The incorporation of fungal to bacterial ratios and plant ecosystem effect traits into a state-and-transition model of land-use change in semi-arid grasslands

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Abstract

Feedbacks between plants, microbes and their growth traits are important in the maintenance of nutrient cycling functions. Despite this, there is little understanding of the role these relationships may play in the transition between alternate vegetation states in semi-arid and arid lands. We investigated the relationships between vegetation, soil nutrients and soil microbes across grasslands and agricultural fields described within an existing conceptual state-and-transition model of agricultural de-intensification in the semi-arid Riverine Plains grasslands of south-eastern Australia. Sites represented the proposed transition from annual exotic pastures to native perennial grasslands with agricultural de-intensification. Microbial community composition was assessed using phospholipid fatty acid analysis (PLFA).

The native grassland state and the native pasture state were characterized by a higher fungal to bacterial ratio (F:B). The native grassland state had a slightly lower bacterial PLFA biomass whilst the native pasture state had a slightly higher fungal PLFA biomass, although these differences were non-significant. Only the recently cultivated, heavily grazed state was characterized by high soil nutrient availability (soil P and K) and leaf traits indicating rapid growth and resource utilization (high SLA, low LDMC). Thus, the association of these ecosystem properties with a lower F:B was not as close as expected. States with a higher F:B were not characterized by higher total soil C or C:N as hypothesised.

This study further extends our knowledge of the association between fungal dominance and agricultural de-intensification to a semi-arid system with relatively

old, nutrient poor soils. It highlights the need for a better understanding of the mechanistics behind this association and the implications for C cycling and storage in such systems.

Key words: Bacteria, fungi, leaf traits, land-use change, soil microbial communities

1. Introduction

With both the intensification and abandonment of agricultural practices continuing worldwide, there is an increasing need to understand the impacts of land use changes on essential ecosystem services including nutrient cycling (Alcamo *et al.*, 2005). Central to the delivery of ecosystem processes associated with nutrient cycling is the link between soil microbes and plant growth, community structure and functional traits (Wardle *et al.*, 2004). While the value of incorporating both vegetation and soil microbes into multi-trophic models of ecosystem change is recognized (Bardgett *et al.*, 2005), this has rarely been applied in the context of land-use change in semi-arid systems.

There are two primary decomposer energy-channels of the detritus based microbial food web, the fungal (F) channel and the bacterial (B) channel (see Moore and Hunt, 1988). With ecosystem transition following the de-intensification of agricultural practices, the ratios of fungi to bacteria (F:B) in soil typically increase toward fungal dominance (e.g. Bardgett and McAlister, 1999; Zeller *et al.*, 2001 & van der Wal *et al.*, 2006). Reductions in fertilizer inputs (de Vries *et al.*, 2007), tillage (Bailey *et al.*, 2002), grazing regimes and acidity (Bardgett *et al.*, 1996) have all been associated with higher soil F:B following agricultural de-intensification. The most widely used method of measuring F:B is by analyzing the main component of cell membranes, phospholipid fatty acids (PLFA). The total concentration of PLFA gives

information on approximate microbial biomass and signature markers provide the possibility of separating large functional groups (see methodological overviews of Joergensen and Wichern, 2008 & Frostegård *et al.* 2010).

The shift toward fungal rather than bacterial dominance with agricultural de-intensification may be indicative of systems with a greater capacity for self-regulation (Bardgett and McAlister, 1999). It has been associated with changes in ecosystem functions including enhanced rates of carbon (C) use efficiency and storage (Ohtonen *et al.*, 1999, although see Theit *et al.* 2006) and decreased nitrogen (N) mineralization potentials (Ferris and Matute, 2003 & Hogberg *et al.*, 2007a). Across European temperate and sub-alpine agro-ecosystems, increases in fungal PLFA biomass and/or ergosterol biomass (the predominant sterol of fungal cell membranes) with agricutural de-intensification have been assoicated with higher organic C (de Vries *et al.* 2012, de Vries *et al.* 2007 & Zeller *et al.* 2001) and soil C:N (Zeller *et al.* 2001 & Hogberg *et al.* 2007b).

It has also been suggested that increases in fungal dominance with agricultural de-intensification will be associated with secondary succession of vegetation communities typified by resource-exploitative traits (including high specific leaf area [SLA], low leaf dry matter content [LDMC] and high leaf N content [LNC]) to communities with resource-conservative traits (including low SLA, high LDMC and low LNC) (Wardle *et al.*, 2004 & Bardgett *et al.*, 2005). These plant traits have been termed 'ecosystem effect traits' and can exert strong effects on ecosystem properties associated with nutrient cycling including decreases in soil fertility, productivity and decomposition rates with agricultural de-intensification (Garnier *et al.*, 2004 & Quétier *et al.*, 2007). High vegetation relative growth rates have been associaed with increased bacterial PLFA biomass, N pools and N mineralisation rates (Orwin *et al.*

2010) and increased vegetation SLA has been assocated with higher F:B and total soil soil C (deVries *et al.*, 2012).

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Australian soils and vegetation contrast with the sub-alpine and temperate meadow and agricultural fields throughout Europe where the theory of these associations has been tested. Generalisations of ecological theory require verification across systems that are relatively old, climatically buffered and relatively infertile, such as many Australian soils (Hopper, 2009). Australian vegetation displays adaptations for survival in low-nutrient conditions and fire is a potential evolutionary force (Hopper, 2009). Furthermore water is likely to co-limit plant productivity in dry-land areas (Hooper and Johnson, 1999). These factors combined may contribute to differences in how C is cycled and stored (De Deyn *et al.*, 2008) and how vegetation responds to the abandonment of modern-day agricultural practices (Hopper, 2009 & Cramer *et al.* 2008).

