Agriculture, Ecosystems and Environment 163 (2012) 14–27

Contents lists available at SciVerse ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

The Ridgefield Multiple Ecosystem Services Experiment: Can restoration of former agricultural land achieve multiple outcomes?

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ARTICLE INFO

Article history:
Received 5 July 2011
Received in revised form 2 February 2012
Accepted 24 February 2012
Available online 22 March 2012

Keywords:
Carbon sequestration, Global change, Novel ecosystem, Old-field, Plant trait approach, Ecosystem service trade-offs

ABSTRACT

The ability of restoration approaches to provide valued ecosystem services needs to be assessed. The emerging carbon market provides an incentive to afforest agricultural landscapes and could potentially achieve multiple outcomes. However, planting monocultures for carbon sequestration may preclude effective delivery of other ecosystem services. Here, we describe the rationale behind the Ridgefield Multiple Ecosystem Services Experiment, a long-term investigation into trade-offs that might prevent the simultaneous provision of high levels of multiple services in the agricultural landscape of south-western Australia. We compare a monoculture treatment of a fast growing native species (*Eucalyptus loxophleba*) with mixtures of 2, 4 or 8 native plant species. We selected species based on different traits that we predict will relate to the provision of different levels of multiple ecosystem services. We ask: Can restoration of multiple ecosystem services be achieved along with carbon sequestration? Nested treatments of nitrogen deposition and weed removal test whether ecosystem service outcomes respond to environmental changes. We show that historical land-use at the site has the potential to affect service delivery via different soil characteristics and survival of planted species. Ridgefield tests the possibility of restoring and managing agricultural landscapes for multiple ecosystem services, providing a much needed experimental investigation of trade-offs among ecosystem functions.

1. Introduction

Large-scale ecological restoration is a vital component of our response to ongoing environmental change and land degradation. The form that restoration takes depends on the specific challenges faced by land managers and goals set to overcome these challenges (Hobbs and Norton, 1996). From an early focus on small-scale projects aiming to restore historic species assemblages, restoration is now evolving to encompass projects that aim to achieve broader goals including the provision of essential ecosystem service outcomes (i.e. the benefits humans derive from ecosystems (Millennium Ecosystem Assessment, 2005)) (Bullock et al., 2011). Carbon (C) sequestration is one ecosystem service that has the potential to drive restoration for multiple outcomes, but there is little guidance available on how to simultaneously sequester C along with providing an array of additional ecosystem services. In this paper, we present one approach for how restoration in the modern agro-ecological landscape could assist in the provision of multiple services, highlighting how simultaneous provision may be prevented by trade-offs among services. We argue that plant traits could inform restoration management decisions of which species to utilize, given the growing awareness of how traits influence ecosystem function and thence service provision. We consider the effects of environmental changes since they may influence restoration outcomes and the ability of ecosystems to deliver multiple services simultaneously. We go on to present the design and early stages of a long term experiment – the Ridgefield Multiple Ecosystem Services Experiment (hereafter Ridgefield) – established to explore the provision of ecosystem services by different plant species in the context of restoration and global change in the wheatbelt of south-western Australia.

1.1. Multiple services and how to achieve them

The emerging C market has the potential to be a driver of ecological restoration across the globe. Currently, there is concern that C projects generally plant single species (monocultures) that are assumed to rapidly sequester C (Diaz et al., 2011). Such projects run the risk of ignoring other ecosystem services through their restricted focus. In the modern agro-ecological landscape, land

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doi:10.1016/j.agee.2012.02.016

managers and the broader public are likely to value the provision of clean water, biotic resistance to invasion (Levine et al., 2004), nutrient cycling, soil erosion control, the continued existence of valued native biological diversity (hereafter "biodiversity"), and pollination services, in addition to solely C sequestration. Consequently, there is increasing interest in managing ecosystems to protect and restore these attributes and processes (Jarchow and Liebman, 2011).

As interest in ecosystem services increases, the connections among ecosystem attributes, processes/functions, services and biodiversity are continually being clarified, especially to make them more easily applied to management (Wallace, 2007; Mace et al., 2012). For example, biodiversity in itself may not be a service, but its maintenance can be. It has recently been argued that biodiversity and ecosystem services have a multi-layered relationship (Mace et al., 2012). Biodiversity can be a good valued in economic terms at one level, while at another biodiversity can be considered synonymous with ecosystem services such that having one necessarily entails having the other (Mace et al., 2012). In addition, as managers increase efforts to manage ecosystems to provide multiple valued services, new complexities are arising, such as the presence of conflicting valuation of individual functions. For example, biotic resistance to invasion is often considered an ecosystem function (Fargione and Tilman, 2005; Hooper et al., 2005; Thompson and Starzomski, 2007), but whether it is a service or dis-service will depend upon the values of the land manager and the restoration targets they are aiming for. Thus, when invading species are unwanted in managed ecosystems, for example because they compromise the maintenance of native biodiversity, invasion resistance will be a service. When however novel components to an ecosystem add to a valued function – for example, increased carbon sequestration (Lugo, 2004; Escobedo et al., 2010; although note Kanowski and Catterall, 2010) – invasion resistance would then be a dis-service, as it prevents the achievement of that intended goal. In other words, a single function can be a service or dis-service at the same time. We suggest that consideration should be given to explicit statement of end goals and the imposing of human values on ecosystem processes and attributes such that the nuances of the relationships among ecosystem services can be elucidated.

Explicit consideration of these goals suggests that the simultaneous provision of high levels of multiple ecosystem services might be prevented by trade-offs (Hector and Bagchi, 2007; Gamfeldt et al., 2008; Bennett et al., 2009). While there is a wealth of literature examining how species richness and composition affects single ecosystem functions and services (e.g. Hooper et al., 2005), few studies have directly addressed trade-offs among services using experimental data (see Zavaleta et al., 2010 for a notable exception). Trade-offs may arise among services for a number of reasons. Firstly, there may be a shared driver that creates apparently causal relationships between two services. For instance, crop productivity and water quality are often negatively related because of the shared driver of nitrogen (N) fertilization, not because they directly interact (Bennett et al., 2009). Other services may be causally related and not be deliverable at high levels simultaneously (Kareiva et al., 2007). For instance, sequestering C requires water and higher C sequestration may therefore lower stream water supply at a regional scale, particularly in drier regions (Farley et al., 2005; Brown et al., 2007; Calder, 2007). This relationship might occur regardless of the species planted, although its exact form would depend on particular species and the landscape context (Farley et al., 2005).

Trade-offs could also occur because of the traits of the species found in assemblages (Zavaleta et al., 2010). Species assemblages could contain functionally similar species that all provide high levels of a few services, or functionally diverse species that all provide high levels of a different service. Within a species, those that have evolved to cope with environmental changes (for example, drought tolerant species) may not be highly productive (Diaz et al., 2004). If the goal is provision of multiple services, understanding how services are related to each other and how different species might affect their simultaneous delivery in the context of global change (Isbell et al., 2011), will increasingly inform restoration projects.

The provision of ecosystem services is underpinned by ecosystem functions and the species traits that determine these functions (van der Putten et al., 2004; McGill et al., 2006). Increasingly, species traits are recognized as the link between the composition and functioning of ecosystems (Lavorel and Garnier, 2002; McGill et al., 2006; Suding et al., 2008). The trait approach is a significant development for ecological restoration of landscapes (Funk et al., 2008), because it offers a guide to species selection and the possibility of restoring and maintaining high levels of ecosystem services with a select group of species. These arguments suggest that land managers, when restoring a particular landscape, might consider the traits of the species mixtures that they plant in order to increase the likelihood of reaching ecosystem service goals.

