Accounting For Soil Microbial Communities During Ecological Restoration

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Declaration of own work:

I, James Listberger, certify that this thesis is my own work, based on my personal research in conjunction with my supervisors and that I have acknowledged all material and sources used in its preparation. I also certify that this thesis has not previously been submitted for assessment, and that I have not copied in part or whole or otherwise plagiarised the work of other persons.

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1. Abstract:

Invasive plants often negatively impact the (a)biotic conditions of ecosystems. These impacts can persist after removal of the invader as 'legacy-effects'. I assessed whether the invasion of native Cumberland Plain Woodland (CPW) by African olive, *Olea europaea* subsp. *cuspidata*, alters soil (a)biotic properties and, if so, the effect it has on the performance of five native CPW species. I then tested whether native soil inocula can mitigate the potential biotic impacts on CPW soils. The five study species were grown in mesocosms under five soil treatments; CPW soil, African olive-invaded soil, and invaded soil inoculated with CPW soil, native rhizosphere soil, or both. The impact of soil treatment on various metrics of native plant performance was determined. Olive-invaded soils were found to have higher pH, total carbon, phosphorus, nitrogen, and nitrate nitrogen but lower ammonia nitrogen. Of the study species, only the biomass of *Indigofera australis* and *Dodonaea viscosa* increased in olive soils. Nodulation of *I. australis* did not differ between treatments. In contrast, *Acacia implexa* biomass and nodulation decreased in invaded soils and responded positively to inocula. Overall, my results suggest that native soil biota may benefit the outcomes of ecological restoration projects only on a species-specific basis.

2. Introduction

Invasive plants pose a significant threat to biodiversity, ecosystem services (Levine *et al*., 2003; Gerber *et al*., 2008; Charles and Dukes, 2008; Vilà *et al*., 2011), agricultural productivity, and human health globally (Pimentel *et al*., 2001; Pimentel *et al*., 2009; Mack and Smith, 2011; Nyasembe *et al*., 2015; Hoffmann and Broadhurst, 2016). To compound the impact of these species, their management is often challenging and resource intensive (Alday *et al*., 2013; Martin and Blossey, 2013; Hoffmann and Broadhurst, 2016). While there are numerous mechanisms by which invasive plants impact ecosystems, a common outcome of most invasions is that they alter the structure and function of the ecosystems they invade (Jordan *et al*., 2008; Iponga *et al*., 2008; Gerber *et al*., 2008; Weidenhamer and Callaway, 2010). This often leads to the formation of positive feedback loops, which can alter seed bank composition, fire regimes, soil nutrients, leaf litter quantity and/or quality, and soil biotic structure and function (Allison and Vitousek, 2004; Grigulis *et al*., 2005; Stevens and Beckage, 2009; Beckstead *et al*., 2011; Elgersma *et al*., 2011; Elgersma and Ehrenfeld, 2011; Arthur *et al*., 2012; Lee *et al*., 2012; Elgersma *et al*., 2012; Gaertner *et al*., 2014; Wagner and Fraterrigo, 2015). For example, invasive gamba grass (*Andropogon gayanus*) in northern Australia lowers available soil nitrogen by rapidly using nitrogen but not depositing nitrogen equally fast via leaf litter decomposition (Rossiter-Rachor *et al*., 2017). Other

invasive species like buffelgrass (*Cenchrus ciliaris*) alters local soil microbiomes, enriching nitrifiers and arbuscular mycorrhizal fungi that increases soil nutrient availability to this grass (Gornish *et al*., 2020). In the western United State, cheatgrass (*Bromus tectorum*) are dominant structural components of degraded rangelands, resulting in an increased fuel load in these areas (Whisenant, 1990; Morris *et al*., 2016; Morris and Leger, 2016). Consequently, this leads to more intense and frequent fires, which may further facilitate the invasion of cheatgrass by reducing the competitive effect of the fire-intolerant native flora (Chambers *et al*., 2007; Bradley *et al*., 2018). These fires also alter soil physiochemical properties through changes in nutrient cycling (i.e. increased carbon and phosphorus and decreased nitrogen levels after intense burns), which may exacerbate the invasion of cheatgrass (Morris *et al*., 2016) and/or other species such as nitrophillic plants (Whisenant, 1990; Bird *et al*., 2000; Lee *et al*., 2012; Morris *et al*., 2016; Pearson *et al*., 2016). Cheatgrass invasion in the United States serves as a powerful example of how the dominance of a single invasive species can profoundly alter both the structural and functional components of the ecosystems it invades.

The impacts of invasive plants on the aboveground component of ecosystems, such as those described above for cheatgrass, are easily observed and quantified, so have unsurprisingly received much research attention (Walker and Smith, 1997; Daehler, 2003; Compagnoni, 2014; Fried *et al*., 2014; Jones *et al*., 2015; Bradley *et al*., 2018). In contrast, far less is known about the impacts of invasive plants on the structure and function of the belowground component of ecosystems, which encompasses the soil microbiome (Callaway and Aschehoug, 2000; Callaway *et al*., 2004; Wolfe and Klironomos, 2005). Despite this, we can predict that the impacts of invasive plants on the soil microbiome are likely to be significant for numerous reasons. Firstly, soil microbial communities are extremely sensitive to changes in abiotic soil physiochemical properties such as moisture content (Wang *et al*., 2006; Brockett *et al*., 2012; Banerjee *et al*., 2016; Cavagnaro, 2016), pH (Rousk *et al*., 2010; Zhalnina *et al*., 2015), nutrient status (el Zahar *et al*., 2008; Carrillo *et al*., 2012; Leff *et al*., 2015), salinity (Rietz and Haynes, 2003), carbon content (Badri *et al*., 2009), structure/texture (Andrew *et al*., 2012), heavy metal concentration (Chaudri *et al*., 2000) and temperature (Wang *et al*., 2006; Waldrop and Firestone, 2006; Fierer and Jackson, 2006). Secondly, soil microbial communities represent a considerable portion of plant-symbiont interaction networks (Elliot *et al*., 2007; Wang *et al*., 2012; Martin *et al*., 2017). Therefore, they can directly or indirectly influence the diversity and structure of the aboveground component of plant communities through plant-soil feedbacks (Stinson *et al*., 2006; Mangla and Callaway, 2008; Zhang *et al*., 2010; Bever *et al*., 2013). For example, microbial community composition in hyper-diverse fynbos soils in South Africa has been shown to be strongly correlated with aboveground plant community composition at

small spatial scales (Slabbert *et al*., 2010; Miyambo *et al*., 2016). This is because growth rates and community composition of microbes are often host-dependent, and the identity of the local hosts will, therefore, to some extent influence soil community composition (Reynolds *et al*., 2003; Mummey and Rillig, 2006; Micallef *et al*., 2009; Chaparro *et al*., 2012). Conversely, the relative abundance of plant species within communities can also be influenced by soil microorganisms and their associated feedback effects, either as positive or negative feedback loops (Klironomos, 2002).

Invasive plants can form dense monocultures, affecting many abiotic components of ecosystems, including soil physiochemical properties (Holdredge and Bertness, 2011; Boughton and Boughton, 2014; Jo *et al*., 2017; Gibbons *et al*., 2017). This, in turn, may have a significant impact on soil microbial community diversity, structure, and function (Kourtev *et al*., 2002; Vogelsang and Bever, 2009; Jo *et al*., 2017). For example, changes to soil physiochemistry (e.g. increased soil carbon through leaf litter deposition) are expected to cause changes in soil microbial diversity, community structure, and microbial metabolic activities such as those stemming from the addition of extracellular enzymes (Gordon, 1998; Broz *et al*., 2007; Elgersma *et al*., 2012; Brockett *et al*., 2012; Suseela *et al*., 2016; Zhang *et al*., 2020), with these impacts increasing with dense monoculture invasions (Zhang *et al*., 2020). These changes in soil (a)biotic properties resulting from plant invasions can then lead to plant-soil feedbacks (PSFs) (Klironomos, 2002). That is, invasive plants can either alter soil properties to favour (i.e. positive PSFs, e.g. by increasing mutualist availability) or inhibit (i.e. negative PSFs, e.g. by increasing pathogen loads) their own growth and performance over successive generations (Klironomos, 2002; Nijjer *et al*., 2007; Zhang *et al*., 2010; Jiang *et al*., 2020). There are many examples of invasive plants causing positive PSFs through changes in soil nutrients (Vinton and Goergen, 2006; Lee *et al*., 2012; Perkins and Nowak, 2013) and enrichment of beneficial mutualists such as rhizobia and mycorrhizal fungi (Zhang *et al*., 2010; Bever *et al*., 2013; Lekberg *et al*., 2013; Le Roux *et al*., 2018). While these mutualists may enhance the performance of the invasive species, they may not be effective when in association with co-occurring native species, as relationships between mutualists and their host plants can be genera or species specific (Vogelsang and Bever, 2009). These impacts may be further compounded by invasive species increasing soil pathogen loads to be more harmful to native species than to themselves (Mangla and Callaway, 2008; Rudgers and Orr, 2009; Zhang *et al*., 2010; Eisenhauer *et al*., 2010; Grman and Suding, 2010; Kulmatiski and Beard, 2011; Jordan *et al*., 2011; Crawford and Knight, 2017; Zhang *et al*., 2019).

Invader-induced changes to (a)biotic habitat conditions may persist long after the removal of the invasive species (Holdredge and Bertness, 2011; Pearson *et al*., 2016; Suseela *et al*., 2016; Pickett *et al*., 2019). These so-called 'legacy effects' have important implications for the recovery

of native ecosystems, whether through passive or active restoration (Heneghan *et al*., 2006; Carrillo *et al*., 2012; Viall *et al*., 2014; Pearson *et al*., 2016). For instance, soils previously invaded by Port Jackson willow (*Acacia saligna*) in South Africa had elevated pH levels compared to uninvaded soils ten years after the removal of this species (Nsikani *et al*., 2017). Similarly, the invasion of the N-fixing bush lupin (*Lupinus arboreus*) in North America led to elevated soil nitrogen levels which persisted for 25 and five years after its removal, at unrestored and restored sites respectively (Maron and Jefferies, 2001). While rarely studied, legacy effects have additionally been observed in biotic components of invaded ecosystems, with some invasive plants altering soil microbial communities to their benefit and/or to the detriment of native competition (Elgersma *et al*., 2011; Lankau *et al*., 2014). For example, chemically induced disruptions between mutualistic mycorrhizal fungi and native plants caused by invasive garlic mustard (*Alliaria petiolata*) reduced the availability of native mycorrhizae for native plants, an affect which persisted for six years after the invader's removal (Lankau *et al*., 2014). As discussed above, the diversity and structure of microbial communities are intricately linked to soil abiotic properties, therefore, it can be expected that the legacy effects of one are likely to cause legacy effects in the other.

This is particularly detrimental to already vulnerable native vegetation communities, e.g. the critically endangered Cumberland Plain woodland – which is the focus of this study, as legacy effects can further reduce their survival. The Cumberland Plain Woodland (hereafter CPW) is a unique vegetation community with its remnants located in Western Sydney and is characterised by an open *Eucalyptus* dominated canopy (e.g. *Eucalyptus moluccana*), a small tree/shrub dominated understory (e.g. *Bursaria spinosa*), and a groundcover comprised of a diverse range of low growing herbaceous species and 26 native grasses, some of which are unique to CPW and essential for local wildlife (Bannerman and Hazelton, 1989; National Parks and Wildlife Service, 2000; Muyt, 2001; Benson and Howell, 2002).