With the abandonment of cropping and fertiliser addition over ~ 100 years across the semi-arid Riverine Plains of Victoria, Australia, grassland communities have shown the capacity to converge toward a subset of the uncultivated grasslands, from initial exotic dominance (predominantly of Mediterranean and Asian origin) to native perennial grass dominance (Wong *et al.*, 2010 & Scott and Morgan, 2011). Soil nutrient availability decreases to reach that of the uncultivated state within ~ 50 years (Scott and Morgan, 2011). State-and-transition models are useful tools for conceptualizing non-linear ecosystem dynamics incorporating multiple stable or transitional states (Westoby *et al.* 1989). A state-and-transition model for this system based on time-since-cultivation (Wong *et al.*, 2010) incorporates these vegetation and soil components, highlighting the multiple states and transitions that could occur under alternate management regimes (see also Westoby *et al.*, 1989 & McIntyre and

Lavorel, 2007).

Using the criteria identified in this model we identified sites that represent the proposed states and transitional states of the semi-arid Riverine Plains grasslands to test the hypotheses that with agricultural de-intensification: 1. Fungal biomass and the ratio of fungi to bacteria (F:B) will be higher. The PLFA markers commonly used in the calculation of F:B ratios were used here to allow comparison with other studies (Frostegård and Bååth, 1996 & Frostegård *et al.*, 2010) and 2. Community weighted means (CWMs) of plant growth traits for slower growth and resource conservation will be higher (higher LDMC, lower SLA) and 3. C sequestration will be higher (Higher total soil C, higher C:N and lower labile C). The findings will be incorporated into the existing conceptual state-and–transition model for grassland management of this system.

2. Methods

2.1 Study area and sampling

The Riverine Plains is classified as semi-arid (Henderson-Sellers and Pitman, 1991). There is high inter-annual variation in rainfall, with the austral-winter months of June and July weakly dominating. Seasonal average temperatures range between 15 °C and 30 °C in summer and 3 °C and 15 °C in winter (Australian Bureau of Meteorology, 2014). The majority of the plains are currently subject to rotational sheep grazing-cropping, with paddocks typically grazed for a period of three to 10 years between cropping cycles (Wong *et al.*, 2010). Alternatively, cropping is ceased and replaced entirely with stock grazing. Long-term grazing levels are approximately 2 – 2.5 dry sheep equivalents per hectare. The soils are low in phosphorus (P) and N and superphosphate is commonly required for pastures and crops (Skene, 1971).

Using the criteria identified in the state-and-transition model based on time-since-cultivation (Wong *et al.* 2010), we identified 23 sites representing three proposed vegetation states and one transitional vegetation state that occur following agricultural de-intensification of Riverine Plains. Soil P ranges from 24.25 mg/kg to 9.5 mg/kg (Colwell P), with no fertilizer additions after the cessation of cropping. The native grasslands are considered to have been impacted by past management regimes and are now dominated by C3 grass tussocks inter-dispersed with perennial and annual forbs mainly of the families Asteraceae and Chenopodiaceae (see Wong *et al.*, 2010). The study was restricted to the brown/orange clay loam over red/yellow clay sub-soils of the 'Red Soil Plains', derived from the deposition of Quaternary alluvial sediments. Site elevations ranged from 90 m – 110 m above sea level (Department of Environment and Primary Industries, 2014). The sites were as follows:

1. 'Native grassland' ('state one'), six sites. Never-cultivated, never fertilized. Characterized by a wide range of native perennials, in particular geophytes and forbs (e.g. *Wurmbea dioica*, *Arthropodium minus*, *Bulbine bulbosa*, *Chrysocephalum* sp. 1 and *Cheilanthes* sp.). Grazing intensity and soil disturbance are very low.
2. 'Native Pasture' ('state two'), six sites. A long-uncultivated state (before 1955). Characterized by a range of native annuals as well as native perennial species (e.g. *Asperula conferta*, *Swainsona* spp. and *Salsola tragus*). Grazing intensity is medium to high and soil disturbance is low.
3. 'Volunteer Pasture' ('state seven'), six sites. A recently cultivated state (last cultivated after 1980). Characterized by exotic annual grasses (e.g. *Avena barbata*), a range of exotic annual forbs and several native species including

the perennial grasses *Rytidosperma* spp. and *Austrostipa* spp. Grazing intensity is high and soil disturbance is low.

4. 'Recently cultivated' (Transition six to seven), five sites. Cultivated between 5 and 10 years ago. Characterized by early invading ruderal species, in particular exotic annual grasses and forbs. Widely distributed native grasses in these sites are absent (e.g. *Rytidosperma* spp. and *Austrostipa* spp.) and cereal and fodder crops are still present (e.g. *Medicago sativa* and *Hordeum vulgare*). Soil disturbance and nutrients are proposed to be intermediate between state six and seven, with high grazing intensity and medium levels of soil disturbance.

Soil was collected and vegetation measurements taken from each site in October 2010 along a 20 m transect. All transects were established at least 50 m or more from fence boundaries. Soil cores were taken using an auger (10 cm in diameter) every 2 m along each transect. Core depth was 0 – 15 cm, sampling only the A_1 horizon. These soil cores were carefully mixed (within transects) and passed through a 2 mm sieve, giving one composite soil sample per site. A 100 g sub-sample of soil from each transect was immediately frozen (-20 °C) for later microbial community analysis (Section 2.4). All remaining soil was placed in sealed plastic bags and stored at 4 °C until returned to the laboratory for immediate physicochemical analysis (Section 2.3).

