		
	Stylosanthes humilis						+		+																	+														+							+																			
	Tephrosia brachyodon var. *brachyodon*									+					+	+		+																																															
	Tephrosia filipes													+										+			+							+																																
	Zornia dyctiocarpa var. *filifolia*																										+																																						
	Zornia muriculata										+																																																						
	Zornia sp.																													+																																		
	Goodeniaceae																																																																	
	Goodenia sp. (Mt Castletower M.D.Crisp ++++)	+					+						+	+	+	+	+		+	+																							+				+		+						+	+				+						+
	Hemerocallidaceae																																																																	
	Dianella longifolia												+															+						+													+	+					+													
	Dianella sp.														+		+						+				+	+		+	+																																		
	Johnsoniaceae																																																																	
	Tricoryne anceps subsp. *anceps*													+																																																			
	Tricoryne elatior	+		+	+								+		+				+		+				+	+				+	+																																		
	Lamiaceae																																																																	
	Clerodendrum floribundum													+																									+	+																										
	Lauraceae																																																																	
	Cassytha filiformis																																									+					+	+						+	+											
	Cassytha glabella forma *glabella*																																																																	
	Neolitsea brassii																																						+	+				+			+			+	+		+													
	Laximinaceae																																																																	
	Eustrephus latifolius	+		+		+	+	+	+		+			+						+			+	+				+	+	+	+	+				+	+				+	+	+			+	+		+							+	+		+									
	Lomandra confertifolia subsp. *pallida*	+																																																																
	Lomandra filiformis subsp. *filiformis*											+	+	+			+																																																	
	Lomandra hystrix																																							+		+		+	+	+	+								+		+			+	+			+	
	Lomandra multiflora subsp. *multiflora*																							+		+	+	+		+																																			
	Lomandra sp.				+	+		+		+	+	+	+						+				+	+			+			+					+		+	+																							+					
	Lecythidaceae																																																																	
	Planchonia careya											+	+																																		+																			
	Malvaceae																																																																	
	Hibiscus divaricatus																														+		+		+	+				+		+	+				+	+	+	+	+	+		+		+					+			+	+
	Hibiscus heterophyllus							+		+																																																	+						
	Hibiscus sp.						+																																																										
	Hibiscus splendens	+				+									+					+		+	+	+	+			+	+																																			

Q, C	Plant name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
*	*Malvastrum americanum* var. *americanum*								+	+	+			+																	
*	*Sida* sp.								+							+					+										
*	*Sida spinosa*												+																		
	Sida subspicata							+																							
	Meliaceae																														
	Turraea pubescens																						+								
	Mimosaceae																														
	Acacia aulacocarpa																						+				+		+		+
	Acacia crassa subsp. *longicoma*							+		+																					
	Acacia decora							+	+																						
	Acacia disparrima subsp. *disparrima*																														
	Acacia excelsa															+															
	Acacia leiocalyx													+																	
	Acacia leptostachya	+		+	+	+	+					+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Acacia sp.								+	+	+																				
	Moraceae																														
	Ficus opposita										+												+								
	Trophis scandens																														
	Myrtaceae																														
	Corymbia dallachiana										+					+															
	Corymbia erythrophloia																														
	Corymbia intermedia															+															
	Corymbia sp.						+																					+			+
V	*Corymbia xanthope*	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Eucalyptus crebra																														
	Eucalyptus fibrosa subsp. *fibrosa*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Eucalyptus melanophloia															+															
	Eucalyptus tereticornis																														
	Gossia bidwillii																														
	Lophostemon confertus										+																				
	Lophostemon grandiflorus																														
	Melaleuca bracteata																														
	Melaleuca fluviatilis																														
	Melaleuca sp. (Marlborough Creek G.N.Batianoff+ MC9+ 8 6)																														
	Oleaceae																														
	Jasminum didymum																														
	Notelaea sp.					+								+																	
	Oxalidaceae																														
	Oxalis chnoodes							+			+					+													+		+
	Passifloraceae																														
*	*Passiflora suberosa*							+	+	+													+	+					+		+
	Pyllanthaceae																														
	Breynia oblongifolia				+				+														+		+						
	Bridelia leichhardtii								+																						
	Phyllanthus sp. aff. Simplex									+				+			+							+							
	Phyllanthus virgatus		+			+		+							+	+							+	+	+	+	+	+		+	+
	Phytolaccaceae																														
*	*Rivina humilis*																														
	Picrodendraceae																														
V	*Neoroepera buxifolia*																						+								
	Petalostigma pubescens								+								+														
	Pittosporaceae																														
V	*Bursaria reevesii*			+													+						+					+			
	Pittosporum spinescens																														
	Poaceae																														
	Alloteropsis semialata			+															+	+											+
	Ancistrachne uncinulata																														
	Aristida calycina var. *calycina*	+			+	+			+	+	+	+					+	+					+	+							+
	Aristida gracilipes								+			+					+						+						+		+
	Aristida personata	+					+							+								+									
	Aristida queenslandicum		+					+					+																		
	Aristida sp.																														
	Arundinella nepalensis		+																												
*	*Bothriochloa pertusa*								+					+																	
	Cymbopogon refractus							+	+	+	+					+		+													