1.2. Global change and novel ecosystems: considering the future

Restoration of multiple ecosystem services needs to be considered in the context of today's rapidly changing world where interactive effects of multiple global change drivers affect the ability of ecosystems to function and deliver the services upon which humanity depends (Steffen et al., 2007; Tylianakis et al., 2008). These global changes (e.g. climate change, biological invasions, N deposition) also affect our ability to restore ecosystems and, perhaps more significantly, affect the ecological relevance of ecosystems that we restore. A rapidly changing world necessitates that land managers consider how to ensure continued provision of ecosystem services over the coming decades. Such consideration may involve choosing species based on their traits such as drought tolerance, or broad distributions that may indicate tolerance of a wide range of environmental conditions.

Persistent alterations such as climate change and N deposition, are superimposed on human-altered landscapes: an estimated 38% of the ice-free land surface of the globe is now covered with novel ecosystems (Ellis, 2011). Novel ecosystems are here defined as new assemblages of native and non-native species that lead to novel interactions and cascading effects on other system components (sensu Hobbs et al., 2006; cf. Hobbs et al., 2009). It is important to consider how such novel ecosystems could affect the provision of multiple ecosystem services. Novel assemblages may be persistent due to the crossing of abiotic and biotic thresholds that prevent the return of a previously extant system (Cramer et al., 2008). Thresholds in former agricultural land include phosphorus (P) fertiliser residues (Barrow, 1980; Sharpley et al., 2004; Walker et al., 2004; Standish et al., 2006), a lack of propagules to allow establishment of native plants (Pywell et al., 2007), and/or the presence of non-native species that can prevent renewed colonization by native species (Standish et al., 2007a). All these thresholds are highly likely to be present within the modern, fragmented agro-ecological landscape. Given that overcoming some of these thresholds may be impractical (Suding and Hobbs, 2009), land managers may have to increasingly rely on novel assemblages for the provision of ecosystem services and consider how historical legacies of prior agricultural land use (McLauchlan, 2006) affect ecosystem service delivery.

1.3. Questions

In order to provide multiple ecosystem services both now and into the future, a number of questions need to be answered. We propose that the following three key questions are particularly crucial to address:

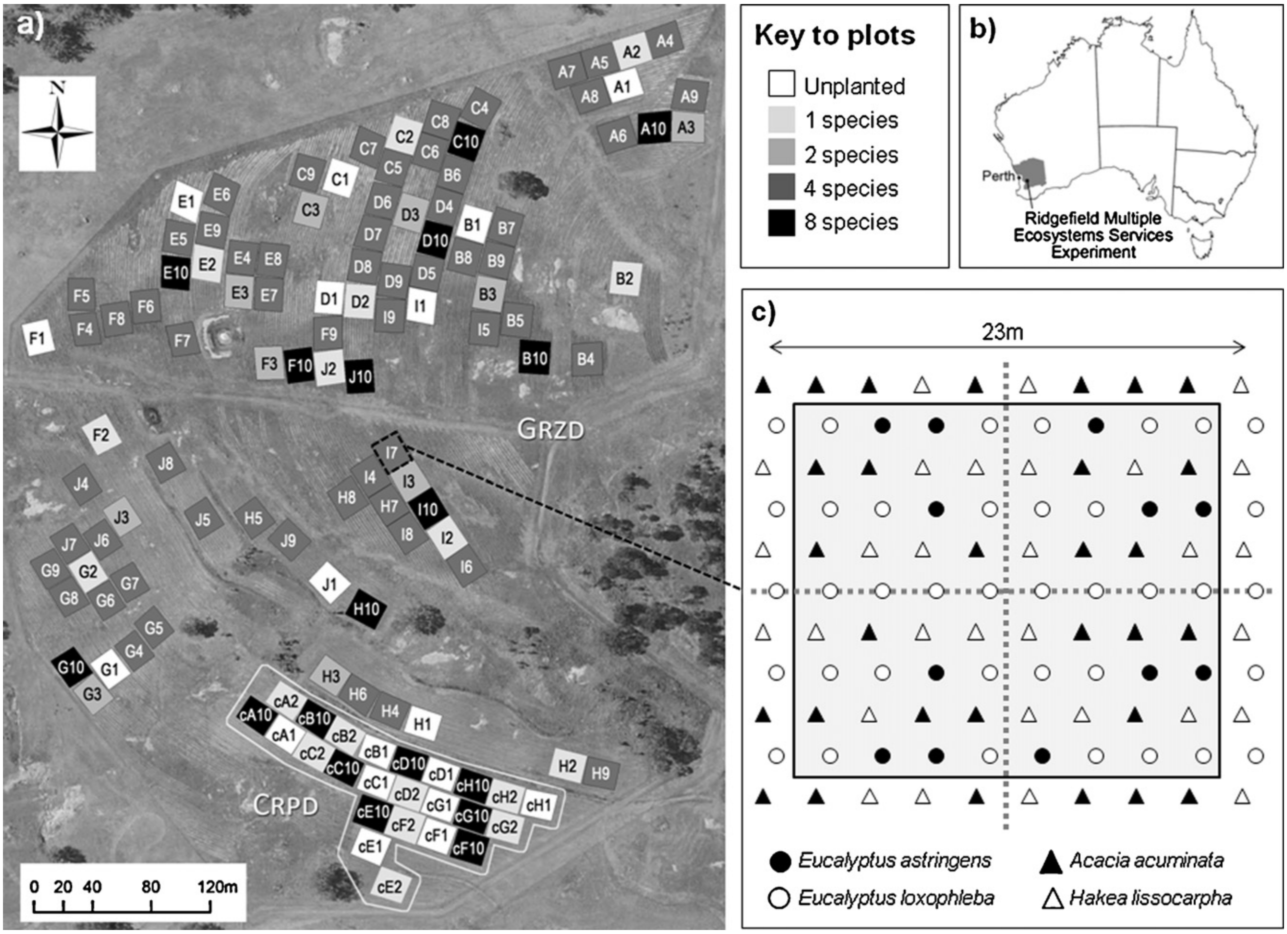

Fig. 1. (a) The Ridgefield Multiple Ecosystem Services Experiment, (b) its location in the wheatbelt (grey shading) of south-western Australia, and (c) an example plot layout. In (a) the white solid line encloses the cropped land use history area; the remainder of the site was previously grazed. Plots are blocked primarily according to soil type and then aspect and soil moisture, with blocks denoted by the letter within plots (see Section 3.1 for further explanation); numbers within plots denote plant assemblage treatments in Table 2. Woody individuals in each plot are arranged in a stratified random manner to maintain composition within each quarter, as shown in (c). Half plots are subjected to N deposition, with weed removal nested in quarter plots within these halves. A buffer zone has been established around the outer edges of the plot and on the middle rip line with measurements taking place on, or in the area enclosed by, the 16 individuals within each quarter.

(i) How is ecosystem service provision influenced by different plant assemblages and the traits of species within them? This includes understanding the occurrence of trade-offs among services and reasons for them.
(ii) How resilient is the provision of these services and will trade-offs be affected by multiple environmental changes?
(iii) How does land use history affect the provision of, and trade-offs among, ecosystem services?

The next two sections detail how the Ridgefield experiment can address these questions.

2. Ridgefield: answering the broad questions

Ridgefield is located at the University of Western Australia's "Future Farm" in the wheatbelt of south-western Australia (Fig. 1). The broad aims of the Future Farm are to develop best-practice sustainable farming methods for cropping and animal production, and to develop prospective future industries such as C farming. Within this broad remit, there is an objective to investigate ways to better conserve native biodiversity and integrate marginalized farmland into remnant vegetation areas. The potential to use native species in C farming provided the initial impetus for our experiment. We developed this idea to consider the provision of multiple services, and how this might be achieved using different plant assemblages, to create the Ridgefield Multiple Ecosystem Services Experiment.

2.1. How is ecosystem service provision influenced by different plant assemblages and the traits of species within them? Are there trade-offs among services?

For our experiment, we focused on a number of ecosystem attributes and processes, including those likely to be valued as services by land managers as well as the broader public. The services we will investigate in the first stages of the experiment are C sequestration, biotic resistance (Levine et al., 2004), nutrient cycling, soil erosion control, biodiversity maintenance, and pollination. We hypothesized that ecosystem service deliveries and potential trade-offs among them will occur due to the trait composition of the species mixtures. We also acknowledge that some trade-offs among services may be inevitable regardless of which species are planted (Bennett et al., 2009).