2.2 Vegetation measurements

For each study site, 100 point quadrats were measured (at 20 cm intervals) using a 4 mm diameter pin. All species touching the pin were recorded. Total cover for native perennials, native annuals, exotic perennials and exotic annuals was

calculated as the percentages of total pin touches per study site. Community weighted means were calculated for the plant traits specific leaf area (SLA) and leaf dry matter content (LDMC). These plant traits have been linked with key biogeochemical processes including productivity, decomposition and soil nutrient status in the context of land-use change intensification (Garnier *et al.*, 2004 & Quétier *et al.*, 2007). Leaf dry matter content (LDMC) is the ratio of leaf dry mass (mg) to water-saturated fresh mass (g), expressed in mg/g^{-1}. Specific leaf area (SLA) is the one sided area of a fresh leaf divided by its oven-dry mass, expressed in m^2kg^{-1} (Cornelissen *et al.*, 2003).

For each individual site, the CWM calculation included trait values for all of the species which, taken together, accounted for 90 % of the total vegetation touches. LDMC and SLA values were taken from data collected between 2005 and 2011 across the Riverine Plains. All measurements were made in accordance with the standardized procedure of Cornelissen *et al.* (2003). CWM calculations for both LDMC and SLA followed Garnier *et al.* (2004):

$$\mathrm{CWMj} = \sum_{k=1}^{n_j} A_{k,j} \times ET_{k,j}$$

Where: n_j is the number of species sampled in community j, $A_{k,j}$ is the relative abundance of species k in community j and $ET_{k,j}$ is the effect trait of species k in community j. Note that effect trait values here were taken as averages from plants sampled across the Riverine Plains, rather than specifically from within each site.

2.3. Soil physicochemical analysis

Upon return to the laboratory, the soils were divided into four sub-samples. The first sub-sample was used for determination of mineral N pools. Extractions were carried out on triplicate 10 g soil samples by adding 20 ml of 2 M KCl and shaking

for 1 h at 200 rpm. The inorganic N content of the extracts (nitrate NO_3^- and nitrite NO_2^-) were determined colourmetrically (TeCan EVO Spectrophotometer, TeCan, Germany) following the methods reported within (Asghari and Cavagnaro, 2011).

The second sub-sample was for determination of the amount of potentially mineralisable N (PMN) following the method of Keeney & Nelson (1982). Triplicate 10 g soil samples were weighed into tubes. To each tube, 10 ml water was added and headspaces were filled with N_2 to establish anaerobic conditions. Samples were then incubated at 37°C for seven days. After incubation, 10 ml of 4 M KCl was added to each tube and NH_4^+-N was extracted and measured as described above. Potentially mineralized N was calculated from the increase in NH_4^+-N between day 7 and day 0.

The third sub-sample was used for determination of gravimetric soil moisture by drying 10 g of soil at 105ºC for 48 hours. On the fourth sub-sample, the following soil physicochemical properties were measured by Southern Cross University Environmental Analysis Laboratory (2013): Total soil C and N by were measured by dry combustion (CHN-2000, LECO Corporation, St Joseph MI, USA), the labile fraction of soil C as permangenate-oxidisable C, plant available (Colwell) P, potassium (K) (Morgan 1), pH and electrical conductivity.

2.4. Microbial community measurements

The ratios of fungi to bacteria and estimates of total, bacterial and fungal PLFA, were determined by the analysis of soil microbial phospholipid fatty acids (PLFA) (Bardgett *et al.*, 1996). Briefly, duplicate 4 g frozen soil samples from each site were ground with a mortar and pestle at 4°C, following removal of visible roots and other plant matter. PLFAs were extracted from the ground soil samples following the method of Frostegård et al. (1991). Lipids were extracted with a one-phase mixture (0.9:1:2) of chloroform, methanol and citrate buffer (0.15 M, pH 4) and then

fractionated through solid phase extraction columns (GracePure SPE Silica 500 mg/6 ml, Grace Davidson, IL, USA). The neutral lipid fractions were eluted with 5 ml $CHCl_3$, glycolipids with 10 ml acetone and phospholipids with 5 ml methanol. The phospholipids were then subject to mild alkaline methanolysis.

The resulting fatty acid methyl esters were separated on a gas chromatograph (CP 3800, Varian, CA, USA) equipped with CP-1177 splitless injector and flame ionization detector. The column was 30 m, 5 % phenyl and 95 % methylsiloxane coated, 0.25 µm film thickness with an internal diameter of 0.25 mm (FactorFour, CP8944, Varian, CA, USA) and helium as the carrier gas. Phospholipid fatty acids were identified by comparing GC retention times with a Bacterial Acid Methyl Ester (BAME) standards mix (product number 47080-U, Supelco, St. Louis, MO, USA) and quantified by external calibration. Markers of interest that were not included in the BAME mix were identified from their mass spectra and relative retention times by GC/MS (CP3800 and Saturn 2200, Varian, CA, USA), using the same injector and column parameters and were quantified using response factors for isomers present in the BAME standard.

Fungal and bacterial PLFAs were used to indicate the relative abundance of these two microbial groups (Bardgett *et al.*, 1996). Bacterial phospholipid markers of interest were those considered to be of bacterial origin (see Frostegård and Bååth, 1996 and references therein). These markers were i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, a17:0 17:0cy, 17:0 and 19:0cy. Linoleic acid (18:2ω6,9) was used as an indicator of fungal biomass (Frostegård and Bååth, 1996 & Frostegård *et al.*, 2010). This is a signature fatty acid for saprophytic and ectomycorrhizal fungi. It has been closely associated with soil ergosterol that is highly specific to these higher fungal

phyla (Joergensen and Wichern, 2008). Total PLFA biomass was taken as the total of bacterial and fungal PLFA.