Q, C	Plant name	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10	NB11	NB12	NB13	NB14	NB15	NB16	NB17	NB18	NB19	NB20	NB21	NB22	NB23	NB24	NB25
*	*Malvastrum americanum* var. *americanum*	+																								
*	*Sida* sp.																			+	+					
*	*Sida spinosa*																									
	Sida subspicata																									
	Meliaceae																									
	Turraea pubescens																									
	Mimosaceae																									
	Acacia aulacocarpa																									
	Acacia crassa subsp. *longicoma*																									
	Acacia decora											+						+	+	+	+	+			+	
	Acacia disparrima subsp. *disparrima*	+	+			+	+		+	+																
	Acacia excelsa																									
	Acacia leiocalyx																									
	Acacia leptostachya	+	+	+	+	+	+	+	+		+	+	+	+				+	+	+		+		+	+	
	Acacia sp.																									
	Moraceae																									
	Ficus opposita	+							+	+			+	+	+		+				+	+				+
	Trophis scandens		+																							
	Myrtaceae																									
	Corymbia dallachiana																+									
	Corymbia erythrophloia																+									
	Corymbia intermedia																									
	Corymbia sp.																									
V	*Corymbia xanthope*	+	+	+	+	+	+	+	+				+					+	+	+	+		+	+	+	+
	Eucalyptus crebra																									
	Eucalyptus fibrosa subsp. *fibrosa*	+	+	+	+	+	+	+			+		+				+	+	+	+	+		+	+	+	+
	Eucalyptus melanophloia																									
	Eucalyptus tereticornis														+					+						
	Gossia bidwillii		+						+																	
	Lophostemon confertus																									
	Lophostemon grandiflorus											+				+							+			
	Melaleuca bracteata															+	+							+	+	
	Melaleuca fluviatilis										+	+				+	+		+			+				+
	Melaleuca sp. (Marlborough Creek G.N.Batianoff+ MC9+ 8 6)										+	+				+		+				+	+	+		+
	Oleaceae																									
	Jasminum didymum		+					+																	+	
	Notelaea sp.																									
	Oxalidaceae																									
	Oxalis chnoodes	+																				+				
	Passifloraceae																									
*	*Passiflora suberosa*	+	+	+			+		+							+	+			+	+	+				+
	Pyllanthaceae																									
	Breynia oblongifolia														+	+		+				+				
	Bridelia leichhardtii																									
	Phyllanthus sp. aff. Simplex	+		+																						
	Phyllanthus virgatus					+																				
	Phytolaccaceae																									
*	*Rivina humilis*								+																	
	Picrodendraceae																									
V	*Neoroepera buxifolia*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Petalostigma pubescens	+															+	+								+
	Pittosporaceae																									
V	*Bursaria reevesii*	+	+					+					+					+	+						+	
	Pittosporum spinescens								+	+																
	Poaceae																									
	Alloteropsis semialata																									
	Ancistrachne uncinulata								+																	
	Aristida calycina var. *calycina*																									+
	Aristida gracilipes																									
	Aristida personata				+	+	+																			
	Aristida queenslandicum												+													
	Aristida sp.																		+							
	Arundinella nepalensis	+		+							+		+		+		+	+		+	+	+	+		+	
*	*Bothriochloa pertusa*																									
	Cymbopogon refractus																	+								