We selected species primarily based on their nutrient acquisition traits but also on morphological traits such as growth form as we considered that these would strongly affect C sequestration as well as other ecosystem services (Table 1). Further, we predicted that a greater diversity in floral and other plant-based resources encompassed by the selected species will contribute to greater plot-scale biodiversity. We utilized nutrient acquisition traits including

Table 1
Nutrient acquisition, morphology and floral traits of native eucalypt woodland species planted at Ridgefield.

Species	Family	Life form[a]	Height (m)[a]	P acquisition strategy	N acquisition strategy	Flower color and bloom months[a]	References[b]
Eucalyptus loxophleba *Eucalyptus astringens*	Myrtaceae	Tree	15	AM/ECM *inferred*	Roots	White, July–Feb White to yellow, Aug–Dec	Brundrett et al. (1996), Chen et al. (2000)
Acacia acuminata *Acacia microbotrya*	Fabaceae	Shrub/small tree	7	AM AM *inferred*	N-fixing bacteria	Yellow, July–Oct Cream to yellow, Mar–Aug	Standish et al. (2007b)
Banksia sessilis *Hakea lissocarpha*	Proteaceae	Shrub	6 1.5	Cluster roots Cluster roots	Roots	Cream to yellow, Apr–Nov White to yellow, May–Sept	Brundrett and Abbott (1991), Roelofs et al. (2001)
Calothamnus quadrifidus *Callistemon phoeniceus*	Myrtaceae	Shrub	5 2	AM/ECM *inferred*	Roots	White, yellow, red, Jun–Dec Red, Sept–Jan	Warcup (1980)

[a] Life forms after Bell et al. (1993), heights and floral traits from Flora Base (http://florabase.calm.wa.gov.au/, accessed 1 June 2011).
[b] References denote source of nutrient acquisition strategy information, where AM: arbuscular mycorrhiza, ECM: ecto-mycorrhiza. *Inferred*: no records of species-specific mycorrhizal associations.

rooting depth, arbuscular and ectomycorrhizal fungal associations, N fixation capacity, and cluster root formation to choose species that potentially acquire soil resources in different ways. Having decided upon these nutrient acquisition trait groups, we then considered the species made available to us and further refined our choice to ensure a range of structure, flower color and bloom time among our final species selection. These traits are broadly representative of those found in the region, but given the high floristic diversity of this region we do not know the extent to which this variation matches the trait variability observed more generally within the Australian flora. The species are not mutually exclusive in their mode of nutrient acquisition, but we expected that different groups would occupy different areas of nutrient-acquisition trait space that may have consequent effects on community dynamics and service delivery (Bever et al., 2010).

In addition to the trait-based choices, we chose these species because they are widespread natives within south-western Australia. We hypothesized that these species would be more likely to survive future environmental change compared with more narrowly distributed species. Furthermore, native shrub species are more prominent in drier *Eucalyptus loxophleba* woodlands (Prober et al., 2011), an important factor given the current and likely ongoing drying trend in south-western Australia (CSIRO, 2007). Finally, these species are available through commercial nurseries and likely to be used by restoration practitioners in the region.

Using the morphology and nutrient acquisition traits outlined (Table 1), and taking into account species' distributions, we chose two tree species and six shrub species. The two tree species were *E. loxophleba* ssp *loxophleba* (hereafter *E. loxophleba*), the dominant woody native on similar soil types prior to its clearing for agriculture (Beard, 1990), and *E. astringens*. The six shrub species consisted of two N fixing Fabaceous species (*Acacia acuminata* and *A. microbotrya*), two cluster root forming Proteaceous species (*Banksia sessilis* and *Hakea lissocarpha*) and two Myrtaceous shrub species (*Calothamnus quadrifidus* and *Callistemon phoeniceus*).

We assembled the plant species into ten treatments along a gradient of species and functional group richness, and structural complexity which will alter with time (Table 2). In treatments involving greater functional and species richness, we "replaced" individuals of *E. loxophleba* with individuals from other species using a substitutive design (Fox, 2005). The substitutive design allows us to maintain planting density while increasing species and functional richness, and structural complexity. We also included bare plots as one of the ten treatments to examine what changes will occur in the absence of deliberate plantings. The gradient will enable us to investigate how provision of multiple services is affected by plant species' traits, whether species identity further modifies this provision, and ascertain what trade-offs exist among ecosystem services.

2.2. *How resilient is the provision of these services and will trade-offs be affected by multiple environmental changes?*

Multiple global environmental changes challenge, and will continue to challenge, the ability of flora and fauna to persist in south western-Australia. In addition to monitoring responses to environmental variability throughout the duration of the experiment, two environmental changes will be manipulated experimentally at the site: N deposition and non-native weed cover, given that both are increasing in importance across the region and the globe. These

Table 2
Plant assemblage treatments at Ridgefield.

Treatment	Species and Number of Individuals in each Treatment Plot[a]								Functional Richness	Species Richness	Anticipated Structural complexity
	E.l.	E.a.	A.a.	A.m.	C.p.	C.q.	H.l.	B.s.			
1 Bare	0	0	0	0	0	0	0	0	0	0	0
2 York gum	110	0	0	0	0	0	0	0	1	1	1
3 Trees	98	12	0	0	0	0	0	0	1	2	1
4 Trees + Myrtaceous shrubs	38	12	0	0	30	30	0	0	2	4	2
5 Trees + Proteaceous shrubs	38	12	0	0	0	0	30	30	2	4	2
6 Trees + Fabaceous shrubs	38	12	30	30	0	0	0	0	2	4	2
7 Trees + Fabaceous + Proteaceous	38	12	30 of one species		0	0	30 of one species		3	4	2
8 Trees + Myrtaceous + Fabaceous	38	12	30 of one species		30 of one species		0	0	3	4	2
9 Trees + Myrtaceous + Proteaceous	38	12	0	0	30 of one species		30 of one species		3	4	2
10 Trees + all shrubs	38	12	10	10	10	10	10	10	4	8	2

[a] Abbreviations in species composition are as follows (nomenclature follows FloraBase (for Banksia sessilis) otherwise Paczkowska and Chapman, 2000): E.l.: *Eucalyptus loxophleba* ssp *loxophleba*; E.a.: *Eucalyptus astringens*; A.a.: *Acacia acuminata*; A.m.: *Acacia microbotrya*; C.p.: *Callistemon phoeniceus*; C.q.: *Calothamnus quadrifidus*; H.l.: *Hakea lissocarpha*; B.s.: *Banksia sessilis*.

will be manipulated in a nested design to test for their effects on the provision of ecosystem services and any trade-offs among them.

Nitrogen deposition is increasing across the globe (Phoenix et al., 2006) but has been somewhat overlooked in Mediterranean-type climate systems and in biodiversity hotspots more generally (Phoenix et al., 2006; Bobbink et al., 2010). The limited data that exist for Mediterranean-climate regions suggest that deposition of 10 kg N ha^{-1} yr^{-1} could be a threshold beyond which ecosystem functions can be adversely affected and vegetation composition altered (Bobbink et al., 2010; Fenn et al., 2010). Agricultural legacies at the site, the composition of the assemblages and the otherwise ancient landscape of south-western Australia may alter this threshold. Investigations of ecosystem service responses to N deposition are thus required. In particular, if a species responds adversely to additional N, the ability of the system to continue to provide ecosystem services may be compromised. To mimic the predominantly dry deposition assumed to occur at the site and likely upper rate of deposition that could be expected in the future (Ochoa-Hueso et al., 2011), dry pellets of ammonium nitrate will be distributed on plots nested within the plant assemblages four times per year at an overall rate of 10 kg N ha^{-1} yr^{-1}.