2.5. Statistical analysis

ANOVA and Tukey's pair-wise comparisons were performed in author SYSTAT (Version 12, 2007, Systat Software Inc.). Data was $\log_{10}$ transformed as required to meet ANOVA assumptions. To assess whether individual PLFAs were indicative of any one vegetation state, comparisons were made using the indicator species analysis of Dufrêne and Legendre (1997) in PC-ORD (McCune and Mefford, 2006). This method combines information on the concentration of marker abundance in a particular group and the faithfulness of occurrence of the marker to a particular group. Prior to this analysis, PLFA data were square-root transformed to reduce skewness and the coefficients of variation. Of the PLFA markers identified in all samples, only those that were present in > 5 % of all samples were included in the indicator species analysis, this included a total of 24 PLFAs. The statistical significance of the indicator values were calculated against 1000 randomizations by Monte Carlo Method.

3. Results

3.1. Vegetation properties

Plant group cover varied across the vegetation states sampled for exotic annual (*F*-stat = 22.15, *P*-value = <0.001), exotic perennial (*F*-stat = 5.44, *P*-value = 0.007) and native perennial cover (*F*-stat = 10.56, *P*-value = <0.001), yet there were no significant differences in native annual cover (*F*-stat = 1.54, *P* = 0.234) (Fig. 1). Exotic annual cover was highest in the recently cultivated sites, with cover decreasing through to native grasslands. In contrast, native perennial cover was highest in the native grasslands, with cover decreasing through to recently cultivated pastures. The

cover of exotic perennials was lower in recently cultivated sites. There were significant differences in community weighted means of both LDMC (*F*-stat = 8.76, *P*-value = <0.001) and SLA (*F*-stat = 16.97, *P*-value = <0.001) (Fig. 2). In the recently cultivated pastures, LDMC was lower whilst SLA was higher.

3.2. Microbial and soil physicochemical properties

There was a significant difference in F:B between the vegetation states (*F*-stat = 6.28, *P*- value = 0.004) with F:B being higher in both the native grasslands and native pastures (Fig. 3a). The higher F:B of native pastures was likely due high fungal PLFA relative to the other vegetation states (Fig. 3b). This was supported by 'indicator species analysis' of individual PLFAs, revealing the fungal biomarker (18:2ω6,9) as a significant indicator of the never-cultivated native pastures (*P* = 0.01, indicator value 31 %). There were no other PLFA biomarkers that were significant indicators of the vegetation states (*P* > 0.05) (See Appendix Table A.1). The higher F:B of the native grasslands was likely attributed to a combination of a slightly higher fungal PLFA (Fig. 3b) and lower bacterial PLFA (Fig. 3c). There was no significant difference in total PLFA between the vegetation states (*P* < 0.05) (Fig. 3d). For soil physiochemical properties, there were significant differences in soil K (*F*-stat = 4.22, *P*-value = 0.018) and plant-available (Colwell) P (*F*-stat = 25.65, *P*-value = <0.001) between the vegetation states, with significantly higher levels in the recently cultivated sites (*P* < 0.05) (Table 1).

4. Discussion

4.1. Overview

We utilized an existing conceptual state-and-transition model for land-use change in this system to summarize the main findings of this study (Fig. 4). There was an increase in F:B at the native pasture and native grassland (reference grassland) state.

At the volunteer state, CWM for SLA was higher and LDMC lower, suggesting more rapid growth and resource utilization. As expected, soil nutrients (plant available P and K) were higher at the recently cultivated state and were lower at the less intensively managed grassland states. Native perennial vegetation was higher with decreased management intensity and exotic annual species lower. These soil and vegetation changes are as previously described in the existing state-and-transition model. The above- and below-ground observations were as hypothesized; however, they did not occur in tandem as predicted (Bardgett *et al.*, 2005). That is, contrary to the hypothesis, increases in F:B were not closely associated with increases plant traits for slower growth and resource ultilisation, or increases soil total C, C:N or decreases in labile C with agricultural de-intensification. The importance of the findings in the context of existing studies follows in the sections below. It should be noted that the proposed model acknowledges that transitions between grassland states are likely to be driven by complex management regimes and multiple biotic and abiotic factors and that transition toward the 'reference grassland' state, if it does indeed occur, will not necessarily be linear or stable (see Wong *et al.* 2010 & Cramer *et al.* 2010 and references therein).

4.2. Vegetation

As hypothesised, high soil nutrient availability was associated with leaf traits for fast growth and resource acquisition (high SLA, low LDMC). These properties were characteristic only of the recently cultivated state. Soil P and K are most likely high at this state due to the only recent cessation of fertilizer inputs (5 to 10 years ago), which would have been predominantly superphosphates. The plant traits observed likely reflect those of the recently sown crops.

The differences in plant leaf traits between the recently cultivated and volunteer pasture state may be a result of reductions in nutrient availability constraining the persistence of resource-exploitative exotic annual species (high SLA, low LDMC), favoring more slower-growing resource-conservative native perennial dominance (low SLA, high LDMC) (see Wright *et al.*, 2004). These shifts have been observed in grasslands with reduced management intensities (e.g. Quétier *et al.*, 2007) and old fields (e.g. Garnier *et al.*, 2004). Time-since-cultivation chronosequence studies in this system have demonstrated that soil nutrient availability can decrease to reach that of the uncultivated state within ~ 50 years (Scott and Morgan, 2011). The importance of soil P in driving the observed differences in the measured plant traits relative to other potential environmental constraints, and trade-offs of plant species responses to these constraints (Tilman, 1990), will require direct investigation in manipulative studies. For example, persistent grazing intensity could similarly drive shifts increases in LDMC (e.g. Quétier *et al.*, 2007).

