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A landscape scale survey of indicators of soil health in grazing systems

Running title: Indicators of soil health

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Abstract

In a broad-scale survey across pasture-based grazing systems in south-eastern Victoria, soil biological and chemical properties were measured in an effort to establish baseline levels for commonly used indicators of soil health. Whereas, soil properties were highly variable among sites and biological properties were difficult to predict, total soil C was found to be closely associated with soil CEC. Importantly, the strength and nature of relationships between soil properties differed among soil textural classes. We also measured a range of soil and vegetation properties in a small number of patches of remnant vegetation and their adjacent grazed pastures. This was done in an effort to assess the sensitivity of these measures when used on samples collected from strongly contrasting land-use types. While some factors, such as mycorrhizal colonization of roots and soil C did differ between the two land-use types, other factors did not. Taken together, this survey provides baseline information on the land-scape scale for commonly used indicators of soil health, explores relationships between these soil properties, and assesses how they differ between two strongly contrasting land-use types. Results are discussed in the context of monitoring soil and vegetation attributes relevant to soil health.

Key Words: Carbon, microbial biomass, mycorrhizas, nutrient cycling, soil survey

Introduction

In recent years there has been an increase in global consumption of animal derived food products and this trend is expected to continue given current projections of global human population growth (Tillman *et al*. 2002). Despite a shift towards feedlots and other intensive livestock production systems, pasture-based grazing systems occupy 25% of the Earth's land surface, and are expected to remain the primary source of animal products on a global scale (Asner *et al.* 2004). If pasture-based systems are to increase in productivity without eroding the natural resource base, we need a clear understanding of the impacts of such farming activities on the soil, and ways in which we can measure these impacts. It is for these reasons that there has been growing interest, especially from farmers, in the assessment of soil health.

As with most agricultural systems, pasture-based grazing systems have profound effects on the soil. Direct impacts of livestock on the soil include soil compaction and redistribution of nutrients (Greenwood and McKenzie, 2001; Gusewell *et al.* 2005). Indirect impacts include effects of above-ground plant herbivory on below-ground resource partitioning (Bardgett and Wardle 2010). These impacts can affect soil biological diversity, nutrient cycling and soil structural stability, and hence, the capacity for the soil to provide ecosystem services essential to agriculture.

The term 'soil health' is increasingly being used to describe the state of the soil resource. Soil health relates to the current condition of the soil, reflecting management effects (Bennett *et al.* 2010; Kibblewhite *et al.* 2008), and encompasses the physical, chemical and biological processes and properties of the soil. A wide range of soil properties have been proposed as indicators of soil health (Cardoso *et al.* 2013). For example, soil chemical indicators include soil carbon, soil C:N ratio, soil nutrient levels, soil pH, among many others. Soil bulk density (as a measure of soil compaction) is a commonly measured physical indicator of soil health. Soil microbiological indicators, such as microbial biomass carbon (MBC), the formation of arbuscular mycorrhizas (AM) and potentially mineralizable nitrogen (PMN), have been regarded as particularly useful (Cavagnaro and Martin 2011; Ross *et al*. 1990) because they can be related to ecosystem functions and are sensitive to changes in soil management (Schloter *et al*. 2003). Despite the strong interest in improving the health of soils in all farming systems (Bell *et al.* 2007), there have been few studies that directly measure indicators of soil health across large scales. For example, whereas both the Victorian and Australian State of the Environment reports recommend accurate information on which to base soil management programs (Australian State of the Environment committee 2011), such data are currently lacking.

Understanding the impact of grazing, or indeed any agricultural practice, on soil properties requires knowledge of ‘typical’ values of those properties in the relevant system. In the case of soil chemical and physical properties, such information is widely available (Peverill *et al.* 1999), and is the cornerstone of soil test interpretation and subsequent land management (Rayment and Lyons 2010). However, there is a paucity of equivalent ‘base-line’ information for soil biological properties used as indicators of soil health. This is in part due to the fact that soil biological properties are typically time-consuming and difficult to measure, as well as being highly variable across scales (Cambardella *et al*. 1994; Parkin 1993; Wilson *et al.* 2010). To this end, the identification of relationships between soil biological properties and other edaphic factors may be useful both in terms of informing management, but also with a view to identifying more easily measured proxies for key soil biological properties, as has been done previously for other soil properties, such as soil C (Smith et al, 2012).