Additionally, we included a non-native comparison in the experiment because novel assemblages of species are increasingly common in global landscapes and may affect ecosystem service delivery. Historically, the landscape has been characterized by nutrient impoverishment and geological quietude (Hopper, 2009), which has led to a flora characterized by traits that confer persistence in the landscape rather than traits that allow dispersal and colonization (Cramer et al., 2008). This evolutionary history together with fragmentation and agricultural practices has led to the crossing of abiotic and biotic thresholds, including high soil P concentrations (Barrow, 1980; Sharpley et al., 2004; Standish et al., 2006) and lack of native propagules (Walker et al., 2004; Standish et al., 2007a). In particular, the presence of non-native species can lead to the creation of novel ecosystems and reinforcing feedbacks that keep the system in a degraded state (Cramer et al., 2008). The crossing of these thresholds generally prevents the return of previously extant native vegetation in the area.

On the other hand, some non-native species may aid the delivery of ecosystem services, and modify trade-offs among them. Hence, the Ridgefield experiment addresses whether a novel component modifies the delivery of ecosystem services. We do this by nesting a non-native removal treatment within the N deposition and woody plant-assemblage treatments. Herbicides (active ingredients haloxyfop and glyphosate) will be applied to early winter herbaceous growth to remove the winter-active non-native species found at the site, including *Erodium* spp., *Bromus* spp., *Avena* spp. and *Arctotheca calendula*. The occasional native grass species found at the site may also be unintentionally removed with this treatment. Summer-active non-natives will also be removed with spot application of glyphosate herbicide.

In combination, the two environmental treatments (N deposition and weed removal) will assess whether service deliveries of the planted assemblages are altered by environmental change. They will test whether any trade-offs among services found within the different assemblages can be changed by these stressors either separately or in concert.

2.3. How does land use history affect the provision of, and trade-offs among, ecosystem services?

Prior land use history may affect the delivery of ecosystem services (Prober and Smith, 2009; Maestre et al., 2012). The landscape around Ridgefield is highly fragmented due to vegetation clearing that occurred from the 1830s on the more fertile soils, and with increasing rapidity across the whole 14 million hectares of the wheatbelt following the end of the Second World War (Prober and Smith, 2009). The 21 ha experimental site was dominated by two prior agricultural land uses, pasture for sheep (hereafter 'grazed'), and a smaller cropped area most recently planted with canola (*Brassica napus*, oil seed rape) in a 3-to-1 rotation (3 years crops, 1 year fallow/grazed) (K. Robertson, personal communication. Yates et al., 2000). It is likely that the area was cropped with wheat (*Triticum* spp.) in the past.

Cropping often leads to a legacy of high soil P availability (Barrow, 1980; Sharpley et al., 2004; Standish et al., 2006). This legacy has differential consequences for native flora: increasing soil P can be toxic to proteaceous species (Shane and Lambers, 2006) but can increase the growth of myrtaceous species (Stoneman et al., 1995). High soil P can also increase the competitive ability of non-native species on old-fields (Standish et al., 2008). The previously cropped and grazed areas of the site therefore provide us with the ability to investigate how ecosystem service delivery, and any restoration trajectory, is influenced by land use history.

3. Ridgefield: Experimental set-up

The Ridgefield experiment (32°29′S 116°58′E) is in a Mediterranean-climate region, at an average elevation of 350 m. The average (summer) January maximum daily temperature is 31.8 °C, with an average minimum (winter) August daily temperature of 5.6 °C at Pingelly (300 m elevation; 1970–2011 averages). Mean annual rainfall is 445.1 mm (1891–2010), with precipitation winter dominant with occasional heavy falls in summer (Bureau of Meteorology, 2012).

3.1. Pre-treatment, blocking and planting design

Most of the experimental site was ripped in July 2010 to a depth of 30 cm with rip lines spaced at approximately 2 m intervals on contour. A rip line is a deep plough line. We located 124 experimental plots (Fig. 1a) of 23 m in length by 11 rip lines across the site. The nature of the site, both in terms of its slope and rocky obstructions, prevented us establishing perfectly square (i.e. 23 m × 23 m) plots; the within plot design takes these slight differences in area into account. We recorded aspect of each plot using a hand-held compass to inform blocking of the experiment.

We collected representative soil samples from each plot between 15 and 19 July 2010, taken to a depth of 10 cm, to establish baseline soil physical and chemical properties. Samples for baseline soil biological properties, including microbial biomass C, N and P, and fungal to bacteria biomass ratios via phospholipid fatty acid analysis, were also collected. Analysis of the biological samples is ongoing and these are not discussed herein. Details of the soil sampling methodology can be found in Supplementary Information. We used clay content to determine blocking of the experiment; we used other physical and chemical properties to confirm the integrity of the design (see Section 3.2). Bulked samples were air dried, weighed, and sieved <2 mm, the gravel content (fraction >2 mm) thus being determined. Half the dried soil was archived, and the remainder was analyzed for physical and chemical properties including soil texture, ammonium N, nitrate N, Colwell P and potassium, sulphur, organic C, conductivity, pH using both $CaCl_2$ and H_2O, exchangeable aluminium, calcium, magnesium, potassium and sodium, and total N, P and C (analyses conducted by CSBP, see Standish et al., 2006 for analytical methods). Bulk density was determined on one sample from the middle of each plot using a metal ring of internal diameter of 48 mm and depth 10 cm. We double bagged soils in the field to prevent moisture loss. In the laboratory, we weighed wet soil and then oven-dried them at 105 °C

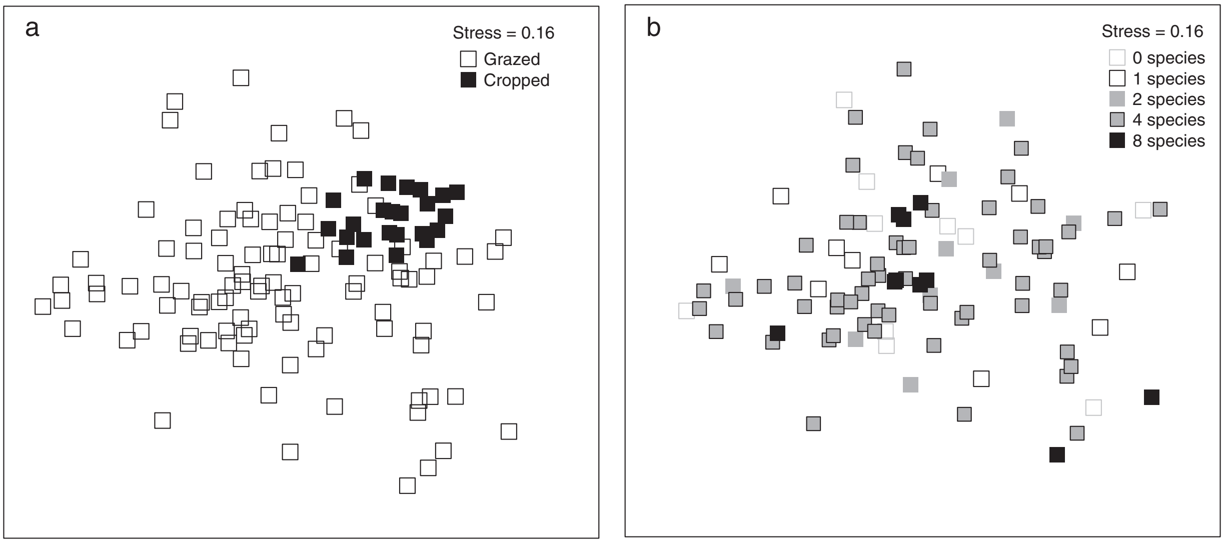

Fig. 2. Multi dimensional scaling ordinations based on physical and chemical environmental variables (Section 3.2) for the Ridgefield Experiment with (a) comparing the formerly grazed and cropped areas of the site and (b) the differences among woody plant assemblage treatments in the formerly grazed area of the site. The grazed and cropped land use areas differ significantly in their underlying soil physical and chemical properties (Global $R = 0.116$, $p = 0.023$ from 999 permutations), as further explored in Table 3. No underlying environmental differences exist between different plant assemblage treatments in the grazed area (Global $R = -0.049$, $p = 0.994$ from 999 permutations), nor in the cropped area (Global $R = -0.043$, $p = 0.724$ from 999 permutations; see also Fig. 4).

for 48 h for dry-weight determination. We calculated soil moisture as ((wet soil mass − dry soil mass)/dry soil mass) × 100%.