That there was no difference in community-level plant traits beyond the volunteer state may have to do, in part, with the lack of difference in soil nutrient availability between the states. This would be in line with the hypotheses, though again requires determination by manipulative studies. The lack of difference in the measured plant traits between the grassland states could also be influenced by seed limitation (Scott and Morgan, 2011) patch grazing (McIntyre and Lavorel, 2007) and the timing, type and degree of disturbances (Cramer *et al.*, 2008). There may be a continuum of multiple plant growth strategies should transition toward the native grassland state occur (Huston and Smith, 1987), for example diversification of nutrient acquisition strategies (Lambers *et al.*, 2010). Negative plant-soil feedbacks may become increasingly important in the maintenance of native plant species

richness (Bever *et al.* 1997). Trait plasticity between the states (Godoy *et al.*, 20110) and percent biomass contribution of species (Garnier *et al.*, 2004) should also be considered.

4.3. Soil microbial communities

It was hypothsised that lower quality and quantity of plant litter input, here assumed by a lower SLA and higher LDMC, would exert strong selection pressure for a more fungal than bacterial based food web (higher F:B). There may be several reasons why these close associations may not have been observed in contrast to other studies (e.g. deVries *et al.* 2012 & Orwin *et al.* 2010). Firstly, the association of SLA and LDMC with the broader suite of plant traits associated with resource acquisition and use (including tissue nutrient content, leaf toughness/decomposability and relative growth rate) requires direct confirmation in this system. Secondly, there is evidence that the capacity of microbial groups to degrade C-substrates can vary between soil habitats (Rinnan and Bååth, 2009). Therefore, whether fungal groups are indeed utilizing more complex or recalcitrant C-continaing compounds requires direct verification, for example through the use of ^{13}C-labeled substrates (Rinnan and Bååth, 2009). Also, very slight changes in F:B may occur that are not detected here due to inherent site varition within the grassland states (van der Wal *et al.*, 2006).

The higher F:B at the native pasture state may be explained by the increase in fungal PLFA, whilst the higher F:B at the native grassland state may be explained by the slightly lower bacteria PLFA, although the differences in fungal PLFA and bacterial PLFA between the states were not significant. There were no clear contrasts in soil nutrients or pH that could clearly be associated with the quantity of fungal and bacterial PLFA as in other studies (e.g. Bardgett *et al.* 1996; Orwin *et al.* 2010 & de Vries *et al.* 2012). Other potential drivers may be hypothesized. For example the

maintenance of grazing could account for the high fungal PLFA of the unfertilised native pasture (Bargett *et al.* 1996). Differences in plant community composition, or the attributes of individual plant species, between the vegetation states could also drive differences. For example, Smith *et al.* (2003) found increases in fungal PLFA and F:B to be assoicated with an increase in vegetation diversity, legumes and stress-tolerator traits.

The ability to detect changes in fungal and bacterial community dominanance across the states and transitions could also be influenced by the choosen method. For example, the amount of PLFA for specfic bacterial and fungal groups can vary within and between species, therefore PLFA provides an approximate extimate for biomass only (Olsson, 1999). The dominance of saprophytic, ectomycorrhizal and arbuscular mycorrhizal fungi AMF can change with conversion from tillage-based agriculture (Allison *et al.*, 2005), with arbuscular mycorrhizae hypothesized to be more dominant in perennial grasslands compared with arable croplands (Joergensen and Wichern, 2008). The marker used here (18:2ω6,9) does not represent arbuscular mycorrhizal fungi. More specific markers (such as the NLFA 16:1ω5) or amino sugars would detect these shifts (Joergensen and Wichern, 2008 and references therein). Further differences in microbial community composition across the states and transitions may be revealed via finer-scale metagenomic approaches (e.g. He *et al.* 2012). The consistency of microbial and soil physicochemical results across various soil depths also warrants further investigation.

4.4. Potential consequences for soil C sequestration and nutrient cycling functions

Increases in soil organic C and soil C:N have been associated with increases in fungal biomass and F:B biomass (Orwin *et al.*, 2010 & de Vries *et al.*, 2012). However, attributing fungal dominance relative to bacteria as the underlying

mechanism for greater C storage and slower C turnover has been cautioned against (Thiet *et al.*, 2006). This may explain, in part, why there were no increases in total soil C and C:N assoicated with the increases in F:B or fungal biomass at the native pasture and native grassland states. Fungi do not always have a greater gowth efficiency than bacteria (Thiet *et al.*, 2006) and C use efficiency can be influenced by a number of factors including soil structure (Six *et al.*, 2006). Furthermore, shifts within the fungal community, particularly from saprophytic to mycorrhizal fungi, can have a stronger influence on soil biological processes than changes in F:B alone, specifically decreasing microbial turnover and metabolic quotents (Joergensen and Wichern, 2008).

It can be predicted that the higher SLA and lower LDMC of the recently cultivated state may be linked with higher productivity and decomposition rates (e.g. Garnier *et al.*, 2004 & Quétier *et al.*, 2007). However, differences in nutrient cycling functions can not be assumed from the differences in F:B and plant traits between the states and transtitions. For example, high fungal biomass and low vegeation relative growth rates have been associated with increases in soil respiration and decomposition rates (Orwin *et al*. 2010). Direct linkage to nutrient cycling rates is required, for example through radioisotope analysis. Again, metagenomic approaches would also provide useful information (e.g. He *et al.*, 2012).

The lack of increase in soil C and C:N with fungal dominance and agricultural de-intensification may have to do with how C is cycled and stored in this semi-arid system. This may contrast to previous studies which have focused largely focused on temperate and sub-alpine European grassland systems (e.g. Orwin *et al.*, 2010; de Vries *et al.*, 2012 & Zeller *et al.* 2001). High temperatures and low preciptiaiton could lead to low inputs of organic C and N into these soils versus European soils (Hassink,

1997). Australian vegetation can be adapted for growth on relativly old, nutrient poor soil. For example, Australian vegeation can have compatativly higher leaf mass that some European vegetation communities (Lambers *et al.*, 2010). This may be associated with growth traits including slower growth rates, lower decomposition rates, higher tissue concentrations of low quality C such as lignin and tannins and higher leaf C:N. Hence, soil C may be held in recalcitrant forms within plant tissues, rather than through direct inputs of high quality C into the soil (De Deyn *et al.*, 2008). Following initial loses of soil C and N with cultivation, low soil N inputs and C3 grass dominance could also limit soil C accumulation (Knops and Tilman, 2000). How and where C is stored and cycled in the proposed transition towards native perennial dominance in this system warrants investigation.