The Cumberland Plain Woodland once covered approximately 125 000 hectares, extending north-west to Kurrajong and south-west to Picton (National Parks and Wildlife Service, 2000). Due to its relative fertility (its characteristic rich clay soils being derived from the underlying Wianamatta shale formation) compared to other Sydney soils (i.e. Hawksbury sandstone derived) (Beadle, 1962; Bannerman and Hazelton, 1989; Thomson and Leishman, 2004), land clearing and development by early European settlers and contemporary Australians, which persisted until the late 20th century (Benson and Howell, 2002), has meant only 9 % of relatively pristine CPW still remains (National Parks and Wildlife Service, 2000). As such, CPW has been labelled as critically endangered, with some of its plant and animal species at risk of extinction (NSW Scientific Committee, 2010).

One of the principal threats to the CPW is the invasion of the exotic African olive, *Olea europaea* subsp. *cuspidata*, which was introduced into Australia in the mid-19th century for use as European olive rootstock and as a garden specimen (Cuneo and Leishman, 2006; NSW Scientific Committee, 2010). Since then, African olive has invaded many areas throughout Australia, its berries spread readily by frugivorous birds who consume and distribute its large and viable seeds (Muyt, 2001; Cuneo and Leishman, 2006; Cuneo *et al.*, 2010). African olive is particularly damaging to native ecosystems due to its habit of forming dense monocultures with large shading canopies, these not only can alter local ambient conditions, such as humidity, but also reduce light to a point where native understory (where most CPW diversity exists) can no longer persist (Cuneo and Leishman, 2013). This, combined with the ease of its dispersal, has led to African olive being classified as a noxious weed in New South Wales and as an environmental weed in Australia overall (Csurhes and Edwards, 1998; Muyt, 2001; NSW Scientific Committee, 2010). African olive monocultures display many of the features (i.e. the replacement of native plant biodiversity, addition of shading, altered local environmental conditions, and increased leaf litter) known to alter soil (a)biotic conditions, with many of these potentially existing as legacy effects (Gordon, 1998; Cooke, 2001; Broz *et al.*, 2007; Arthur *et al.*, 2012; Brockett *et al.*, 2012; Elgersma *et al.*, 2012; Suseela *et al.*, 2016; Zhang *et al.*, 2020). In fact, African olive has already been shown to alter some of the physiochemical conditions of CPW soils during invasion (Cooke, 2001; Cuneo and Leishman, 2014).

Despite a clear link between plant community structure, abiotic soil properties, soil microbial diversity, and their legacy effects, our understanding of the role this complex relationship may play in the success of ecological restoration remains rudimentary. This study aims to address this knowledge gap by examining the soil-mediated impacts of invasive African olive on CPW plant species, and whether soil microbial interventions during restoration can mitigate these impacts. This was achieved by comparing the performance of native CPW species grown in CPW soils and African olive-invaded soils inoculated with native soils.

Specifically, the aims of this study were to: (1) assess whether African olive invasion alters the physiochemical conditions of CPW soil; (2) ascertain whether African olive-invaded soils negatively impact native plant performance; (3) investigate whether the performance of native CPW species grown in invaded soils is improved under different inoculum treatments; and (4) determine whether specific native plant-soil mutualist interactions (i.e. legume-rhizobium associations and mycorrhizal infection) can be improved under inoculation. It was hypothesised that: 1) soil nutrients and pH would be elevated in African olive-invaded soils(IS), 2) that native species grown in African olive invaded soils would have reduced performance measures (i.e. biomass,

mycorrhization, and nodulation), 3) that all inocula treatments would mitigate these negative impacts and result in improved native plant performance (all species), mycorrhization (all species), and nodulation (legumes only) compared to invaded soils and similar performance to CPW soils.

3. Materials and Methods

3.1. Soil and seed collection

CPW and IS soils were collected from The Australian Botanic Garden, Mount Annan (-34.056384, 150.774354), NSW, Australia on the 20/02/2020. Each soil type was collected from one site. Collection sites were in close proximity, thus accounting for the potentially confounding effects of climate, soil geological origin, etc. The CPW soils were collected from semi-pristine CPW (-34.067855, 150.765131) while the African olive invaded soils were collected from underneath a dense monoculture of African olive (-34.079188, 150.766452). At each site, 2 m^3 of topsoil (first 20 cm) was collected from a number of different locations beneath vegetation. After removing the leaf litter, sticks, and other overlaying organic detritus, soil was gathered using shovels and stored in individual sterilised plastic tubs which were covered and sealed with a clean plastic cover to prevent cross-contamination during transportation. Care was taken to ensure soil was taken from CPW in a way which did not impact the native species growing there and which minimised disturbance. Leaf litter was replaced after soil collection. All equipment was sterilised with 80 % EtOH prior to soil collection at each site to prevent microbial cross-contamination. In addition to these bulk soil collections, ten independent 100 g soil samples were collected from the rhizospheres ($\leq$ 5 mm directly around root surfaces) of ten healthy individuals of the five native study species (*Acacia implexa*, *Bursaria spinosa*, *Dodonaea viscosa*, *Einadia nutans*, and *Indigofera australis*) as well as ten 100 g topsoil samples from non-vegetated areas within the CPW site. Once again, all equipment was sterilised using 80 % EtOH prior to collecting each soil sample. Seeds of the five native study species were obtained from the Australian Seedbank at The Australian Botanic Garden, Mount Annan, which had been previously collected on-site.

3.2. Experimental design and setup

CPW and IS soils were separately sieved (5 mm) and then mixed with perlite (1:2 perlite-to-soil ratio). These mixtures were used to fill 22 L planting tubs (L395 mm x W285 mm x H210 mm – tapering to L340 mm x W230 mm). Ten tubs were filled with the CPW soil mixture and 40 tubs with the African olive-invaded soil mixture. 50 g of one rhizosphere soil sample from each of the

five native species were then combined to make up ten 'combined species' rhizosphere inocula (i.e. 250 g each, hereafter referred to as rhizosphere inoculum or RI). A 60 g subsample of each RI was then paired and combined with a 60 g CPW inoculum soil to make up ten mixed rhizosphere +CPW inocula (i.e. 120 g each, hereafter referred to as CPWI+RI). The remaining CPW inoculum soils were kept separately to serve as CPW inocula (hereafter referred to as CPWI). Prior to planting, seeds of *A. implexa* and *I. australis* were submerged in boiling water for two minutes, while *D. viscosa* seeds were lightly scarified with sandpaper, in order to promote germination. All 50 tubs were sown with 10 seeds of each native species (i.e. 50 seeds total/tub). Ten tubs filled with African olive-invaded soils were each inoculated with either RI, CPWI+RI, or CPWI, by evenly scattering 120 g of inoculum over the soil surface. This resulted in ten replicates of each positive control (i.e. uninoculated CPW soils), negative control (i.e. uninoculated African olive-invaded soils - IS), and three inoculum treatments (IS+RI, IS+CPWI+RI and IS+CPWI; Fig. 1).

The tubs were arranged in ten randomised blocks in a glasshouse at Macquarie University's Plant Growth Facility (-33.774917, 151.116811) and plants allowed to grow between 10/12/2019 to 15/02/2020. The temperature of the glasshouse was set at night/day of 19 ˚C and 25 ˚C, respectively. The mean photosynthetically active radiation (PAR) and relative humidity recorded at 14:00 h was 574.98 μmolm^{-2}s^{-1} (SE = 42.92) and 90.12 % respectively (SE = 0.22). Tubs were mist-watered for 2 min three times daily (at 06:00, 12:00, and 18:00). Seedlings of non-target species were removed weekly, the majority (> 90 %) of which were *Solanum nigra, Conyza bonariensis* or *Phytolacca octandra*.
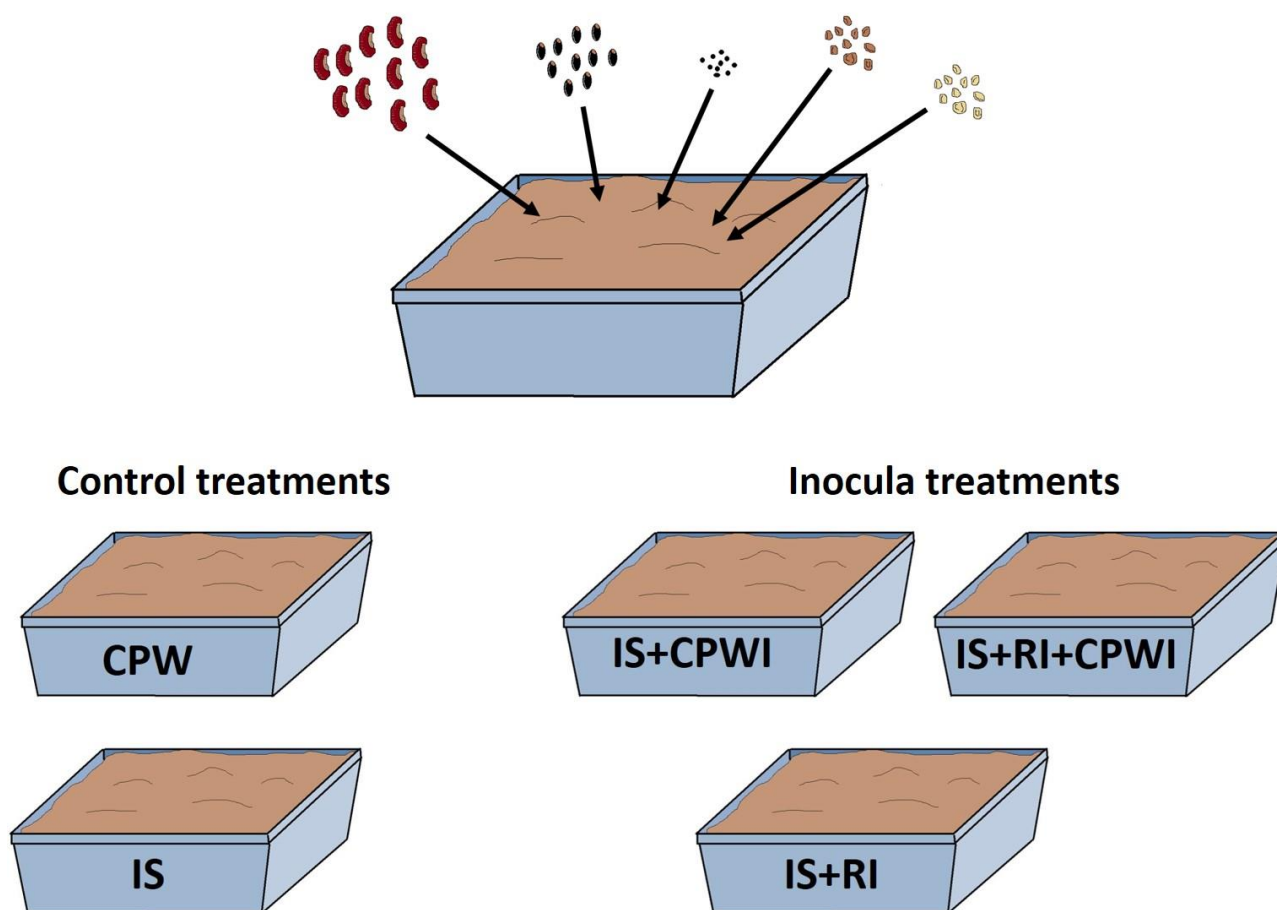
Figure 1. Ten seeds of each native species (*Acacia implexa, Bursaria spinosa, Dodonaea viscosa, Einadia nutans, Indigofera australis*) were added to growth tubs. Ten replicate tubs were used per treatment, including tubs filled with Cumberland Plain woodland soils only (CPW), African olive-invaded soils only (IS), IS + Cumberland Plain woodland bulk soil inoculum (IS+CPW), IS + Cumberland Plain woodland bulk soil and rhizosphere inoculum (CPW + RI + CPWI) and IS + rhizosphere inoculum (IS + RI).