To reduce weed competition prior to planting, herbicide was applied on 5 and 7 August 2010, as a mix of simazine and glyphosate at rates of 2 L ha^{-1} and 1 L ha^{-1} respectively. To exclude rabbits from the experimental area, we fenced the perimeter of the site using 1.4 mm gauge wire mesh of hole size 40 mm and height 900 mm. Approximately 450 mm was attached to the pre-existing stock fence and the remaining 450 mm was pegged down facing outwards, with rocks placed on top. We check the fence at least once per month and maintain its integrity. Establishment methods such as herbicide spraying are standard industry practice, although more practical solutions exist for putting in rabbit fences particularly where a new fence-line is being put in as opposed to adding to an existing fence. Some practitioners may accept loss of some individuals to herbivores and not fence areas. It remains to be seen whether the ecosystem services will pay back the cost of investment and that will depend on whether and how ecosystem services become valued, but it is possible that afforesting marginal agricultural land comes at little opportunity cost (Crossman et al., 2011).

There were two prior land use histories on the site—formerly cropped and formerly grazed (hereafter referred to as 'cropped' and 'grazed'). One hundred plots were located in the grazed area, and 24 were located in the cropped due to its smaller area. All plots, within the land use histories, were blocked on the basis of soil type, aspect, and soil moisture, following a rule-based system. We chose these factors given their likely importance in determining plant growth and thus the responses of the ecosystem services of interest, taking primary account of soil type. Soil types were classified via hand texturing, and thus based on clay content. The range of clay content found at the site was from 5–10% to 40–45%, corresponding to the range of clay classes in Supplementary Information, Table S.I.2.1. All blocks, except A and F, are composed of plots that vary by at most one soil texture class. Block A's northerly aspect and location meant it was logical to include one plot of slightly higher clay content compared to other plots within the block, while Block F contained plots having more than 20% clay content, including 4 texture classes. The majority of plots, and all in the cropped area, had clay contents of 5–10%, and were classified as either loamy sand (LS) or clayey sand (CS). All plots within a block have aspects within 55° of each other, with the majority of blocks having plots within 30° of each other.

In the grazed area, the ten plant assemblage treatments were assigned to plots within blocks at random (Fig. 1a). The different representations of Treatments 7, 8, and 9 were randomly allocated to blocks such that there were three repeats of two of the representations, and two repeats of the other two representations (see Supplementary Information, Table S.I.2.2. for details). In the cropped area we established 8 blocks of Treatments 1, 2 and 10, i.e. control, tree monoculture and the most diverse treatment plots.

The within plot design takes account of the nesting of N deposition and weed removal treatments within the woody plant assemblage treatments (Fig. 1c). We varied the locations of individual plants among quarters and among plots with the same plant assemblage treatment. This design maintains plant assemblage composition, rather than location of individuals, as the treatment of interest to prevent hidden treatment effects (Huston, 1997).

All species were planted from 14 to 16 August 2010. Shrubs and trees were planted on alternate rip lines, with a gap of 2.5 m between adjacent individuals on the same rip line, resulting in 10 individuals per rip line in each plot. The outer rip lines always have shrubs, apart from in the bare and tree only plots (Treatments 1, 2 and 3). Each plot consists of a buffer zone, such that the outer rip lines and the first and last individual on all other rip lines, will be excluded from measurements. Numbers of individuals in each plot are shown in Table 2. All plots were checked immediately after planting to ensure all 11,660 plants had been placed correctly, with the few mistakes rectified. We replaced individuals that died in the first month using remaining plant stock. As in similar experiments (Scherer-Lorenzen et al., 2007), individuals that died during the first year of establishment were replaced the following planting season (August 2011) to maintain plant assemblage composition at the outset of environmental change treatments. Thereafter, the trajectory of plant community response, and patterns of mortality, will become a response variable in our experiment.

3.2. Statistical analysis

We analysed soil physical and chemical properties not used to inform blocking (bulk density, ammonium N, nitrate N, Colwell

Table 3
Mean soil physical and chemical properties between grazed and cropped land-use histories at Ridgefield.

Soil physical/chemical property	Grazed	Cropped	p-value
Total N (%)	0.165 (0.005)	0.202 (0.004)	<0.0001
Total P (mg P/kg)	253.6 (9.77)	408 (12.26)	<0.0001
Total C (%)	2.35 (0.065)	2.93 (0.08)	<0.0001
Nitrate (mg N/kg)	9.86 (0.38)	15.25 (0.93)	<0.0001
Ammonium (mg N/kg)	2.59 (0.12)	2.5 (0.22)	0.72
Available (Colwell) P (mg P/kg)	39.77 (1.92)	72.96 (2.38)	<0.0001
Available (Colwell) K (mg K/kg)	158.7 (7.76)	168.4 (6.35)	0.34
S (mg S/kg)	4.56 (0.12)	7.26 (0.2)	<0.0001
Organic C (%)	1.73 (0.05)	2.19 (0.05)	<0.0001
Exchangeable Na ($cmol_c$/kg)	0.13 (0.01)	0.09 (0.002)	<0.0001
Exchangeable K ($cmol_c$/kg)	0.4 (0.02)	0.42 (0.02)	0.41
Exchangeable Al ($cmol_c$/kg)	0.16 (0.008)	0.23 (0.01)	<0.0001
Exchangeable Ca ($cmol_c$/kg)	5.32 (0.19)	5.5 (0.1)	0.41
Exchangeable Mg ($cmol_c$/kg)	0.9 (0.05)	0.6 (0.01)	<0.0001
Conductivity (dS/m)	0.05 (0.001)	0.06 (0.002)	<0.0001
Bulk Density (g/cm^3)	1.25 (0.01)	1.25 (0.02)	0.68
pH ($CaCl_2$)	4.91 (4.9–4.93)	4.88 (4.86–4.91)	0.29
pH (H_2O)	5.92 (5.91–5.95)	5.78 (5.75–5.81)	0.0005

Values in parentheses denote ±standard error of the mean, apart from pH where range of the standard error is given because of the log scale.

P and potassium, sulphur, organic C, conductivity, pH using both $CaCl_2$ and H_2O, exchangeable aluminium, calcium, magnesium, potassium and sodium, and total N, P and C; see also Section 3.1) using Primer version 6 (Clarke and Warwick, 2001) to assess differences between land-use histories and to test whether there were differences in underlying soil conditions among plant assemblage treatments within land use history. PRIMER allows the analysis of multiple variables through the investigation of distances between sample points in multi-variate space. Environmental data were log transformed where necessary, and Euclidean distance measures were calculated on normalized data before multi-dimensional scaling was used to visualize the environmental space (Fig. 2). ANOSIM was used to indicate whether there were differences among the plant assemblage treatments within land-use histories, and whether there were differences between land-use histories. No differences were found among plots assigned to different plant assemblage treatments (Fig. 2, although note Fig. S6) which will allow us to attribute any subsequent differences in plant or service response to the experimental plant assemblages and environmental treatments, rather than to measured underlying soil property differences. However, neighbor effects and distance to edges of the site will also likely need to be considered for some response variables such as invertebrate colonization. Differences between grazed and cropped land-uses, and among blocks within the grazed and cropped areas, were examined by comparing individual soil properties, noting that we can compare the particular grazed and cropped areas at our site but are unable to make broader inferences about grazed and cropped land uses more generally.

Table 4
Percentage of dead individuals in July 2011 given number planted in August 2010 in the two land-use histories at Ridgefield.