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Table 1 Soil physicochemical properties of the vegetation states (means ± S.E)

Soil physicochemical properties	Vegetation states				
	Reference grasslands	Native pastures	Volunteer pastures	Recently cultivated	*P* - value
Total N (%)	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	0.10 ± 0.01	0.20
NO_3^- -N(mg/kg)	2.86 ± 1.05	3.77 ± 0.19	4.09 ± 0.42	4.10 ± 0.64	0.51
NH_4^+-N (mg/kg)	4.44 ± 1.78	2.29 ± 0.27	2.75 ± 0.49	3.03 ± 0.39	0.70
PMN (µg NH_4^+-N/g^{-1})	19.38 ± 4.79	14.59 ± 2.83	14.88 ± 2.46	17.73 ± 4.57	0.76
Total C (%)	0.87 ± 0.06	0.95 ± 0.06	0.95 ± 0.07	1.09 ± 0.09	0.23
Labile C (%)	0.20 ± 0.02	0.22 ± 0.01	0.22 ± 0.02	0.24 ± 0.03	0.56
C:N	10.06 ± 0.31	10.95 ± 0.38	10.58 ± 0.33	10.56 ± 0.41	0.38
P (Colwell) (mg/kg)	6.83 ± 0.88[a]	6.18 ± 0.47[a]	9.01 ± 0.63[a]	24.26 ± 3.62[b]	< 0.001
K (mg/kg)	154.5 ± 6.43[a]	154.5 ± 9.28[a]	148.0 ± 5.66[a]	219.2 ± 34.51[b]	0.03
Conductivity (dS/m)	0.06 ± 0.01	0.080 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.33
pH	6.60 ± 0.13	6.86 ± 0.13	6.48 ± 0.12	6.76 ± 0.22	0.30

n = 6 sites per vegetation state, with the exception of 'recently cultivated' (*n* = 5). Superscript letters indicate significant differences (P = Colwell phosphorus, $P < 0.001$; K = Potassium, $P < 0.018$).

Fig. 1 Plant group cover of the vegetation states (means ± S.E) for exotic annuals ($P <$ 0.001), exotic perennials ($P = 0.007$), native annuals ($P = 0.234$) and native perennials ($P = <$ 0.001). $n = 6$ sites per vegetation state and $n = 5$ 'recently cultivated'. Comparisons are only valid within the plant groups

Fig. 2 Plant community functional parameters (CFPs) of the vegetation states a) leaf dry matter content, LDMC ($P < 0.001$) and b) specific leaf area (SLA) ($P < 0.001$). Means ± S.E. $n = 6$ sites per vegetation state and $n = 5$ 'recently cultivated'

Fig. 3 a) Fungal to bacterial ratios, F:B ($P = 0.004$), b) fungal ($P < 0.07$), c) bacterial ($P =$ 0.75) and d) total PLFA ($P = 0.08$) of the vegetation states (means ± S.E). $n = 6$ sites per vegetation state, $n = 5$ 'recently cultivated'.

Fig. 4 State-and-transition model for cultivated and grazed grasslands of southeastern Australia. Adapted from Wong *et al.* (2010) and McIntyre and Lavorel (2007). Bold text indicates results of this study. N pools = total soil N, NO_3^--N (mg/kg), NH_4^+-N (mg/kg) and potential mineralized N (µg NH_4^+-N/g^{-1}); F:B = ratio of fungal PLFA to bacterial PLFA and SLA and LDMC = community weighted mean specific leaf area and leaf dry matter content respectively.

Figure 1

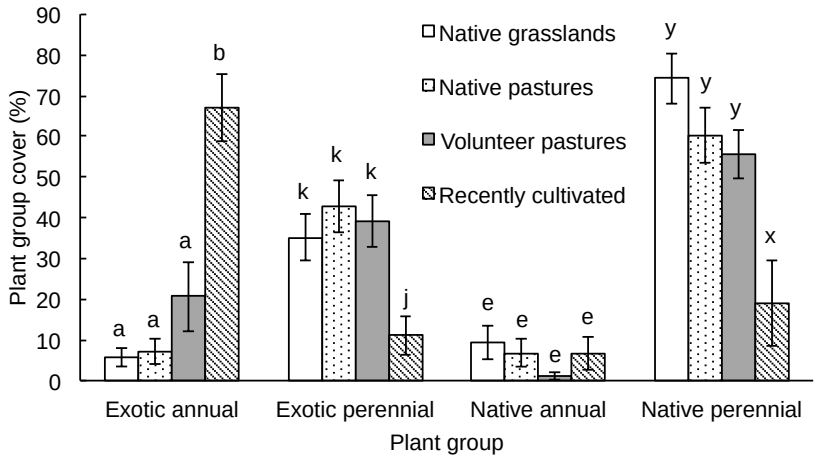

Plant group cover (%)
90
80
70
60
50
40
30
20
10
0
Native grasslands
Native pastures
Volunteer pastures
Recently cultivated
a
a
a
b
k
k
k
j
e
e
e
e
y
y
y
x
Exotic annual
Exotic perennial
Native annual
Native perennial
Plant group