3.3. Harvesting

Following 10 weeks of growth, the abundance (number of individual plants) of each of the five native species was recorded for each tub. Plants were washed free of soil and separated into their above and belowground biomass, with approximately 15 cm of fine root material collected from each plant (where possible and excluding *B. spinosa* plants) and stored in 70 % EtOH for later mycorrhizal staining (see below). For the two legumes (*A. implexa* and *I. australis*), the number of root nodules and their fresh weight (g) were recorded at the mesocosm-level. Collected root nodules were dehydrated after weighing and stored in 50 mL Falcon tubes containing silica gel until further

use. Plants were then bulked by species for each tub and oven-dried at 70 °C for 48 hrs before being weighed using an electronic balance (Mettler Toledo, Port Melbourne, VIC, Australia).

3.4. Soil analyses

While harvesting was being conducted, 100 g of soil was collected from differing depths at multiple points in each tub and air-dried at room temperature. These soil samples were then passed through a 0.15 mm sieve to remove the perlite. For each sieved soil sample, 0.2 mg was weighed out using an XPR Microbalance (Mettler Toledo, Port Melbourne, VIC, Australia) to be used for total carbon and nitrogen analysis. Total soil carbon and nitrogen content was determined by combustion using a TruSpec CHN analyser (LECO, St Joseph, MI, United States). Soil pH was determined using the method of Mclean (1982), using 16 g of soil from each sample in 0.01 M $CaCl_2$ at a soil to solution ratio of 1:2.5 and a pH meter (sensIONTM pH3, Hach, US). Total phosphorus in soil digestion solution was determined photometrically at 882 nm as a blue phosphate molybdate acid complex (Olsen and Sommers, 1982). Each soil sample (1 g/tub) was digested using concentrated sulphuric acid (H_2SO_4) and perchloric acid (70 % - 72 % $HClO_4$) at 350 ˚C for 50 min in a block digestion system (AIM600, Seal Analytical, UK). Total phosphorus concentration in soil extracts was measured at 882 nm using a spectrophotometer (UV mini-1240, Shimadzu, Japan). Available phosphorus was determined using 1 g soil/tub and by extraction. For this, a mixture of 0.03 N NH_4F and 0.025 N HCl solutions were used for five minutes at an extraction ratio of 1:7. The phosphorus fraction extracted is known as Bray P1 and available phosphorus concentrations in soil extracts were measured at 882 nm using a spectrophotometer (UV mini-1240, Shimadzu, Japan) (Bray and Kurtz, 1945; Menage and Pridmore, 1973; Bartlett *et al*., 1994). Nitrate (NO_3^--N) and ammonia nitrogen (NH_3-N) were determined using a soil nitrate test kit and soil ammonia test kit, respectively (Geruisi, Suzhou, China). Nitrate nitrogen concentration was measured at 220 nm and 275 nm (using 0.1 g of soil/sample), respectively, and ammonia nitrogen concentration was measured at 625 nm (using 1 g of soil/sample) using a spectrophotometer (UV mini-1240, Shimadzu, Japan).

3.5. Mycorrhizal staining and quantification

The root samples collected during harvesting (see above) were stained to assess levels of arbuscular mycorrhizal colonization according to the method described by Ho-Plágaro *et al*. (2020), with some modifications. Roots stored in 70 % EtOH at harvest were dried on a paper towel and cut

into 1 cm long segments. Using a fume hood, root segments were then submerged in 2 mL 10 % KOH and placed in a boiling water bath for 45 min to remove the cytoplasm and nuclei of the root cells. The KOH was drained, and root segments rinsed twice with water. Root segments were then submerged in 2 mL of 0.1 N HCl and left for 60 min at room temperature. After HCl was drained off, roots were covered with 2 mL of 0.05 % trypan blue in lactic acid (88 %) and placed into a boiling water bath for 30 min. The staining solution was poured off and roots rinsed in water before being submerged in 88 % lactic acid to remove excess stain and preserve the roots until microscopic observation. To determine levels of mycorrhizal colonization for each plant, three stained 1 cm root lengths per root sample were placed lengthways, and parallel, on a microscope slide and covered with a coverslip. Using a light microscope (BX53, Olympus, Japan) with digital photo capture capabilities and a 100 x optic lens, three separate non-overlapping images were taken along each root segment (i.e. nine images per root sample) (example - Fig. 9). Images were analysed using ImageJ (ImageJ Core Development 2012; Schneider *et al.*, 2012), with the proportion of total mycorrhizal colonisation recorded and assessed using ImageJ's bandpass filter and colour thresholding function (Dark-blue stained mycorrhizal structures are screened from the overall image). The selected area of colonisation was manually selected per image to ensure only mycorrhizal structures were selected. If non-mycorrhizal structures were unable to be unselected, then the image would be under-selected to avoid false positives. By subtracting the colonised area from the total field of view area, a proportion of mycorrhizal colonisation could be determined for each image.

3.6. Statistical analyses

Differences in soil physiochemical properties (pH, C, P, and N) between treatments were assessed using one-way ANOVAs. If significant differences were found, Tukey post-hoc analyses were used to determine where the differences were.

All plant performance data [i.e. abundance, above and belowground biomass, R:S, mycorrhization, nodule weight (legumes only)] was checked for normality using Shapiro-Wilk tests and log-transformed if necessary. One-way ANOVAs or Kruskal-Wallis tests (depending on data normality) were used to test whether the soil treatments significantly affected plant performance at both the species- and individual-plant-level (i.e. species mesocosm total / species mesocosm abundance). If significant differences were found, Tukey HSD (ANOVA) or Dunn-Bonferroni (Kruskal-Wallis) post-hoc analyses were used to determine where the differences were.

Nodule count data (legumes only) were analysed at the species-level using Generalised Linear Models (GLMs with a Poisson distribution). If significant differences were found, Tukey

HSD post-hoc analyses were used to determine where the differences were. Pearson correlation analyses were used to determine the relationships between nodule numbers and above- and belowground biomass, and R:S.

Mycorrhizal data was not normally distributed, so differences between treatments for each species were assessed using Kruskal Wallis tests in conjunction with Dunn-Bonferroni post hoc analyses. All statistical analyses were performed in the R statistical environment (R Core Development 2019) using the packages 'ggplot2' (Wickham, 2016), 'ggpubr' (Kassambara, 2020), 'dunn.test' (Dinno, 2017), 'dplyr' (Wickham *et al*., 2020), 'moments' (Komsta and Novomestky, 2015), 'multcomp' (Hothorn *et al*., 2008), and 'emmeans' (Lenth, 2020).

4. Results

4.1. Soil physiochemistry

CPW soils were found to have significantly lower pH, total carbon, and nitrate nitrogen than all other soils (Fig. 2). CPW soils contained significantly less total phosphorus than all other soils except IS+CPWI. Furthermore, available phosphorus was significantly lower in CPW soils compared to IS+CPWI and IS+CPWI+RI. IS+RI contained significantly less available phosphorus than all treatments, except CPW. Ammonia nitrogen was higher in CPW soils than all African olive-invaded soils, and significantly higher than inoculated African olive-invaded soils. Surprisingly, inoculum treatment occasionally appeared to affect the abiotic conditions of African-olive invaded soils.
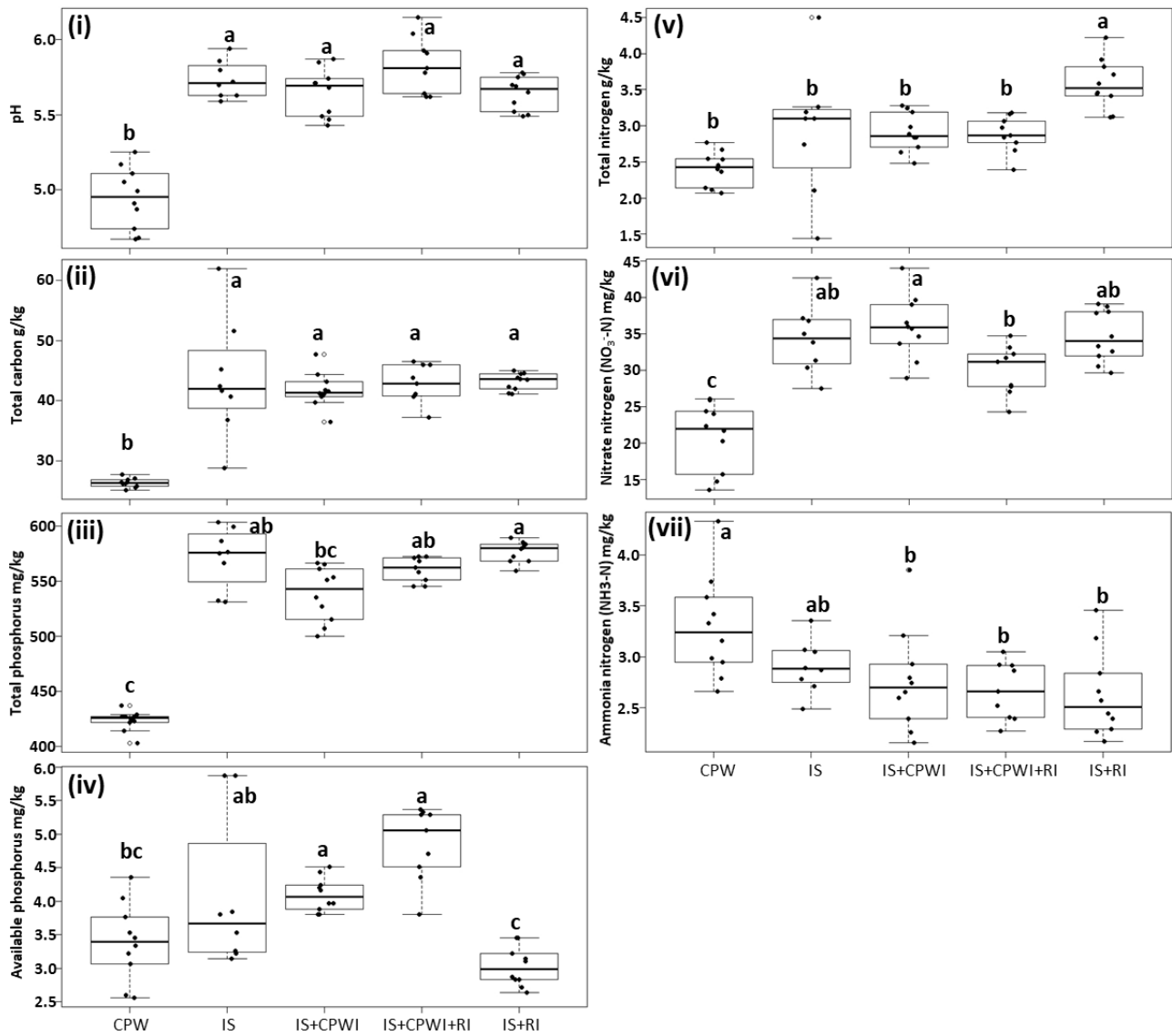
Figure 2. pH (i), total carbon (ii), total phosphorus (iii), available phosphorus (iv), total nitrogen (v), nitrate nitrogen (vi), and ammonia nitrogen (vii) for each of the soil and inoculum treatments. Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq$ 0.05). Shared letters imply similarity.