In addition to establishing baseline information on soil properties, it is also important to determine the suitability and sensitivity of potential measures of soil health to changes in land management. Land-use change provides an opportunity to test such responses. For example, strong changes in potentially mineralizable N have been found along the transition from grazed pasture to restored native vegetation in riparian zones (Smith *et al.*, 2012), indicating a fundamental change in soil biological processes. Similarly, changes in land-use may be expected to alter soil biological properties due to changes in the composition of the plant community and levels of soil disturbance. For example, whereas intensely grazed pastures are typically dominated by fast growing plants, producing high quality litter (low C:N ratio) that favours bacterial growth (Orwin *et al.* 2010; Vries *et al.* 2012), remnant vegetation is typically dominated by slow growing plant species, producing low quality litter (high C:N ratio) that is preferentially decomposed by fungi (Orwin *et al.* 2010; Vries *et al.* 2012). This exerts a strong effect on the amount and composition of the soil microbial biomass (Aerts and Chapin 2000; De Deyn *et al.*2008). Furthermore, it is well established that the formation of arbuscular mycorrhizas (AM) can be strongly influenced by soil nutrient concentrations, soil disturbance, and vegetation type (Abbot and Robson 1994; Watts-Williams and Cavagnaro 2012; Cavagnaro and Martin, 2011). Together, these examples serve to highlight the potential to use land-use change as a context to assess soil biological properties as indicators of soil health.

Here we present results of a study in which we sought to increase our understanding of selected soil biological properties often considered to be synonymous with soil health in grazed pasture systems, and to identify differences in these properties between two distinct land-use types (pasture and remnant vegetation). A major goal of this work was to provide currently lacking baseline information on soil *biological* properties in these important farming systems. Specifically we aimed to:

1. Quantify, to provide currently lacking baseline information on, key indicators of soil biological activity in pasture-based grazing systems across an entire production region, spanning three soil textural classes;
2. Identify relationships between soil biological and physicochemical properties in an attempt to identify more easily measured proxies for soil biological indicators of soil health; and
3. Determine if commonly used indicators of soil health differ between strongly contrasting types of land-use.

To address our aims, we undertook a large-scale survey of rotational grazing systems in south-eastern Victoria, Australia. This survey, which included 32 pasture sites, spanned three soil textural types (clay, clay-loam and loam) and three geographic regions: West Gippsland, South Gippsland and Bass Coast. We also included five paired pasture-remnant vegetation sites in our survey to address Aim 3. Results are discussed in the context of the grazing effects on soil properties and soil health.

Materials and methods

Field sites

Here we present results of a field survey of soil physicochemical and soil biological properties on 32 pasture sites in south-eastern Australia (Table 1). Our focus was on the West Gippsland, South Gippsland and Bass Coast regions of Victoria. Working with the local Landcare group, we were able to identify, and gain access to, 32 sites on 17 separate farms with grazed (beef or dairy cows) perennial pastures, on soils with one of the three dominant textural classes in the regions (clay, clay-loam or loam). Samples from this survey of 32 sites were used to assess Aims 1 and 2. In order to assess the sensitivity to land-use change of soil biological and physicochemical properties (Aim 3), we also collected soil samples from patches of un-farmed remnant vegetation adjacent to five of the pasture sites (indicated in Table 1) included in the main survey. Vegetation at the remnant sites was comprised of open woodland dominated by Eucalyptus species, and with a grassy understory. A pasture-remnant vegetation comparison was selected to address Aim 3 as it provided the strongest contrast in land-use in the region (excluding urban and industrial land-use) we were able to identify. Again working with the local Landcare group, and using aerial photographs coupled with ground truthing, we were able to identify five pasture-remnant vegetation pairs in the region.

Soil and vegetation sampling

Field sampling took place during October to November 2010. In each sampling paddock, or fenced off patch of remnant vegetation, a 20m x 20m sampling area was delineated. The sampling area, which was positioned in the center of each paddock, so as to avoid watering troughs, feeding areas and gates, was divided into four (10 m x10 m) plots. Vegetation and soil samples were taken from each plot as follows. In each 10 m × 10 m plot, percentage of ground cover was estimated visually in a randomly positioned 1 m × 1 m quadrat (Burger et al., 2010). Ground cover biomass was also measured in each plot by clipping all above-ground biomass from three 50 cm × 50 cm quadrats randomly positioned within each plot. All biomass was dried at 60^{o}C and dry weights determined. Soils were sampled from each of the (10 m × 10 m) plots within each sampling area, by taking six randomly located soil cores from the 0-200 mm soil layer using a 100 mm-diameter soil corer. The six cores from each plot were combined to provide one composite soil sample per plot. Thus for each site, there were four soil samples (i.e. one from each 10 × 10 m plot). All soil samples were placed in air-tight bags and immediately stored at 4 °C, to minimise biological activity (Cavagnaro *et al.* 2006), and returned to the laboratory for processing and further analysis (see below).