Species	Grazed	Cropped
	%	
Eucalyptus loxophleba	2.3	1.8
Eucalyptus astringens	5.2	8.3
Acacia acuminata	1.1	2.5
Acacia microbotrya	4	0
Banksia sessilis[a]	33	10
Hakea lissocarpha	17	6.3
Calothamnus quadrifidus	4.8	7.5
Callistemon phoeniceus	3.3	6.3

[a] The figure for *B. sessilis* refers to individuals that were not frost damaged when planted in the grazed area; no frost damaged individuals were planted in the cropped area.

Table 5
Ecosystem services, their components, and their proxy/absolute measurements at Ridgefield.

Ecosystem Service	Components	Proxy/Absolute Measurements
C sequestration	Live and dead above- and below-ground biomass	Destructive harvest of woody individuals planted in experimental surrounds.
	Soil C pools	Sub sampling of herbaceous biomass within experimental plots
	Litter C pools	Drilling to depth for soil C
		Litter collection
Soil erosion control	Soil erosion control	Water stable aggregates
		Sediment traps/Surface microtopography
Biotic resistance	Non-native weed load	Surveys of non-native herbaceous recruits
	Non-native fauna	Direct seed addition experiments
		Resistance to non-native ants
Nutrient cycling	Net N and P mineralization rates	In situ resin probes
	Plant available N and P	P-fractionation of soil samples
	Soil P fractions	Litter bags
	Decomposition	Lysimeters
Pollination	Pollination	Seed set in *E. loxophleba*
		Floral visits to *E. loxophleba*
		Measurement of floral traits
Biodiversity	Invertebrate diversity	Pitfall traps
	Soil fauna and flora	Soil sampling and community level physiological profiling
	Herbaceous species	Surveys of herbaceous species
	Volunteer woody species	Recording of volunteers

4. Land use history comparison: implications for ecosystem services

The MDS plot of grazed and cropped land uses suggests that the cropped area clusters within the variation exhibited by the grazed plots (Fig. 2a) although we found a significant difference in physical and chemical soil properties between these land uses at the site (Global $R = 0.116$, $p = 0.023$ from 999 permutations). Subsequent analysis (Table 3) showed that the previously cropped area had higher concentrations of total N, total P and total C. Colwell (extractable) P concentrations were high across the site, and were an order of magnitude lower than total P concentrations. Importantly, mean values of Colwell P (Table 3) were one to two orders of magnitude higher than might be expected in remnant native *E. loxophleba* woodlands (Prober et al., 2011), or more generally in *Eucalyptus* woodland remnants, even when these have been grazed by livestock (Yates et al., 2000; Gibb and Cunningham, 2010). Nitrate and ammonium concentrations were also high across the site, with nitrate being higher in the cropped area than in the grazed area (Table 3). Concentrations of most cations were higher in the cropped area than in the grazed area, although magnesium and sodium concentrations were lower. Potassium had the same concentration, on average, between formerly grazed and cropped areas. The bulk density of the soil was not different between cropped and grazed areas, while cropped areas were slightly more acidic on average than grazed areas (Table 3).

We found variation in the concentrations of plant available nutrients within the grazed area (Fig. 3 and Supplementary Information, Figs. S3–S5), but not among treatments in this area (Fig. 4 and Table S.I.3.1). By comparison, there was less variation among plots within the cropped area, which is to be expected given its smaller size and close proximity of these plots to each other (see Supplementary Information). While blocking accounts for the variation in soil type, it did not account for the variation we measured in other soil properties within the grazed area i.e. one block does not always have consistently high concentrations of all nutrients (Fig. 3 and Supplementary Information). Despite these inconsistencies, there are some strong correlations between the different soil properties (Supplementary Information, Table S. I.3.2).

The high soil P in the cropped area could be expected to prevent the establishment of *A. acuminata* (Standish et al., 2007b) and the proteaceous species (Shane and Lambers, 2006), while aiding the growth of the myrtaceous species (Stoneman et al., 1995). In spite of these predictions, we did not observe signs of P toxicity on seedlings of the proteaceous species and their survival in the high-P soils of the cropped area was similar to that in the lower P soils of the grazed area (Table 4). However, they have suffered large mortality compared with the other planted species across the site as a whole, particularly in the grazed area. *A. acuminata* seedlings did have a slightly higher mortality, together with the seedlings of the myrtaceous shrubs, in the cropped area (Table 4). The first year after planting is critical for establishment in Mediterranean-type ecosystems, and thus these initial results can be used to predict the trajectory of restoration. Competitive exclusion of those species adversely affected by the high nutrient concentrations by species that benefit from the higher nutrient loadings may also occur, particularly in the all species treatment (Treatment 10). The greater nutrient availabilities in the cropped area may allow a more rapid growth of *E. loxophleba* compared to the grazed area providing sufficient water is available, allowing greater C storage. Additionally, the higher organic C content of the cropped plots may aid in the prevention of soil erosion given the importance of organic C, in particular its contribution to water stable aggregates (Tisdall and Oades, 1982). Our experiment will continue to assess how these different land use histories, and the variation in nutrients across the site will affect the delivery of ecosystem services and trade-offs among them.

5. Ecosystem measures

The capacity of the plant assemblages to provide services will be measured over time, and one would expect differences between young and mature stands. A variety of measures are possible, particularly for nutrient cycling. We outline here the proposed set of measurements (see also Table 5).

5.1. C storage

Carbon at the plot scale is found in live and dead woody biomass, above- and below-ground, herbaceous biomass (again above- and below-ground, and live and dead), coarse woody debris, litter (that has been abscissed from plants), and soil, associated with microbes, soil organic matter and minerals (Snowdon et al., 2002). Compartments will be measured following standard procedures outlined in