4.2. Plant abundance

At the mesocosm-level (i.e. all species per mesocosm), soil treatment did not have a significant effect on overall plant abundance (i.e. number of individuals/mesocosm). At the species x mesocosm level (all individuals/species/mesocosm), there were significant differences in

abundance between soil treatments for some species. *Acacia implexa* and *I. australis* had higher abundance in the IS+RI and IS+CPWI soils compared with African olive-invaded soils, respectively. *Einadia nutans* had greater individual abundance in CPW soils compared with IS+CPWI+RI and IS+RI soils (for species abundance see supplementary table S1).

4.3. Plant biomass and allocation

At the species x mesocosm level, *Acacia implexa* produced significantly more above- and belowground biomass when grown in CPW, IS+CPWI+RI, and IS+RI soils compared with African olive-invaded soils (Fig. 4-5). In contrast, *I. australis* produced significantly more aboveground biomass when grown in African olive-invaded soil, IS+CPWI, and IS+CPWI+RI soils compared with CPW soil. Furthermore, *I. australis* produced more belowground biomass when grown in IS+CPWI and IS+CPWI+RI soils compared with CPW soil. There were no significant differences in biomass production between soil treatments for *D. viscosa, B. spinosa* or *E. nutans* (Fig. 4-5).

On average, *Acacia implexa* individuals produced significantly more above- and belowground biomass when grown in IS+CPWI+RI and IS+RI soils respectively compared to African olive-invaded soil. In contrast, *I. australis* produced significantly more above- and belowground biomass when grown in IS, IS+CPWI, and IS+CPWI+RI soils compared with CPW soil. Similarly, *E. nutans* produced significantly more belowground biomass in African olive-invaded soil than CPW soil (Fig. 7). *Dodonaea viscosa* and *B. spinosa* showed no significant difference in above- and below-ground biomass between soil treatments at the individual plant-level.