Q, C	Plant name	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
*	*Malvastrum americanum* var. *americanum*										
*	*Sida* sp.						+				
*	*Sida spinosa*										
	Sida subspicata										
	Meliaceae										
	Turraea pubescens										
	Mimosaceae										
	Acacia aulacocarpa										
	Acacia crassa subsp. *longicoma*										
	Acacia decora				+		+				
	Acacia disparrima subsp. *disparrima*	+				+					
	Acacia excelsa										
	Acacia leiocalyx										
	Acacia leptostachya	+	+	+	+	+	+			+	+
	Acacia sp.										
	Moraceae										
	Ficus opposita										
	Trophis scandens										
	Myrtaceae										
	Corymbia dallachiana						+				
	Corymbia erythrophloia				+		+				
	Corymbia intermedia										
	Corymbia sp.										
V	*Corymbia xanthope*				+		+				
	Eucalyptus crebra					+	+				
	Eucalyptus fibrosa subsp. *fibrosa*	+	+		+	+	+			+	+
	Eucalyptus melanophloia										
	Eucalyptus tereticornis	+					+				
	Gossia bidwillii										
	Lophostemon confertus										
	Lophostemon grandiflorus										
	Melaleuca bracteata	+	+								
	Melaleuca fluviatilis										
	Melaleuca sp. (Marlborough Creek G.N.Batianoff+ MC9+ 8 6)										
	Oleaceae										
	Jasminum didymum			+							
	Notelaea sp.										
	Oxalidaceae										
	Oxalis chnoodes										
	Passifloraceae										
*	*Passiflora suberosa*	+			+					+	
	Pyllanthaceae										
	Breynia oblongifolia				+						
	Bridelia leichhardtii										
	Phyllanthus sp. aff. Simplex										
	Phyllanthus virgatus										
	Phytolaccaceae										
*	*Rivina humilis*										
	Picrodendraceae										
V	*Neoroepera buxifolia*										
	Petalostigma pubescens				+	+					+
	Pittosporaceae										
V	*Bursaria reevesii*		+								
	Pittosporum spinescens	+									
	Poaceae										
	Alloteropsis semialata										
	Ancistrachne uncinulata										
	Aristida calycina var. *calycina*										
	Aristida gracilipes										
	Aristida personata										
	Aristida queenslandicum										
	Aristida sp.	+									
	Arundinella nepalensis		+			+				+	
*	*Bothriochloa pertusa*										
	Cymbopogon refractus										

Q, C	Plant name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10	NB11	NB12	NB13	NB14	NB15	NB16	NB17	NB18	NB19	NB20	NB21	NB22	NB23	NB24	NB25	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
	Dicanthium fecundum	+	+		+	+						+	+	+	+		+						+					+			+																									+										
	Digitaria minima			+				+	+		+					+																																																		
	Enneapogon gracilis								+								+																	+																																
	Enteropogon unispiceus								+																																																									
	Entolasia sticta	+																																																																
	Eragrostis brownii		+					+						+																																																			
	Eragrostis sp.																																																+										+							
	Heteropogon contortus	+	+			+	+										+	+						+					+	+	+	+								+		+	+				+	+										+	+				+			+	+
	Melinis repens						+		+	+				+			+				+			+							+	+		+		+	+						+	+	+	+	+	+	+	+	+					+		+			+	+				+
	Mnesithea formosa													+																																																			
	Oplismenus aemulus																																											+	+																					
	Panicum effusum	+						+		+		+		+					+													+		+		+	+	+					+	+						+																
	Panicum larcomianum						+																																																											
	Panicum queenslandicum subsp. *queenslandicum*		+						+																																																								
	Panicum simile												+						+	+	+	+	+																																											
	Panicum sp.			+	+	+	+																	+		+	+	+	+	+	+									+		+				+		+	+	+	+	+	+	+		+	+									
	Paspalidium caespitosum																																																									+							
	Paspalidium criniforme			+	+	+	+	+	+			+						+			+	+	+	+		+			+						+		+	+	+						+				+	+						+										
	Paspalum dilatatum																																+																																
	Sarga leiocladum												+					+					+	+			+	+	+	+				+								+			+			+		+	+	+				+				+		+				
	Sehima nervosum		+																																																															
	Sorghum halepense																																																									+								
	Sorghum nitidum forma aristatum																																										+																							
	Themeda quadrivalvis																																															+							+											
	Themeda triandra														+										+	+	+	+		+	+																																			
	Triodia mitchellii	+								+	+	+	+		+				+	+	+		+		+	+	+	+		+	+	+		+	+	+	+	+					+	+										+	+	+	+										
	Polygalaceae																																																																	
	Polygala linariifolia				+														+								+																																							
	Proteaceae																																																																	
V	*Hakea trineura*												+		+	+	+	+	+	+	+	+		+	+	+		+							+	+								+					+				+	+												
	Putranjivaceae																																																																	
	Drypetes deplanchei																																+					+				+				+																				
	Ranunculaceae																																																																	
	Clematis pickeringii				+												+							+								+				+									+					+						+		+		+						
	Rhamnaceae																																																																	
	Alphitonia excelsa	+		+	+	+	+	+	+	+	+	+	+	+		+	+	+				+	+	+					+	+	+	+	+	+	+	+	+		+		+		+		+	+	+	+	+	+	+	+				+		+	+	+		+			+	+
	Rubiaceae																																																																	
	Pavetta australiensis																																		+			+	+						+														+							
	Pogonolobus reticulatus								+	+				+			+																																																
	Psychotria daphnoides	+			+	+	+	+	+	+		+		+		+	+	+		+			+	+				+	+			+		+		+	+						+	+	+			+	+	+	+					+				+						
	Psydrax attenuata						+																+								+																																			
	Psydrax lamprophylla form lamprophylla																															+																																	
	Psydrax longipes																																						+																										
	Psydrax odorata form buxifolia																															+						+																				+							
	Psydrax saligna					+							+	+																																																			
	Spermacoce brachystema																			+			+				+																																							
	Rutaceae																																																																	
	Acronychia laevis																															+					+										+																		
	Geijera parviflora																																				+																													
	Santalaceae																																																																	
	Exocarpos latifolius																																							+	+			+	+		+				+								+						
	Sapindaceae																																																																	
	Arytera divaricata																															+																								+		+							
	Dodonaea triquetra	+	+	+			+					+		+	+		+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+					+	+											+	+		+	+			+					
	Jagera pseudorhus var. pseudorhus																															+						+	+																											
	Sapotaceae																																																																	
	Niemeyera antiloga					+	+													+			+									+	+	+		+	+	+		+		+	+		+	+		+	+	+	+	+	+		+		+		+		+	+				+
	Solanaceae																																																																	
	Solanum elipticum								+																																																									
	Solanum nigrum																																						+																										
	Solanum seaforthianum																																						+					+																					
	Solanum sp.							+	+	+				+																								+																											
	Sparrmanniaceae																																																																	
	Grewia latifolia				+	+	+	+	+	+	+		+	+		+	+	+						+	+			+	+	+	+	+	+					+										+			+				+	+										
	Stackhousiaceae																																																																	