Figure 2

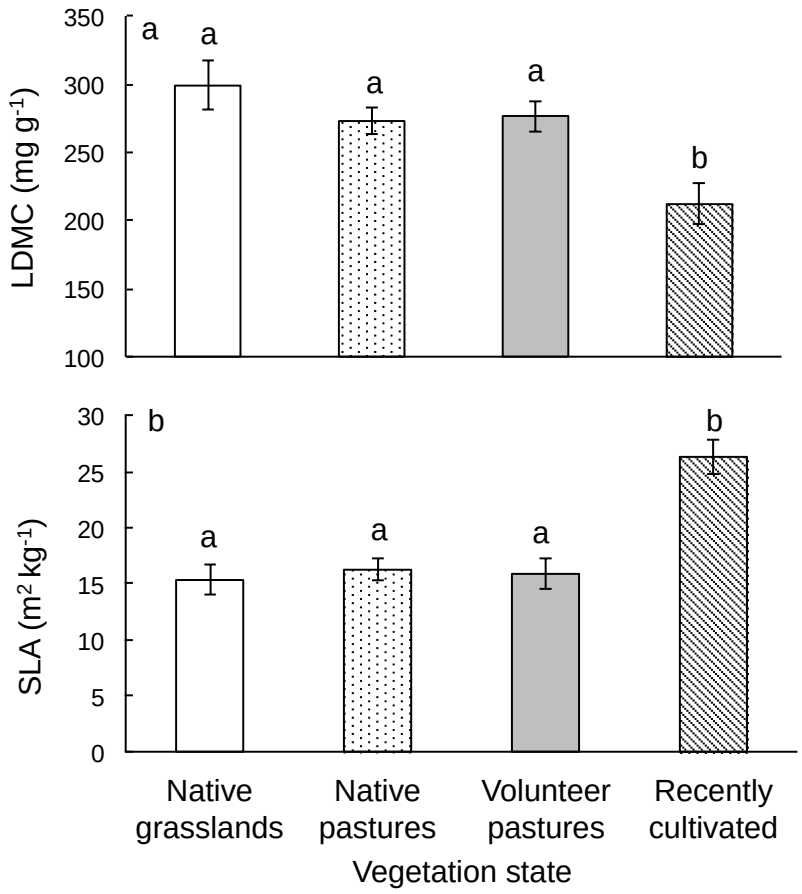

LDMC (mg g-1)
350
300
250
200
150
100
a
a
a
a
b
SLA (m2 kg-1)
30
25
20
15
10
5
0
b
a
a
a
b
Native grasslands
Native pastures
Volunteer pastures
Recently cultivated
Vegetation state