Biomass allocation (R:S) differed between soil treatments for *E. nutans* and *I. australis*, with the former having a significantly higher R:S when grown in IS+CPWI soil compared to CPW soil and the latter having significantly higher R:S when grown in CPW soil compared to African olive-invaded soil (Fig. 6). Aboveground biomass production of *I. australis* was significantly positively correlated with pH, total carbon, total phosphorus, total nitrogen, and nitrate nitrogen (Fig. 3). With the exception of nitrate nitrogen, these results were mirrored for belowground biomass. The aboveground biomass of *D. viscosa* was also positively correlated with soil pH and total phosphorus. Biomass production of *A. implexa* and *E. nutans* were not correlated to any of the abiotic soil factors. The R:S of *I. australis* was significantly negatively correlated with pH, total phosphorus, and total carbon. The remaining three species displayed no significant correlations between biomass allocation and any of the abiotic soil factors.
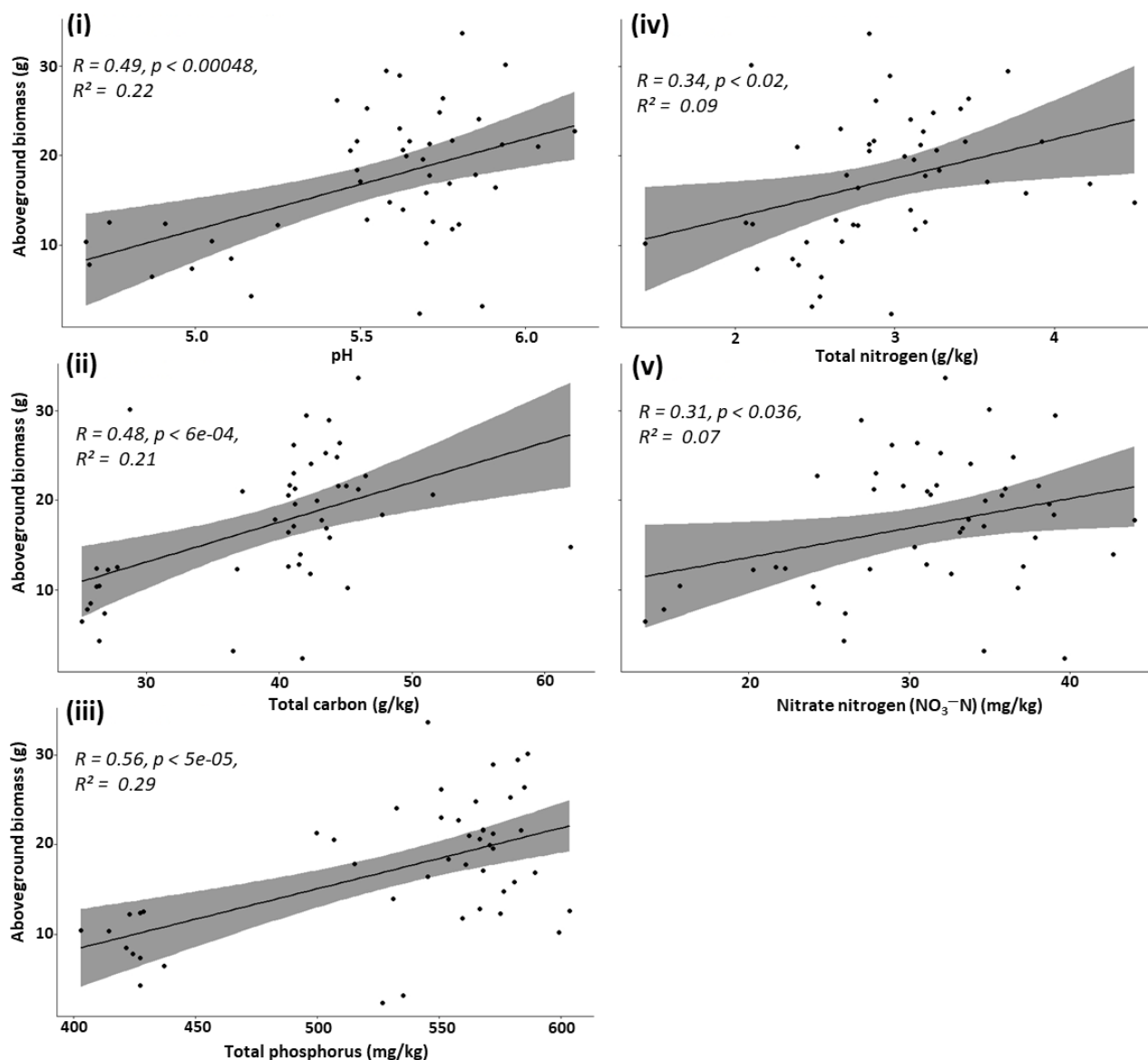
Figure 3. Relationships between aboveground biomass of *Indigofera australis* and soil physiochemical conditions: pH (i), total carbon (ii), total phosphorus (iii), total nitrogen (iv), and nitrate nitrogen (v). All correlations are significant ($p \leq 0.05$).
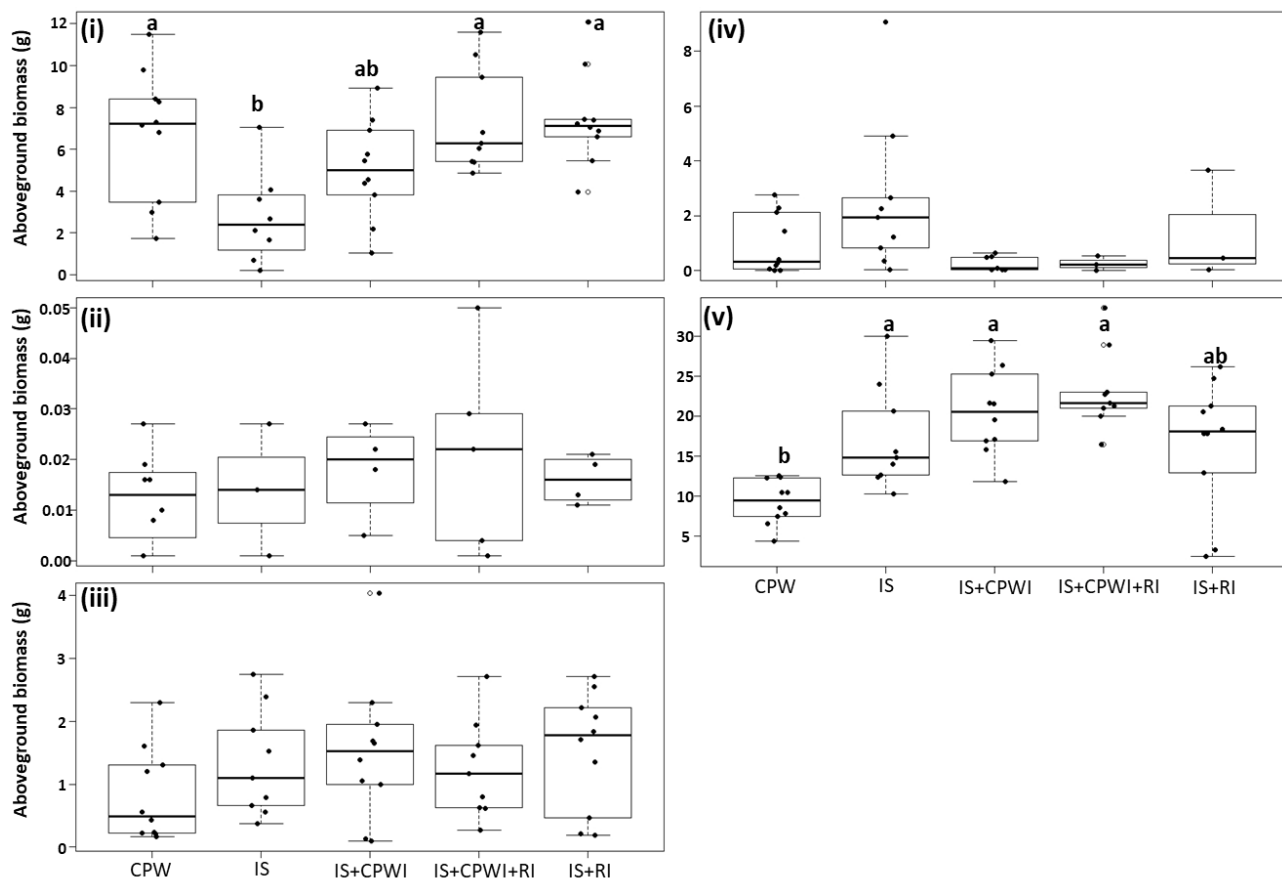
Figure 4. Aboveground biomass of *Acacia implexa* (i), *Bursaria spinosa* (ii), *Dodonaea viscosa* (iii), *Einadia nutans* (iv), and *Indigofera australis* (v) in response to soil inoculum treatments at the mesocosm level. Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq 0.05$). Shared letters imply similarity.
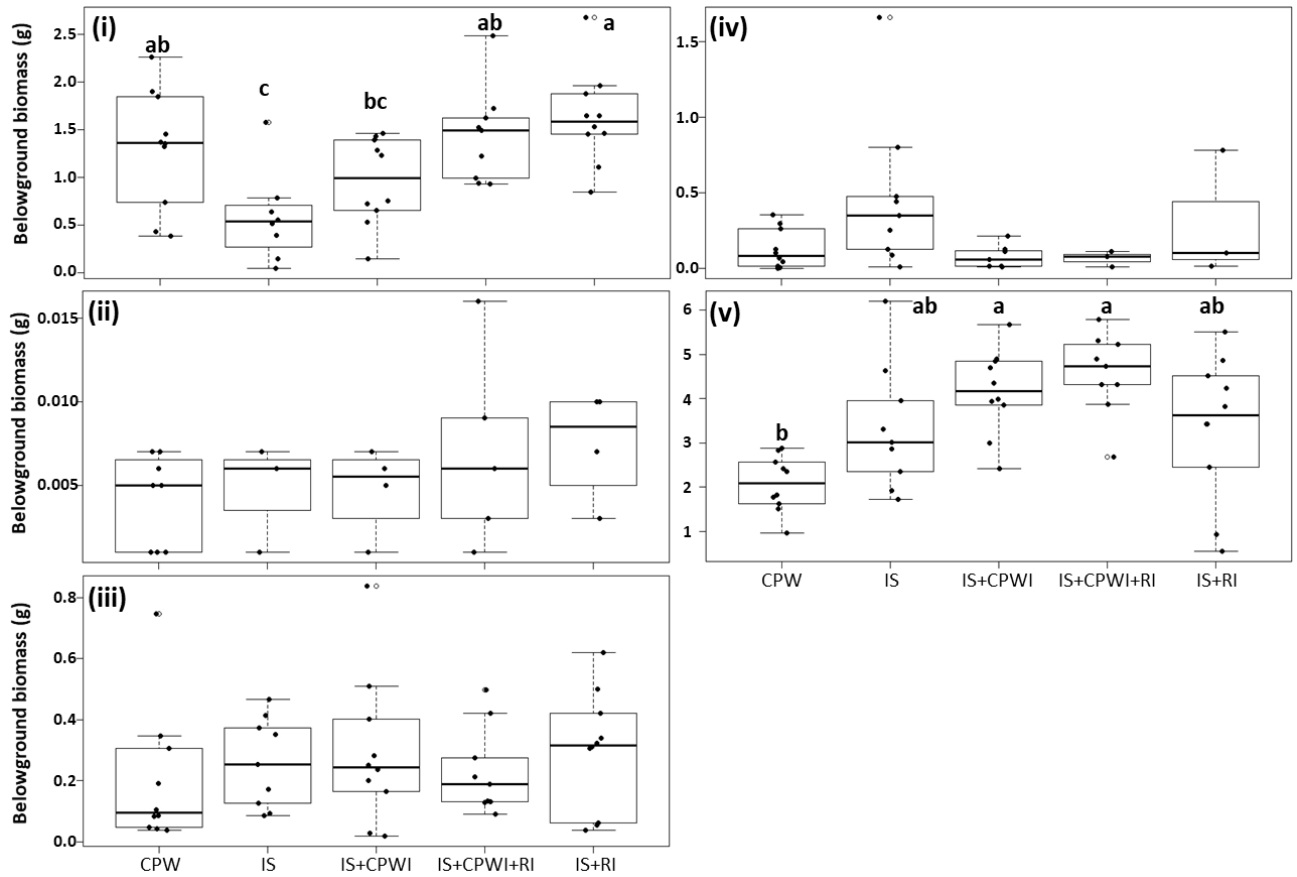
Figure 5. Belowground biomass of *Acacia implexa* (i), *Bursaria spinosa* (ii), *Dodonaea viscosa* (iii), *Einadia nutans* (iv), and *Indigofera australis* (v) in response to soil inoculum treatments at the mesocosm level. Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq 0.05$). Shared letters imply similarity.
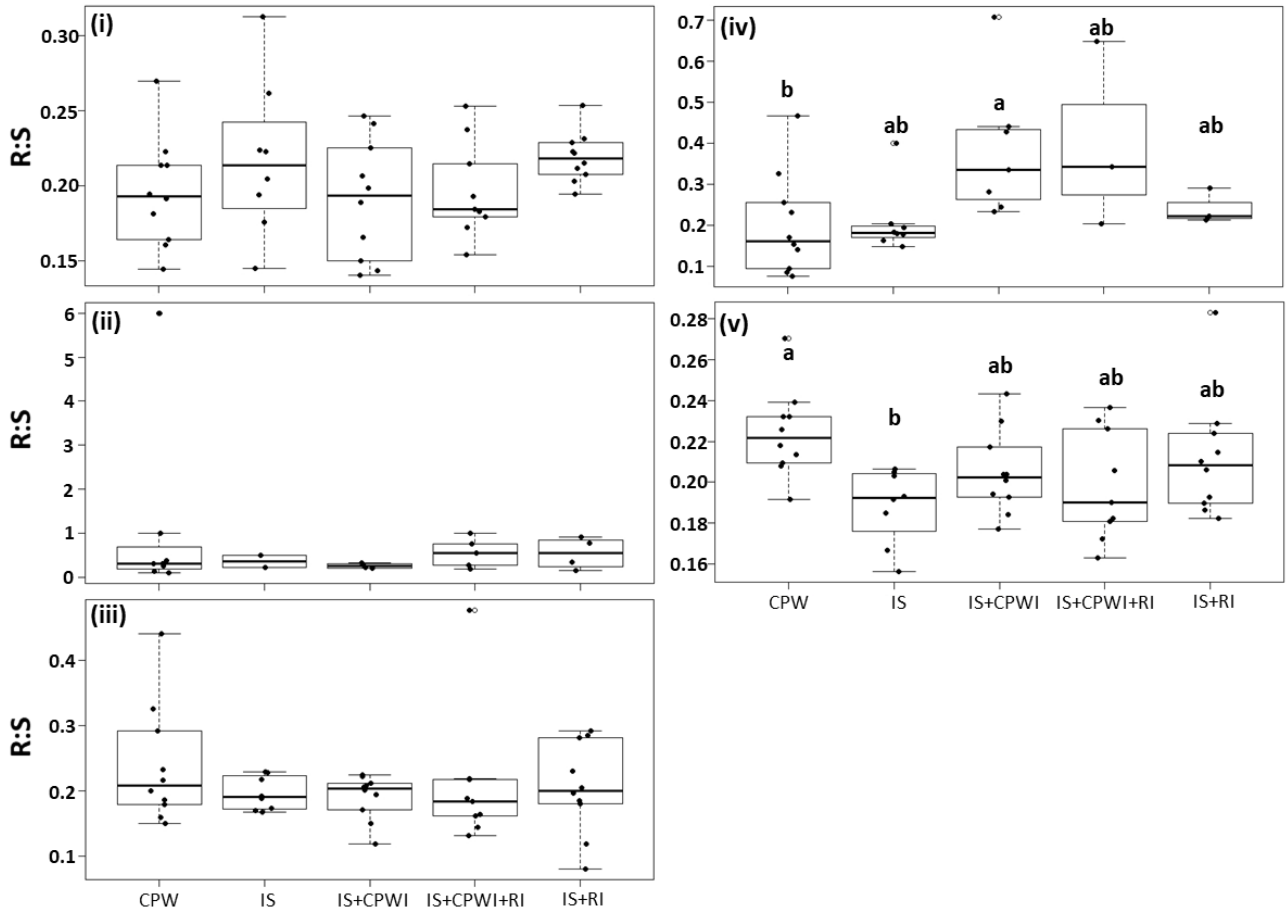
Figure 6. Root to shoot ratio (R:S) of *Acacia implexa* (i), *Bursaria spinosa* (ii), *Dodonaea viscosa* (iii), *Einadia nutans* (iv), and *Indigofera australis* (v) in response to soil inoculum treatments at the mesocosm level. Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq 0.05$). Shared letters imply similarity.
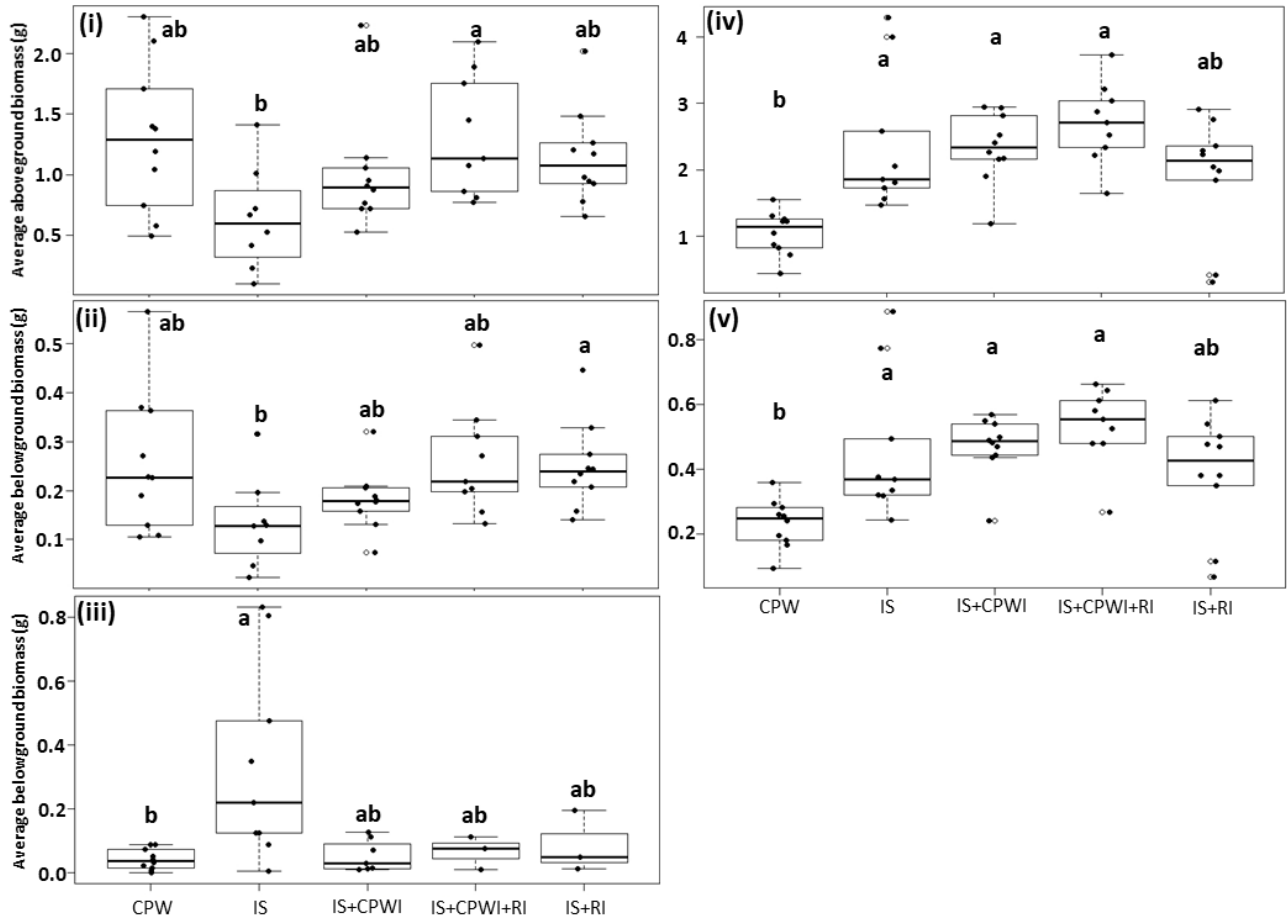
Figure 7. Average aboveground biomass of *Acacia implexa* (i) and *Indigofera australis* (iv). Average belowground biomass of *A. implexa* (ii), *Einadia nutans* (iii), and *I. australis* (v). Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq 0.05$). Shared letters imply similarity. Belowground biomass for *Einadia nutans* and *Dodonaea viscosa* and above- and belowground biomass for *Bursaria spinosa* have been omitted due to the lack of significant differences between treatments.