Q, C	Plant name	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
NT	*Stackhousia tryonii*																										+				
	Sterculiaceae																														
	Brachychiton bidwillii					+		+																							
	Thymelaeaceae																														
NT, V	*Pimelea leptospermoides*	+		+	+	+	+		+	+		+			+		+				+	+					+				
	Ulmaceae																														
	Trema tomentosa																														
	Verbenaceae																														
*	*Lantana camara*								+																				+		
*	*Stachytarpheta jamaicensis*													+																	
	Violaceae																														
	Hybanthus enneaspermus subsp. *stellariodes*								+	+																		+			+
	Hybanthus stellarioides							+	+		+			+		+															
	Xanthorrhoeaceae																														
	Xanthorrhoea johnsonii		+	+	+		+				+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
	Zamiaceae																														
E	*Macrozamia serpentina*	+		+	+	+		+		+	+	+	+		+	+	+	+		+	+	+	+	+	+	+		+	+	+	+

Q, C	Plant name	NB1	NB2	NB3	NB4	NB5	NB6	NB7	NB8	NB9	NB10	NB11	NB12	NB13	NB14	NB15	NB16	NB17	NB18	NB19	NB20	NB21	NB22	NB23	NB24	NB25	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
NT	*Stackhousia tryonii*			+																				+		+										
	Sterculiaceae																																			
	Brachychiton bidwillii																																			
	Thymelaeaceae																																			
NT, V	*Pimelea leptospermoides*			+					+		+	+	+					+			+	+				+	+	+		+						
	Ulmaceae																																			
	Trema tomentosa	+		+					+		+					+																				
	Verbenaceae																																			
*	*Lantana camara*								+	+					+	+		+		+	+	+				+		+				+				
*	*Stachytarpheta jamaicensis*									+		+			+	+	+	+		+	+	+				+		+		+		+			+	+
	Violaceae																																			
	Hybanthus enneaspermus subsp. *stellariodes*																																			
	Hybanthus stellarioides																									+										
	Xanthorrhoeaceae																																			
	Xanthorrhoea johnsonii	+		+		+	+	+					+				+	+	+		+		+	+	+	+				+	+	+				+
	Zamiaceae																																			
E	*Macrozamia serpentina*	+	+	+	+	+	+	+						+				+	+		+				+				+		+					+

Mean elemental concentrations of *Eucalyptus fibrosa* subsp. *fibrosa* foliage (minimum and maximum in parentheses following each mean).

Element	**Concentration**
B (µg g^{-1})	22.68 (5-37)
Ca (mg g^{-1})	3.4 (1.5-5.6)
Cu (µg g^{-1})	1.14 (0.6-3)
Fe (µg g^{-1})	6.83 (2-12)
K (mg g^{-1})	1.5 (0.9-3.5)
Mg (mg g^{-1})	1.1 (0.8-1.9)
Mg / Ca	0.36(0.22-0.63)
Mn (µg g^{-1})	120.43 (33-268)
Na (mg g^{-1})	1.2 (0.5-2.2)
% N	0.92 (0.8-1.3)
Ni (µg g^{-1})	55.14 (12-254)
P (µg g^{-1})	275.26 (194-448)
S (µg g^{-1})	347.87 (258-519)
Zn (µg g^{-1})	4.00 (2-9)