Figure 3

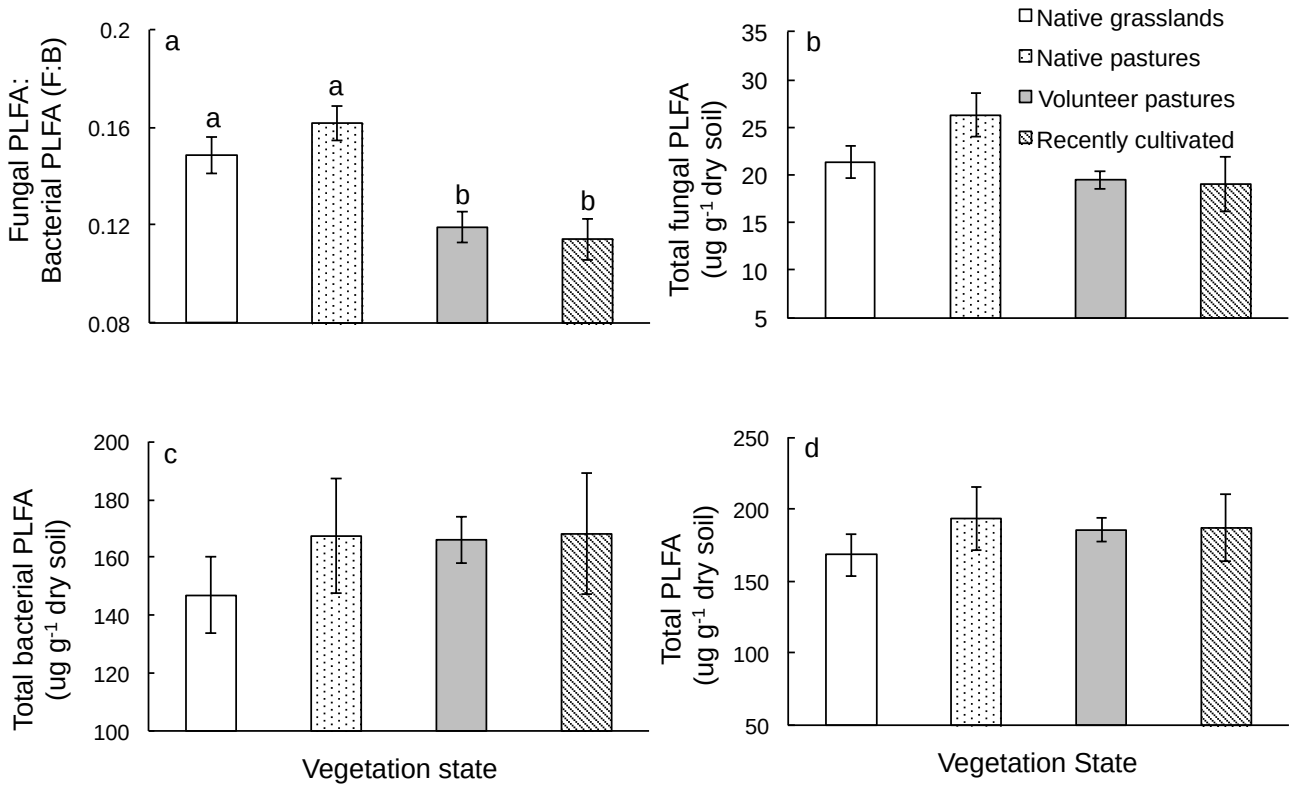
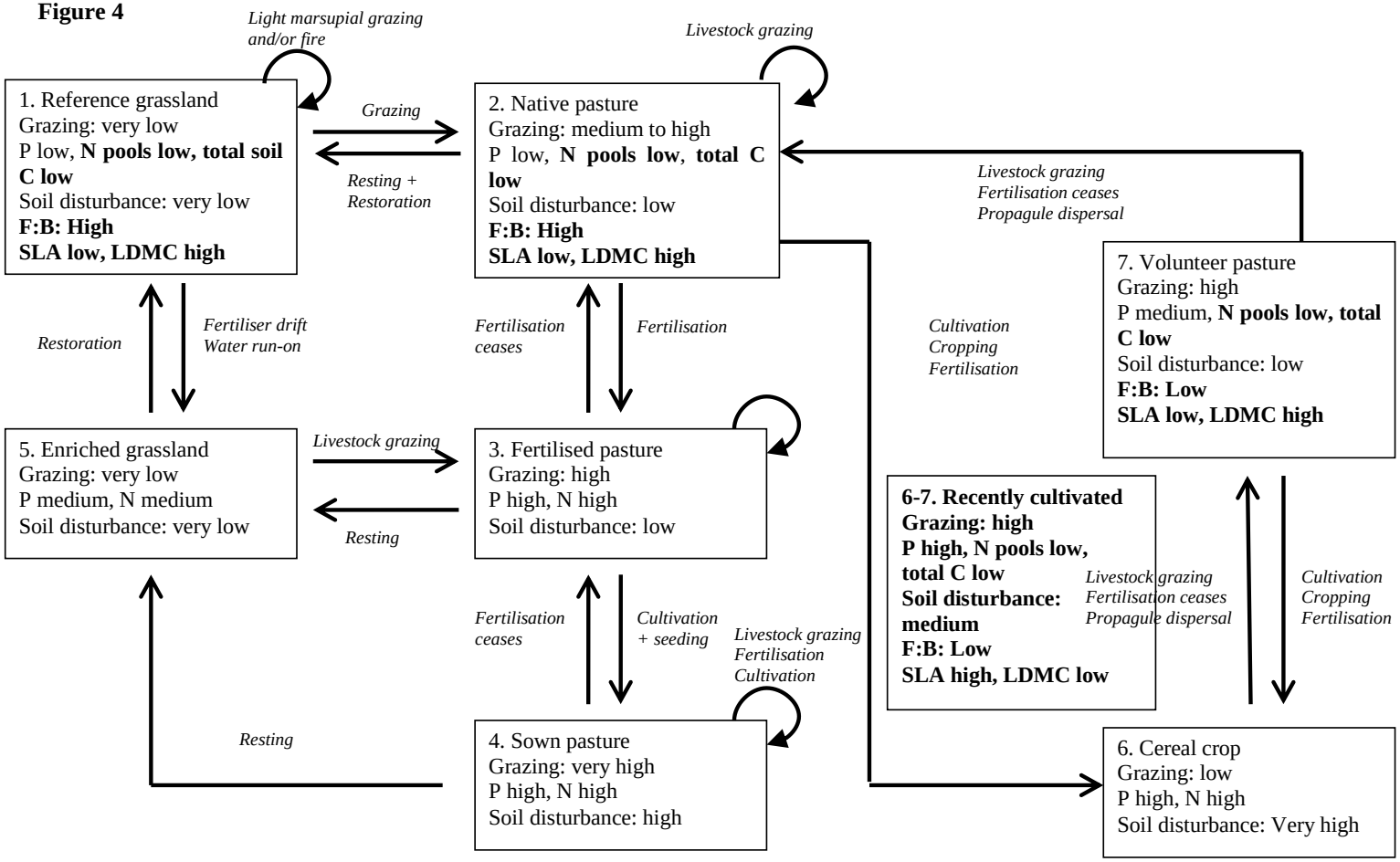

a
b
c
d
Native grasslands
Native pastures
Volunteer pastures
Recently cultivated
Fungal PLFA: Bacterial PLFA (F:B)
Total fungal PLFA (ug g-1 dry soil)
Total bacterial PLFA (ug g-1 dry soil)
Total PLFA (ug g-1 dry soil)
Vegetation state
Vegetation State

Figure 4


Light marsupial grazing and/or fire
1. Reference grassland
Grazing: very low
P low, **N pools low, total soil C low**
Soil disturbance: very low
F:B: High
SLA low, LDMC high
Grazing
Resting + Restoration
Livestock grazing
2. Native pasture
Grazing: medium to high
P low, **N pools low**, **total C low**
Soil disturbance: low
F:B: High
SLA low, LDMC high
Livestock grazing
Fertilisation ceases
Propagule dispersal
7. Volunteer pasture
Grazing: high
P medium, **N pools low, total C low**
Soil disturbance: low
F:B: Low
SLA low, LDMC high
Restoration
Fertiliser drift
Water run-on
Fertilisation ceases
Fertilisation
Cultivation
Cropping
Fertilisation
5. Enriched grassland
Grazing: very low
P medium, N medium
Soil disturbance: very low
Livestock grazing
Resting
3. Fertilised pasture
Grazing: high
P high, N high
Soil disturbance: low
6-7. Recently cultivated
Grazing: high
P high, N pools low, total C low
Soil disturbance: medium
F:B: Low
SLA high, LDMC low
Livestock grazing
Fertilisation ceases
Propagule dispersal
Cultivation
Cropping
Fertilisation
Fertilisation ceases
Cultivation + seeding
Livestock grazing
Fertilisation
Cultivation
Resting
4. Sown pasture
Grazing: very high
P high, N high
Soil disturbance: high
6. Cereal crop
Grazing: low
P high, N high
Soil disturbance: Very high