4.4. Mycorrhizal quantification

Of all the species tested for mycorrhization, only *Dodonaea viscosa* showed differences in mycorrhizal colonisation between treatments, having significantly (or near-significantly) higher colonisation when grown in inoculated African olive-invaded soils compared with uninoculated African olive invaded soils only (Fig. 8).
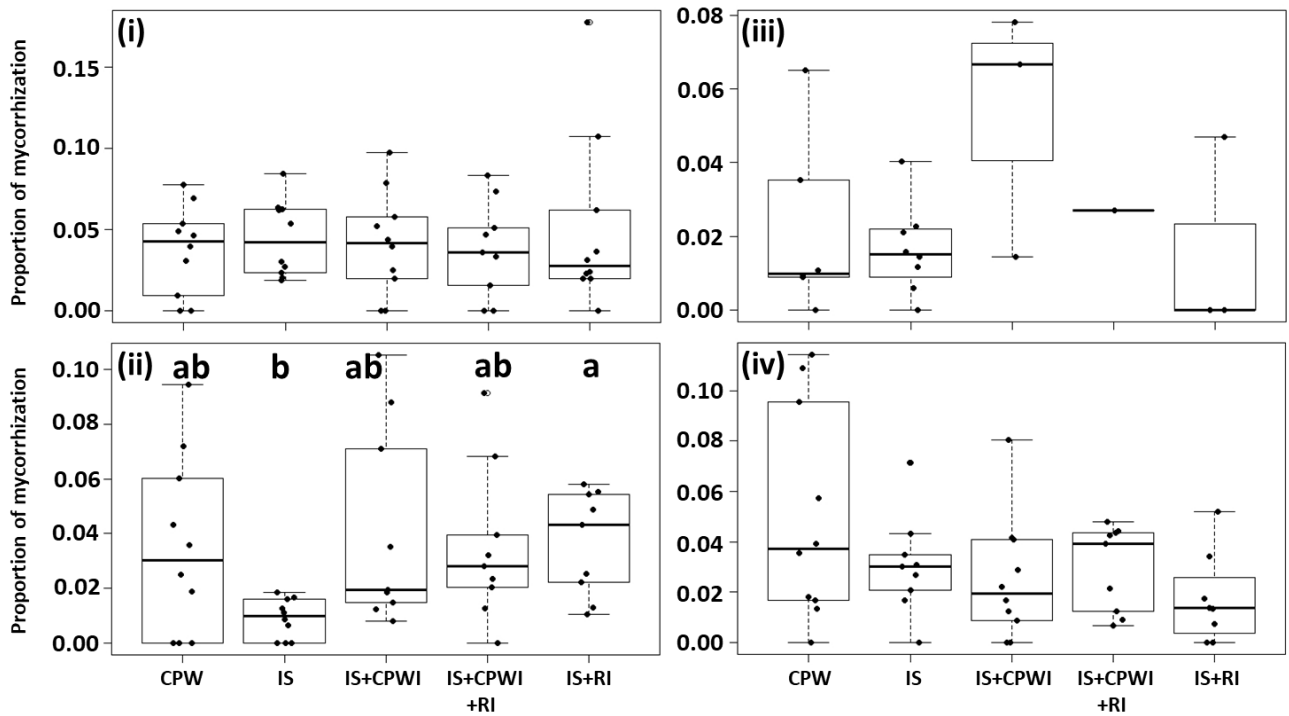
Figure 8. The proportion of mycorrhization of *Acacia implexa* (i), *Dodonaea viscosa* (ii), *Einadia nutans* (iii), and *Indigofera australis* (iv) roots across different soil treatments. Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq 0.05$). Shared letters imply similarity.
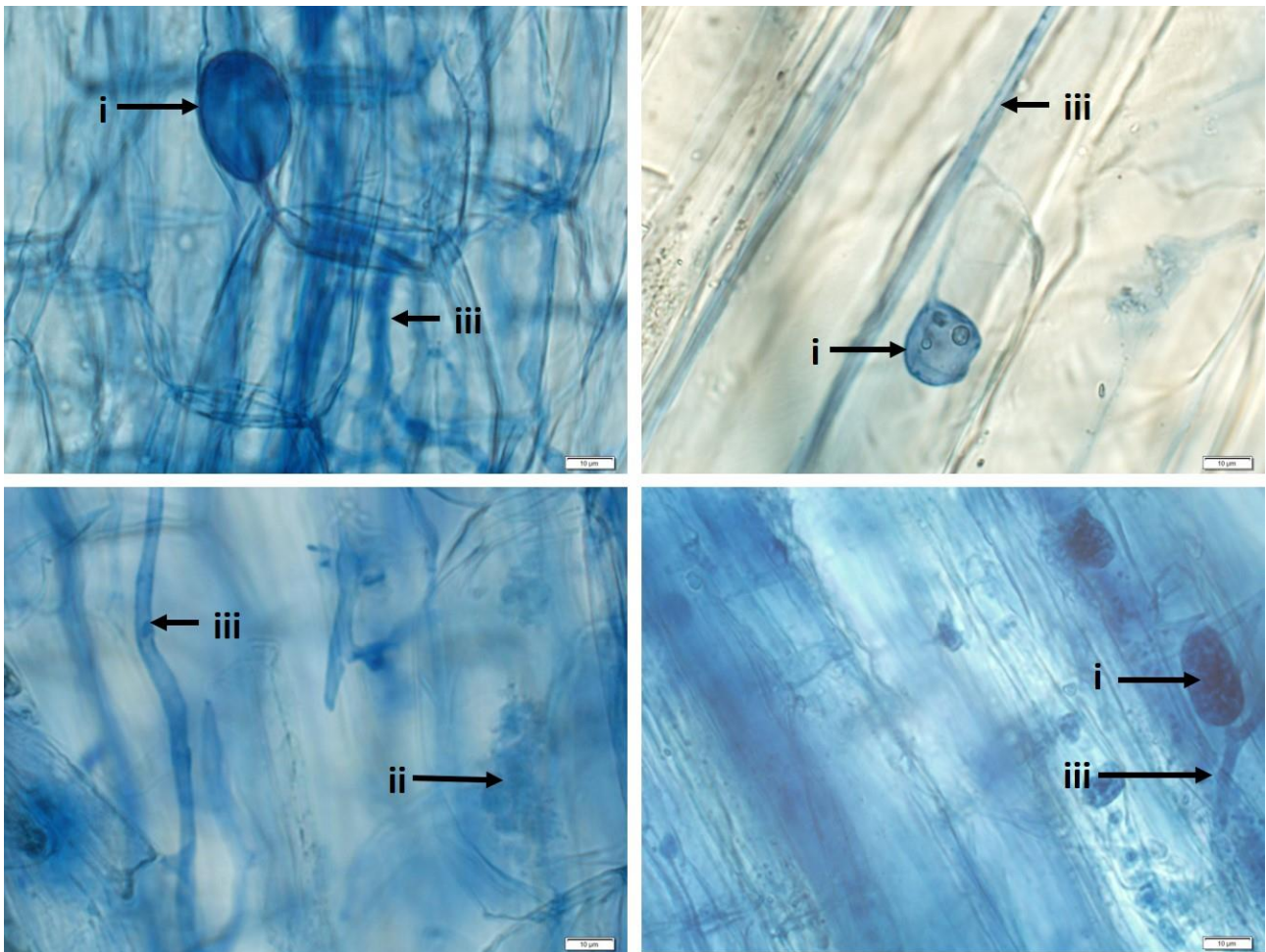
Figure 9. Examples of arbuscular mycorrhization including vesicles (i), arbuscules (ii), and hyphae (iii) [mycorrhizal organs stained dark blue] in the roots of *Acacia implexa* (top left), *Dodonaea viscosa* (bottom left), *Einadia nutans* (top right), and *Indigofera australis* (bottom right) under 100 x magnification.

4.5. Nodulation

For *A. implexa,* plants grown in CPW soil produced the most nodules while those grown in African olive-invaded soil produced the least nodules (Fig. 10). Nodulation for this species was increased when inocula were added, with plants producing more nodules than when grown in African olive-invaded soil, although these differences were not significant. Of the three inoculum treatments, the addition of both CPW soil and rhizosphere soil inocula had the greatest benefit for *A. implexa* nodulation. The nodulation of *I. australis* did not differ between treatments at a species x mesocosm or individual plant level (Fig. 10).

At the individual-plant level, *A. implexa* nodulation mirrored the species x mesocosm-level results (see above). Similarly, *A. implexa* nodule mass per plant was significantly greater in CPW

soil than in African olive-invaded soil. Average individual nodule mass for *A. implexa* did not differ between soil treatments. For *A. implexa*, nodule count at both the individual plant- and species x mesocosm-level was significantly negatively correlated to total carbon (both), total phosphorus (both) and/or total nitrogen (plant-level only). For both legumes, nodulation (nodule count) was found to be highly positively correlated with nodule weight and above- and belowground biomass (Fig. 11).
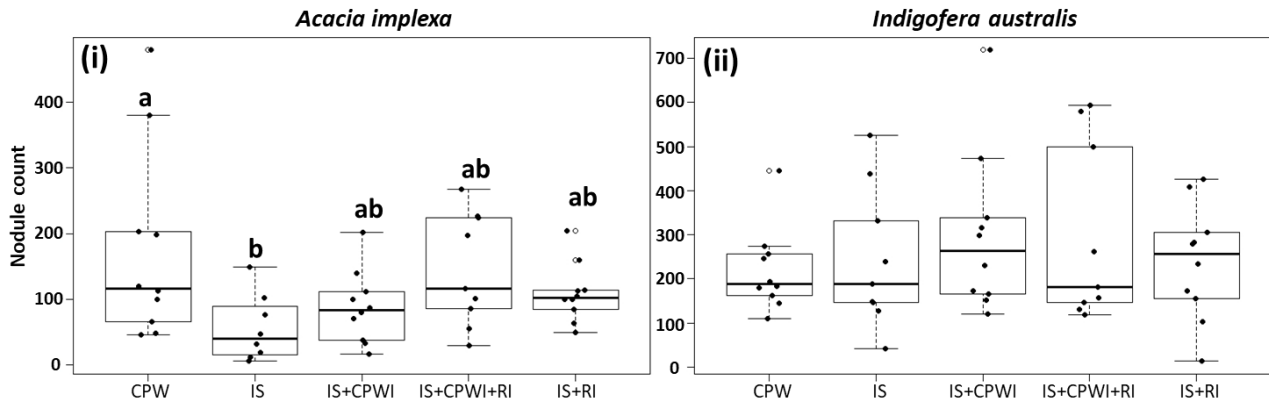
Figure 10. Overall number of root nodules (nodulation) of *Acacia implexa* (i) and *Indigofera australis* (ii) plants across different soil treatments at the mesocosm-level. Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq 0.05$). Shared letters imply similarity.
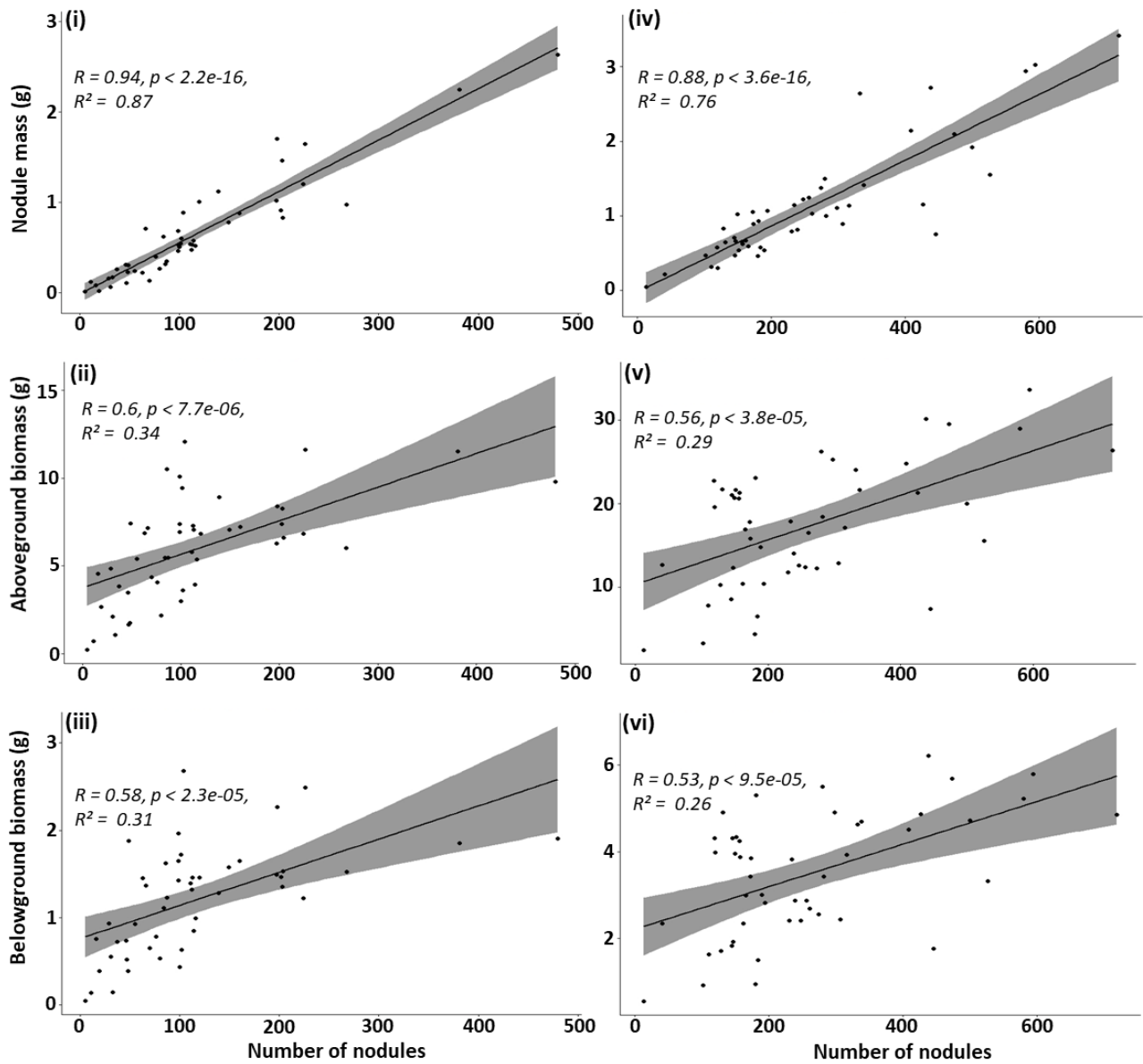
Figure 11. Correlations between nodulation (nodule numbers) of *Acacia implexa* (right) and *Indigofera australis* (left) and nodule mass (i and iv, respectively), aboveground biomass (ii and v, respectively), and belowground biomass (iii and vi, respectively). All correlations were significant ($p \leq 0.05$).