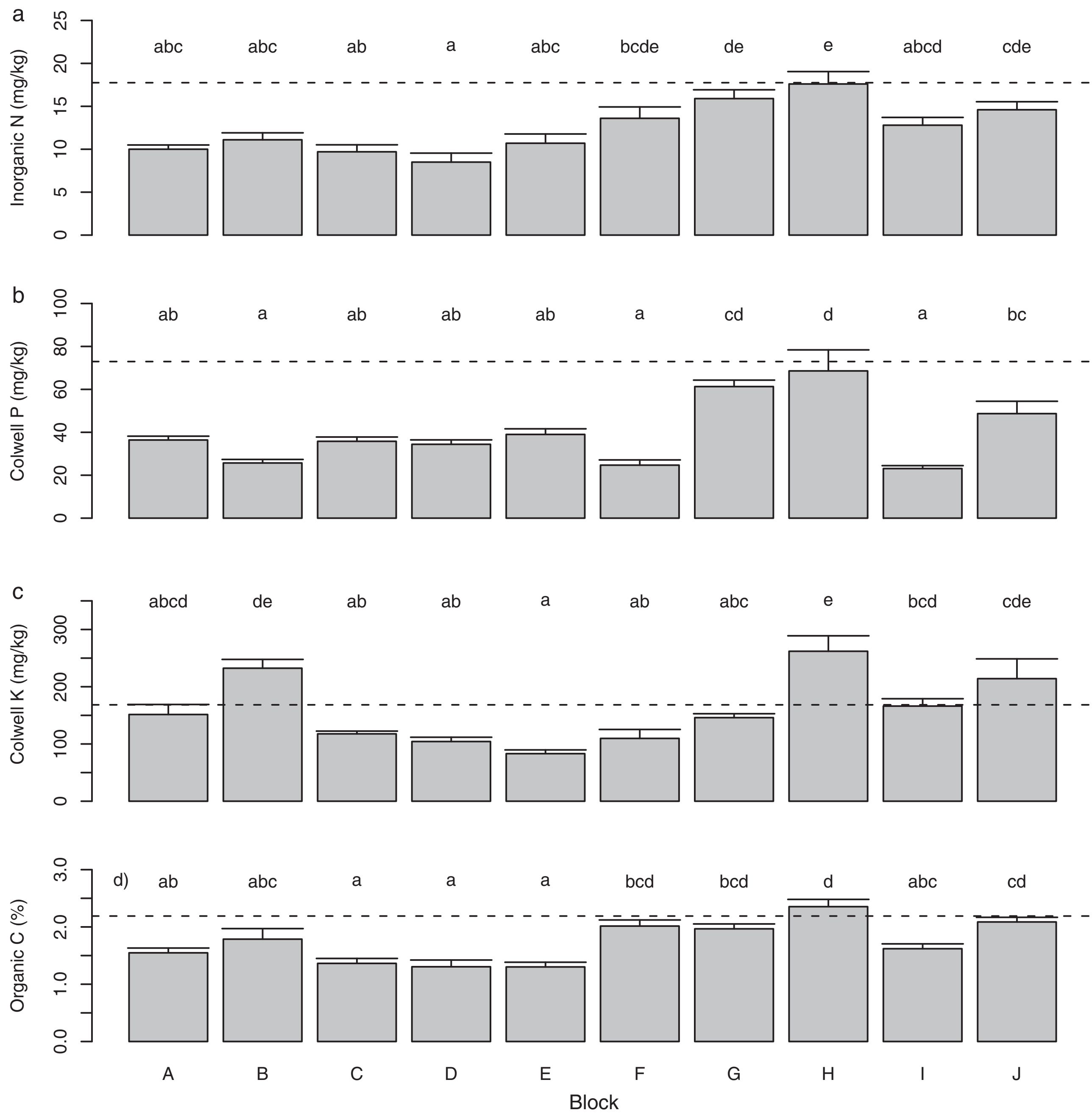

Fig. 3. Mean (+1SE) plant available nutrient and soil carbon concentrations by block within the formerly grazed area at Ridgefield (dashed line shows mean concentration in the cropped area for comparative purposes) for (a) total inorganic nitrogen; (b) Colwell extractable phosphorus; (c) Colwell extractable potassium; and (d) organic carbon. The same letter above different blocks denotes that these block means are not significantly different (at $p = 0.05$), using a Tukey HSD test (Zar, 1999). Other physical and chemical properties are shown by block in Supplementary Information, Figs. S3–S5.

Australia's National C Accounting Scheme (e.g. Eamus et al., 2000; Keith et al., 2000) and using information from Montagu et al. (2005) and Coomes et al. (2002).

Carbon in woody plant biomass will be determined using destructively harvested individuals of the species in the experiment that have been planted in separate allometry plots. This approach is necessary as published information generally only considers harvestable timber species and tends to ignore other trees and shrubs (Grierson et al., 2000; although see Jonson and Freudenberger, 2011), which make up the bulk of the species in the experiment. Since relationships may vary with nutrient availability (although note Montagu et al., 2005), control and N addition plots are included in the allometric plot design.

Sampling for dead above-ground biomass and C content will take place as and when required i.e. when dead biomass is present in the plots. Destructive harvesting to estimate fine root biomass and hence C content will not be possible within the allometric areas. Instead, the methodology of Barton & Montagu (2006) will be followed within our experimental plots giving us fine root estimates for the plot scale. Methods for assessing soil C are continually being improved and will likely change over the course of this long term experiment. However, soil samples have been taken, including at depths to approximately 4 m, to monitor the changes in soil C engendered by the different treatments. The effect of species mixtures on below ground C inputs, particularly in these woody systems, remains untested and unknown;

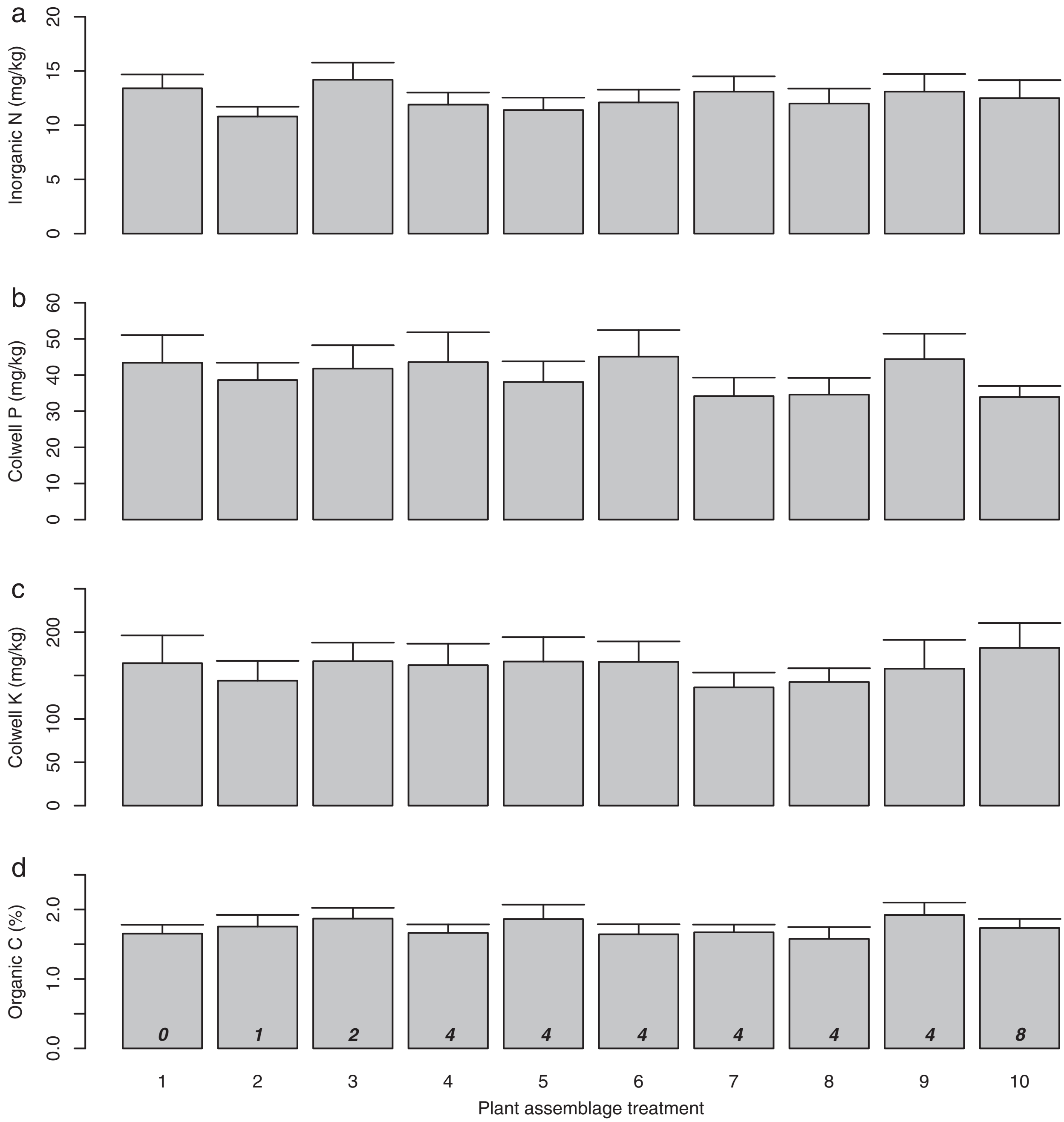

Fig. 4. Mean (+1SE) plant available nutrient and soil carbon concentrations in the different plant assemblage treatments in the formerly grazed area at Ridgefield for (a) total inorganic nitrogen; (b) Colwell extractable phosphorus; (c) Colwell extractable potassium; and (d) organic carbon. Treatment numbers as per Table 2, with species richness in the different treatments noted within the bars in (d). There are no significant differences in underlying nutrient concentrations among plant assemblage types within the different land use types, despite the variation in soil nutrients across the formerly grazed area of the site (as shown in Fig. 3 and Supplementary Information, Table S.I.3.1).

our experiment will be a vital contribution to knowledge in this area.