5. Discussion

Invasive plants may dramatically alter the (a)biotic components of the ecosystems they invade (Suseela *et al*., 2016). These changes can not only directly impact native species but can also persist as 'legacy effects' long after the invaders removal (Holdredge and Bertness, 2011; Pearson *et al*., 2016; Suseela *et al*., 2016; Pickett *et al*., 2019). For example, there is a growing body of evidence suggesting that invasive species can increase soil nutrient levels and alter soil pH (raising or lowering) (Allison and Vitousek, 2004; Vanderhoeven *et al.*, 2005; Dassonville *et al*., 2007; Gibbons *et al*., 2017; Stefanowicz *et al*., 2019; Zhang *et al*., 2020). The findings of this study are consistent with this literature, as soils under African olive were found to possess higher nutrient concentrations and pH than undisturbed Cumberland Plain Woodland soils. These findings are consistent with other available studies performed in the CPW and which assessed the impact of African olive on CPW (Cooke, 2001; Leishman and Thomson, 2005; Cuneo and Leishman, 2015).

The alterations to soil chemistry under African olive invasion may have occurred through various mechanisms. For example, dense African olive thickets may lead to changes in litter fluxes (i.e. high leaf litter input) that differ in chemistry, quantity, and quality from litter inputs of native CPW plants (Allison and Vitousek, 2004; Ashton *et al*., 2005; Arthur *et al*., 2012; Incerti *et al*., 2018). Further, soil physiochemical changes may have resulted from the excretion of chemical compounds released by fallen litter and/or root exudates of African olives (Neumann, 2007; Olanrewaju *et al*., 2019). These changes often facilitate positive invader-soil feedbacks and therefore increase the vulnerability of habitats to further invasion once the original invader has been removed (Symstad *et al*., 2004). Land-use history could also offer an explanation, as farming and grazing in areas that are now invaded by African olive may have contributed to the increased soil nutrients and pH we observed and also may explain why African olive could have preferentially invaded these areas.

Ammonia nitrogen (NH_3-N) and available phosphorus were not consistently elevated in soils under African olive canopy (Fig. 2). It is possible that the higher levels of ammonia nitrogen in CPW soils reflects the presence of native N-fixing legumes like acacias, which are known to elevate ammonia nitrogen in soils (e.g. see Rodríguez-Escheverría *et al*. (2009)). Legumes gain NH_3-N via biological nitrogen fixation through their symbiotic relationships with rhizobia (Bernhard, 2010). Organic nitrogen in legume leaf litter is then decomposed by soil microbes to produce ammonium, a process known as nitrogen mineralization (Clarholm, 1985; Zahran, 1999; Giller, 2001; Patriarca *et al*., 2002). A complete lack of N-fixing legumes under dense African olive canopies (personal observation; Cuneo and Leishman, 2006) may, therefore, in part explain the lower levels of ammonia nitrogen observed in these soils. This, however, does not account for the lower ammonia

nitrogen levels found in inoculated soils compared to invaded, with the former perhaps increasing rhizobial activity to a minor extent as may be the case when observing the non-significantly increased nodulation of *A. implexa* under all inocula treatments.

The increased levels of nitrate nitrogen found in the African olive-invaded soils is a typical feature of invasion (Stefanowicz *et al*., 2017), the abundant, nutrient-rich invasive leaf litter often leading to increased decomposer activity (Eaton, 2001; Allison and Vitousek, 2004; Arthur *et al*., 2012; Eisenhauer *et al*., 2018). Additionally, large dense African olive monocultures increase shading, which may reduce evaporation, resulting in moister soils (Kidron *et al*., 2009; Cuneo and Leishman, 2013). When combined with high leaf litter production, these features are expected to increase decomposer activity, soil carbon and nitrification rates, resulting in increased nutrient concentrations (Cortez, 1998; Allison and Vitousek, 2004; Wardle *et al*., 2004; Lee *et al*., 2014; McTee *et al*., 2017; Zhang *et al*., 2019; Zhang *et al*., 2020). Indeed, this may explain why ammonia was highest in CPW soils, as it may have been converted into nitrate nitrogen by the increased presence of decomposer microbes in African olive-invaded soils. The presence of increased available phosphorus under two of the inocula treatments may be explained by the presence of efficient native rhizosphere and free-living phosphatase producing micro-organisms, which typically mineralise (often-scarce) phosphorus (Richardson and Simpson, 2011; Spohn and Kuzyakov, 2013). Therefore, the significant reduction of available phosphorus in the rhizosphere inocula was unexpected. Although some rhizosphere microbes can decompose P-mobilising root exudates, thereby reducing available phosphorus, the fact that available phosphorus was so much lower in the rhizosphere soils compared to the other inocula treatments, suggests this to be highly unlikely in my case (Deubel and Merbach, 2005). Indeed, the increased carbon content observed in all African olive invaded soils would be expected to facilitate microbial mediated phosphorus retention (Xu *et al*., 2020). Further, alkaline soils are known to bind available phosphorus into phosphates i.e. apatite minerals, but the similar pH across all inocula treatments also makes this unlikely (Deubel and Merbach, 2005).

Overall, I found that the physiochemistry of African olive-invaded soils did not tend to negatively impact the performance of the CPW study species. In fact, *I. australis* and *D. viscosa* biomass positively correlated to increases in soil nutrients and pH, suggesting tolerance and even benefit to higher pH and nutrient levels, this being consistent with the findings of previous studies (Thomson and Leishman, 2004; Wang *et al*., 2018). The responsiveness of these two species to increased soil nutrients and pH may be a result of their 'pioneer' nature (Knox and Clarke, 2006). That is, both species generally respond strongly to post-fire soil nutrient flushes and increases in pH (Wright and Clarke, 2007; Morris *et al*., 2016; Chungu *et al*., 2020). Unlike the neighbouring

nutrient-poor Sydney sandstone soils, which contain phosphorus and nitrogen concentrations between 30-100 mg/kg^{-1} and 180-600 mg/kg^{-1} (Leishman 1990, Hannon, 1956), respectively, CPW soils are derived from relatively nutrient-rich Wianamatta shale (Bannerman and Hazelton, 1989; Benson and Howell, 2002) and typically contain phosphorus and nitrogen concentrations between 74-425 mg/kg^{-1} (Beadle, 1962; Cuneo and Leishman, 2015) and 800-1660 mg/kg^{-1}, respectively (Fitzgerald, 2009). CPW soils are therefore home to plant communities adapted to higher nutrient loads. For example, Sydney sandstone species can experience high mortality rates when grown in soils containing only half the amount of phosphorus that is typically found in CPW soil, while CPW species grow readily (Thomson and Leishman, 2004), as observed in this study.

Alterations to soil physiochemical properties by invasive plant species have been repeatedly linked to dramatic changes in soil microbial communities (Zhalnina *et al.*, 2015; Zhang *et al.*, 2019; Zhang *et al.*, 2020). While not explicitly tested here, it is reasonable to expect African olive-invaded soil to harbour substantially different soil microbial communities than those in uninvaded CPW (Batten *et al.*, 2006; Batten *et al.*, 2008). In general, soil pH, nitrogen availability, soil organic carbon content, temperature, and redox status, in order of importance, have been shown to be important modulators of soil microbial community diversity and structure (Fierer, 2017). The first three of these physiochemical conditions were significantly altered in African olive -invaded soils. One of the ways in which these changes may negatively impact native CPW species is through the reduction of effective mutualists (Mangla and Callaway, 2008; Lankau *et al.*, 2014). Impacts on rhizobium symbionts were evident for *Acacia implexa* which showed reduced growth and nodulation when grown in African olive-invaded soil. These negative impacts were only somewhat mitigated by the use of inocula (RI and CPWI + RI). Nevertheless, this suggests that the inocula were successful in introducing beneficial rhizobia into the African olive-invaded soils. In contrast, *I. australis* showed no difference in nodulation between any of the soil treatments.

Differing nodulation responses of *A. implexa* and *I. australis* may reflect differences in their levels of mutualist specificity. That is, *A. implexa* is likely to have more specialised rhizobial requirements than *I. australis*. Rhizobial associations generally involve higher levels of mutualist-host specialisation than mycorrhizal associations (Albrecht *et al.*, 1999; Bidartondo and Bruns, 2002; Garg *et al.*, 2006; Radutoiu *et al.*, 2007; Smith and Read, 2008; Wang *et al.*, 2012; Andrews and Andrews, 2017). From a rhizobium mutualism viewpoint, acacias are known to mainly form effective symbioses with members of the genus *Bradyrhizobium* (Turk *et al.*, 1992; Murray *et al.*, 2001; Lafay and Burdon, 2001; Le Roux *et al.*, 2018). *Acacia implexa* is no exception, only performing well when in association with its own bradyrhizobial strains (Burdon *et al.*, 1999; Thrall *et al.*, 2000; Lafay and Burdon, 2001). *Indigofera australis*, on the other hand, is capable of forming

symbioses with a wide range of rhizobial genera (Burdon *et al*., 1999; Thrall *et al*., 2000; Thrall *et al*., 2011). Invasive African olive may impact the availability of CPW soil mutualists in two ways. First, changes in soil abiotic conditions such as pH may impact the abundance of *Bradyrhizobium* strains (Fujihara and Yoneyama, 1993; Lafay and Burdon, 2001; Bååth and Anderson, 2003). Second, the absence of native CPW legumes in dense African olive infestations may lead to the disappearance of CPW rhizobia (Jensen and Sørensen, 1987; Depret *et al*., 2004; Bartelt-Ryser *et al*., 2005; Vogelsang and Bever, 2009; Lekberg *et al*., 2013; Lankau *et al*., 2014; Zubek *et al*., 2016).

The impacts of invasive African olive invaded soil on soil mutualists were not so evident for mycorrhizal fungi, with all native plants, except *Dodonaea viscosa,* showing no difference in levels of mycorrhization between treatments. In general, though exceptions do exist, invasive plants reduce mycorrhizal richness and abundance, which may negatively impact native plant mycorrhization and performance (Vogelsang and Bever, 2009; Lekberg *et al*., 2013; Lankau *et al*., 2014; Zubek *et al*., 2016). My observations do not conform to these general observations, though the increase in *Dodonaea viscosa* mycorrhization in rhizosphere inocula soil compared to African olive invaded soil could imply some impact of African olive on the mycorrhization of this species. While the absence of native host plants under dense African olive thickets, rather than African-olive induced soil chemical changes, may be responsible for lower mycorrhizal diversity and/or abundance, leading to reduced mycorrhization of *D. viscosa* in African olive invaded soil (also see Lankau *et al*., 2014), the lack of any response from the other species or indeed in other inocula treatments for *D. viscosa,* suggest this is not the case and that in general mycorrhizae are not greatly impacted by African olive invasion.

While this study shows inoculation to be a successful method of reintroducing native microbes into invaded soils, we did not see a marked increase in biomass performance for most plant species under inocula treatments, *A. implexa* being the only exception. Indeed, while inoculation can be a useful tool for microbial restoration, there are many factors (e.g. species responses, soil physiochemistry, etc.) which can dictate its success (Harris, 2009, Smith *et al*., 2018), and these need to be considered prior to implementation. For example, inoculation success can be improved by removing invaded topsoil prior to application (Wubs *et al*., 2016), or can be reduced when invasive species respond positively to inocula applied for natives (Smith *et al*., 2018). Inocula which are beneficial to some natives may also be ineffective for others, in some cases even hindering native plant performance and survival (Middleton and Bever, 2012). Such factors are therefore important to consider when assessing whether inoculation is a viable restorative measure for specific species, soils, and sites.

In conclusion, the addition of soil inocula to African olive-invaded soils did not, in general, prove beneficial to the selected native study species. From these findings, it is suggested that the introduction of native soil inocula may not be useful as a broad-scale restoration intervention for CPW but may have benefits at the species-level. It should be noted that the lack of responsiveness of most study species to inoculum treatments (i.e. *B. spinosa, D. viscosa,* and *E. nutans*), may be because of the intense competitive effects they experienced from *A. implexa* and *I. australis*, which alone made up 91.6 % of all biomass across all treatments. This research suggests that land managers should place an emphasis on the invasion history of a site when selecting native plants for restoration purposes, as individual species may respond differently to new soil (a)biotic conditions.

Future studies should also assess the plant-soil feedbacks associated with African olive invasion and the effect this species has on specific components of soil microbiota. For instance, growing African olive in sterilised and unsterilised soils will provide valuable insights into how the build-up of nutrients, mutualists and/or pathogens influence the species' invasive performance and impacts on native CPW species (Klironomos, 2002; Levine *et al*., 2006; Nijjer *et al*., 2007; Stricker and Harmon, 2016). Similarly, assessing how the effectiveness of specific native plant mutualists (i.e. rhizobia and mycorrhizae) respond to different (a)biotic conditions, such as pH or nutrients (Zhalnina *et al*., 2015), may be useful in determining the specific ways in which African olive impacts specific components of the soil microbiome.

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7. Supplementary materials:

Table S1: Mean and standard error for performance measures of CPW species, soil physiochemistry, and community metrics across soil treatments. All values shown are rounded to three decimal places unless unable to do so. Note: CPW - Cumberland Plain Woodland soil, IS - African olive invaded soil, IS+CPWI - African olive-invaded soil with CPW inoculum added, IS+CPWI+RI – African olive-invaded soil with CPW and rhizosphere inoculum added, and IS+RI -African olive-invaded soil with rhizosphere inoculum added. Significant differences between treatments are indicated by different letters ($p \leq 0.05$). Shared letters imply similarity.

		Soil Treatment				
		CPW	IS	IS+CPWI	IS+CPWI+ RI	IS+RI
Acacia implexa	Aboveground biomass (AGB) (g)	Mean	6.742 **a**	2.755 **b**	5.039 **ab**	7.37 **a**	7.41 **a**
		SE	0.985	0.772	0.754	0.831	0.715
	Belowground biomass (BGB) (g)	Mean	1.308 **ab**	0.58 **c**	0.961 **bc**	1.437 **ab**	1.621 **a**
		SE	0.198	0.167	0.144	0.166	0.157
	Nodule count	Mean	175.4 **a**	55 **b**	87.4 a**b**	144.67 **ab**	108.9 **ab**
		SE	46.433	17.858	17.462	28.492	14.233
	Root:Shoot	Mean	0.196	0.218	0.191	0.197	0.219
		SE	0.012	0.018	0.013	0.011	0.005
	Nodule mass combined (g)	Mean	1.129 **a**	0.265 **b**	0.452 **ab**	0.747 **ab**	0.585 **ab**
		SE	0.267	0.104	0.111	0.168	0.072
	Abundance	Mean	5.3 **ab**	3.875 **b**	5.2 **ab**	5.889 **ab**	6.6 **a**
		SE	0.473	0.35	0.712	0.484	0.306

Indigofera australis	Aboveground biomass (AGB) (g)	Mean	9.273 **b**	17.151 **a**	20.554 **a**	23.187 **a**	16.551 **ab**
		SE	0.881	2.159	1.709	1.699	2.571
	Belowground biomass (BGB) (g)	Mean	2.071 **b**	3.33 **ab**	4.165 **a**	4.572 **a**	3.374 **ab**
		SE	0.199	0.475	0.303	0.306	0.515
	Nodule count	Mean	219.7	218.9	298.4	296.56	237.9
		SE	29.856	52.842	57.808	67.278	41.202
	Root:Shoot	Mean	0.224 **a**	0.191 **b**	0.205 **ab**	0.199 **ab**	0.212 **ab**
		SE	0.007	0.006	0.006	0.009	0.009
	Nodule mass combined (g)	Mean	0.842	1.222	1.230	1.352	1.007
		SE	0.115	0.301	0.291	0.34	0.18
	Abundance	Mean	9 **a**	7.4 **b**	8.9 **a**	8.667 **ab**	8.5 **ab**
		SE	0.333	0.377	0.433	0.289	0.224
Bursaria spinosa	Aboveground biomass (AGB) (g)	Mean	0.012	0.014	0.018	0.021	0.016
		SE	0.003	0.008	0.005	0.009	0.002
	Belowground biomass (BGB) (g)	Mean	0.004	0.005	0.005	0.007	0.008
		SE	0.001	0.002	0.001	0.003	0.002
	Root:Shoot	Mean	1.06	0.574	0.255	0.551	0.542
		SE	0.713	0.228	0.027	0.151	0.177
	Abundance	Mean	1.2	0.33	0.7	0.556	0.6
		SE	0.249	0.153	0.335	0.176	0.306

Dodonaea viscosa	Aboveground biomass (AGB) (g)	Mean	0.822	1.333	1.537	1.243	1.53
		SE	0.234	0.282	0.359	0.256	0.298
	Belowground biomass (BGB) (g)	Mean	0.199	0.26	0.293	0.231	0.298
		SE	0.07	0.049	0.077	0.047	0.062
	Root:Shoot	Mean	0.238	0.201	0.191	0.21	0.205
		SE	0.029	0.01	0.011	0.035	0.022
	Abundance	Mean	3.9	4.778	4.9	4.778	4.3
		SE	0.64	0.542	0.547	0.465	0.367
Einadia nutans	Aboveground biomass (AGB) (g)	Mean	0.959	2.586	0.261	0.262	1.384
		SE	0.343	0.944	0.102	0.154	1.139
	Belowground biomass (BGB) (g)	Mean	0.142	0.664	0.079	0.066	0.299
		SE	0.041	0.17	0.029	0.03	0.243
	Root:Shoot	Mean	0.201 **b**	0.212 **ab**	0.382 **a**	0.398 **ab**	0.243 **ab**
		SE	0.039	0.025	0.063	0.131	0.025
	Abundance	Mean	2.3 **a**	1.44 **ab**	1 **ab**	0.33 **b**	0.7 **b**
		SE	0.367	0.221	0.298	0.167	0.423
Shannon, Evenness, Richness	Shannons (H)	Mean	1.36 **a**	1.271 **ab**	1.218 **ab**	1.173 **b**	1.17 **b**
		SE	0.03	0.045	0.042	0.033	0.057
	Richness	Mean	4.8 **a**	4.333 **ab**	4.1 **ab**	3.889 **ab**	3.7 **b**
		SE	0.133	0.167	0.3	0.261	0.277
	Evenness	Mean	0.87	0.871	0.885	0.886	0.923
		SE	0.014	0.026	0.018	0.025	0.018

Soil physiochemistry	pH	Mean	4.944 **b**	5.749 **a**	5.647 **a**	5.833 **a**	5.643 **a**
		SE	0.065	0.036	0.05	0.063	0.036
	Total carbon (g/kg)	Mean	26.367 **b**	43.168 **a**	41.767 **a**	42.756 **a**	43.157 **a**
		SE	0.242	2.785	0.932	1.03	0.447
	Available phosphorus (mg/kg)	Mean	3.395 **bc**	4.131 **ab**	4.096 **a**	4.855 **a**	3.029 **c**
		SE	0.181	0.364	0.079	0.182	0.093
	Total phosphorus (mg/kg)	Mean	423.405 **c**	571.335 **ab**	538.083 **bc**	560.504 **ab**	576.735 **a**
		SE	2.905	8.579	7.864	3.736	2.979
	Total nitrogen (g/kg)	Mean	2.404 **b**	2.959 **b**	2.906 **b**	2.878 **b**	3.581 **a**
		SE	0.075	0.255	0.085	0.084	0.109
	Nitrate nitrogen (NO_3^--N) (mg/kg)	Mean	20.868 **c**	34.439 **ab**	35.913 **a**	29.996 **b**	34.625 **ab**
		SE	1.469	1.37	1.368	1.133	1.122
	Ammonia nitrogen (NH_3-N) (mg/kg)	Mean	3.294 **a**	2.881 a**b**	2.757 **b**	2.667 **b**	2.625 **b**
		SE	0.158	0.093	0.157	0.094	0.133