5.2. Biotic resistance

Biotic resistance to weed invasion will be measured in our different treatments by recording the identity and cover of weed species that are present, and the change in cover over time. Biotic resistance of other trophic groups (e.g. ants) may also be investigated and compared among treatments.

5.3. Soil erosion control

The preservation of soil is vitally important to the continued functioning of ecosystems, and is regarded as being particularly important in south-western Australia, due to the nutrient impoverished soils that characterize the area (Hopper, 2009). Agricultural practices alter soil properties compared to remnant areas, thus preventing soil biodiversity persistence and the delivery of other ecosystem services (Yates and Hobbs, 1997; Prober and Smith, 2009). Soil loss will be monitored through the use of sediment traps, and changes in soil condition will be investigated through

Table 6
Example experiments set up using functional traits of woody species to inform restoration practitioners, scientists and policy makers more broadly as to the impact of species mixtures upon ecosystem functions and services.

Experiment Name	Location	Treatments	Service Measures	Additional notes
Ridgefield Multiple Ecosystem Services Experiment (Ridgefield)	Western Australia, Australia	0,1,2,4,8 species planted according to functional diversity and structural complexity 10 treatments overall	See Table 5	N deposition and weed removal treatments nested within assemblages. Land use history comparison
BIOTREE	Thuringia, Germany	4 species mixtures planted over a gradient of functional diversity 24 treatments overall	Carbon pools and fluxes Soil water chemistry Nutrient cycling Biodiversity	See Scherer-Lorenzen et al., 2007 for further details
Biodiverse Carbon Study	Tasmania, Australia	0,1,2 and 3 species mixtures, 16 treatments overall	Above/below ground C before, immediately after and following planting	8 replicate blocks in 3 sites Genetic provenances also being tested Understorey shrub presence/absence nested within plant assemblages Neil Davidson, personal communication
The Thiaki Creek Reforestation Project	Queensland, Australia	1, 6 or 24 species with the 6 species from 6 different families, and 24 species incorporating 4 species from each of the 6 families	Above/below ground C Soil compaction Soil nutrients Plant and insect biodiversity	2 density treatments 1.75 m and 3 m spacing Margie Mayfield, personal communication

For further examples of the effect of woody species richness on ecosystem function, see TreeDivNet: www.treedivnet.ugent.be.

measuring water stable aggregates, indicative of the ability of soil to avoid erosion (Tisdall and Oades, 1982).

5.4. Nutrient cycling

We have chosen to examine an index of nutrient availability, with lower plant available nutrients being considered to provide a better level of ecosystem service outcome, as there will be less potential for available nutrient losses and consequent eutrophication downstream. Further, we will investigate different loss pathways (dissolved organic losses, gaseous losses) from our treatments to indicate the efficiency of nutrient cycling. The level of ecological knowledge surrounding nutrient cycling in woodlands in the wheatbelt is relatively poor (Yates and Hobbs, 1997), although has been increasing in recent years (Prober and Smith, 2009). In particular, we will establish which treatments most effectively decrease available P, and its supply from reactive P pools, as this biochemical pathway is usually less important than organic supply of available P through mineralization in most native systems (Walbridge et al., 1991; Perring et al., 2008, 2009). We will compare data from the assemblages with reference sites (remnants) of *E. loxophleba* woodland (Yates and Hobbs, 1997; Prober et al., 2011).

5.5. Pollination

E. loxophleba is pollinated by a range of insects and birds (Sampson and Byrne, 2008). We will measure seed set of *E. loxophleba* in each of the different treatments as a proxy for pollination. We will also record numbers and richness of floral visitors to *E. loxophleba* across treatments. In combination, floral visitor observations and seed set measurements will elucidate whether other flowering plants in the assemblage compete for, facilitate, or have no effect on *E. loxophleba* pollinators. We will also measure floral traits that affect pollinator attraction (e.g. flower number, size, nectar availability) across treatments to test whether N addition (e.g. Burkle and Irwin, 2010) or non-native groundcover affect these interactions via effects on floral traits. We are aware that pollination services depend on mobile insects whose foraging will likely occur at scales greater than the size of our experimental plots. The measures outlined above will allow us to determine if multi-species plots attract a greater diversity of insect pollinators and result in increased pollination of a common species to all plots compared to single-species plots.

5.6. Biodiversity maintenance

The plant assemblage treatments, together with the imposed environmental changes will likely affect the invertebrate fauna, soil biodiversity, and herbaceous flora. Invertebrate responses will be monitored through a network of pitfall traps. Soil microbial assays will be carried out. Above-ground herbaceous plant diversity will be recorded using quadrats to sub-sample the experimental treatment plots; any woody recruits in experimental plots will also be recorded. We will note whether native biodiversity is allowed to persist in the different plant assemblage treatments, under the experimentally imposed conditions.

6. Outlook

Restoration projects that contribute to the provision of multiple ecosystem services are likely to become more common as various ecosystem functions become increasingly valued by society. The likely demand for C sequestration projects provides an opportunity to investigate how multiple services can be supplied and what trade-offs exist among services, particularly on former agricultural land because agricultural landscapes are likely places for such projects. Trade-offs may arise due to interactions between species and the traits contained within assemblages, and potential trade-offs may be altered by ongoing environmental changes. Although theoretical analyses suggest more species are required to provide increased levels of multiple services compared with the levels provided by single species (Hector and Bagchi, 2007; Gamfeldt et al., 2008), experimental confirmation of these theoretical predictions is scant (but see Zavaleta et al., 2010), and, as far as we are aware, non-existent for mixes of native woody species in a restoration context. Ridgefield investigates how species traits found within assemblages contribute to the provision of multiple ecosystem services.

The Ridgefield experiment is one of a growing network of experiments in Australia and across the globe (see Table 6 for some examples) that are using a trait framework to ascertain the contribution of different species to the provision of ecosystem services. Ridgefield's unique contribution is to simultaneously investigate trade-offs among services, environmental change impacts and the contribution of novel components of the flora to the provision of multiple ecosystem services. Notwithstanding this, there is clearly scope for syntheses of information and comparative analyses of responses both across Australia and more widely. In addition, the array of experiments is filling a need for long term study sites and rigorous experimental designs to assess the resilience of ecosystem service provision to ongoing environmental changes and variability. Such experiments will increase the evidence base for policy set in the context of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES; http://ipbes.net/), and should therefore improve management and restoration for multiple ecosystem services in the future.

The emerging C market, continued environmental change, and the presence of novel ecosystems give us an obligation to reassess the goals of ecological restoration in agricultural landscapes. Increasing awareness of the value of ecosystem services, and the dependence of their effective delivery on species traits and biodiversity, suggests a need to experiment with methods to afforest with diverse mixes of woody species. Experimental evidence is lacking for whether diverse mixtures will indeed simultaneously provide high levels of multiple ecosystem services, and what trade-offs exist among services, under continued environmental change. The lower survival of some species in our experiment and variation in land-use legacy across the site may affect service delivery, and trade-offs among services, into the future. In combination with other studies, essential scientific knowledge and guidance to restoration practitioners and policy makers in the shorter and longer term will be provided by the Ridgefield Multiple Ecosystem Services Experiment as we attempt to mitigate ongoing environmental change and maintain the ecosystem services that underpin human well-being.

Acknowledgements

KBH, LL, TM, RP, MPP and RJS were funded through an ARC Laureate Fellowship awarded to RJH, and by the University of Western Australia; RKD was funded by an ARC Future Fellowship award. Funding bodies had no further influence on the contents of this manuscript. We thank Georgina Holbeche for assistance with soil texture analysis. We thank Nancy Shackelford for creating the R code for determining the placement of individual plants in our experimental plots, and NS and Heather Gordon for drawing Fig. 1. We further thank NS, HG, Kristy Robertson, Ian Williams and numerous volunteers for their help at the site. In particular, we thank Jo Wheeler and her colleagues at Men of the Trees for assisting with site preparation and planting. We thank two anonymous reviewers and the editors for constructive comments on previous versions of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.agee.2012.02.016.

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