Soil analysis

Soil samples were sieved (<2 mm) to remove coarse roots and rocks, prior to analysis of a range of soil physicochemical and biological properties commonly used as indicators of soil health. Gravimetric moisture was determined after drying 50 g sub-samples at 105°C for 48 h. Triplicate soil samples (10 g moist soil) were taken, extracted with 2 M KCl, and inorganic N content determined colorimetrically using a modification of Miranda *et al.* (2001) for NO_3^--N (plus NO_2^--N) and Forster (1995) for NH_4^+-N. Potential mineralizable N (PMN) was determined (on 7 g sub-samples) by anaerobic incubation (Waring and Bremner, 1964; Potthoff et al., 2005), followed by colorimetric analysis of NH_4^+-N, as above. Triplicate soil samples (5 g dry soil equivalent) were taken and analyzed for microbial biomass carbon (MBC) following the fumigation extraction method of Vance *et al.* (1987). Another set of triplicate soil samples (5 g dry soil equivalent) were taken and analyzed for microbial biomass nitrogen (MBN) by fumigation extraction and colourimetric N determination (Jones *et al.,* 2002). Composite soil samples at the plot level were analyzed for physicochemical properties using the Albrecht and Reams suite of soil tests. This analysis included pH and EC (1:5 soil:water), Total C and N and C:N ratio (by dry combustion), Labile (permanganate oxidizable) C, Plant available (Colwell) P, Cation Exchange Capacity (CEC), texture class, extractable (Morgan) Ca, Mg and K, extractable (KCl) S and Al, extractable (DTPA) Zn, Mn, Fe and Cu, and extractable ($CaCl_2$) B and Si. These analyses were performed by the Environmental Analysis Laboratory, Southern Cross University (see for details of analytical methods: http://scu.edu.au/eal/index.php/dds?cat_id=718#cat718, last accessed May, 2014).

Samples were also collected for analysis of root biomass and soil bulk density twice within each 10 × 10 m plot by gently tapping a stainless steel ring into the top 70 mm of the soil until level with the surface, before removing it and leveling off any soil that extended beyond the base of the ring (following, Minoshima *et al.* 2007). Upon return to the laboratory the soil was removed from the cores, weighed, and divided into two sub-samples. The first sub-sample was used to determine soil gravimetric moisture content as described above, and bulk density calculated. Roots were extracted from the second sub-sample by wet sieving (Cavagnaro *et al.* 2006). The extracted roots were weighed and divided into two sub-samples. The first sub-sample was dried for 48 hours at 60°C, and root biomass (dry) per g dry soil determined. The second root sub-sample was cleared with KOH and stained Trypan Blue (following Phillips and Hayman, 1970, omitting phenol from all reagents), and mycorrhizal colonization determined using the line intersect method (Giovannette and Mosse, 1980).

Data analysis

Statistical analyses were performed for all 32 pasture sites, separated by textural classes. This analysis did not include the remnant sites (see below). The overall patterns were assessed for soil and vegetation properties, by conducting one-way ANOVA's (by GLM), with Tukey's post-hoc tests performed where the ANOVA indicated that two or more means were significantly different at the $P<0.05$ level (Zar, 1999). The relationship between soil and vegetation properties was investigated using simple linear regression, both among and within the three soil textural classes. All soil and vegetation properties were regressed against one-another for each soil textural class in an effort to identify relationships between the variables measured.

Classification and Regression Trees (CART) were constructed in an effort to identify potential multi-variate relationships between key soil biological properties (MBC, PMN, and mycorrhizal colonization) and the other physicochemical and vegetation properties measured. In order to provide a comparative assessment of the CART methodology for predicting non-biological properties, we also constructed a tree for the prediction of total organic carbon (concentration) in soil. A total of 30 soil properties, across all 128 plots (i.e. 32 sites × 4 plots/site) on three different textural classes were used as predictors in the analysis. CARTs were constructed using all 30 variables for predicting biological properties but total N and labile C were removed from predictions for total organic carbon because of their inherent correlation. Individual trees were pruned back to an optimum number of splits at which the cross-validation (leave-one-out) error was minimized. All CART analyses were conducted using the RPART package (Therneau *et al.*, 2013) via R statistical software (R Development Core Team, 2005).

Soil and plant properties for pastures and their adjacent remnant sites were analyzed using one-way analysis of variance (ANOVA) using JMP (JMP® version 9. SAS Institute). Significant interactions and differences in properties between the two land-use types were determined by performing Tukeys HSD tests.

Results

Patterns in soil physicochemical and biological properties in pasture soils with contrasting soil texture (Aim 1)

Soil physicochemical properties varied considerably among the sites surveyed (Table 2). The soils in the region, irrespective of soil texture, were acidic, with $pH_{1:5\ water}$ values ranging from 4.9 to 6.8, with a mean (± SE) of 5.5 ± 0.08 across all sites. The mean $pH_{1:5\ water}$ of the soil did not differ significantly among soil textural classes (P>0.05). The concentration of mineral nitrogen species (NO_3^-N and NH_4^+-N) in the soil was also not significantly different among soil textural classes (P>0.05).

Plant available (Colwell) P was highly variable among the sites, with P levels ranging from 6.2 to 116 μg/g dry soil, with a mean of 30.5 ± 4.7 μg/g dry soil across all pasture sites. Importantly, 19 of the 32 pasture sites were found to have plant available (Colwell) phosphorus concentrations (Fig 1a) above those recommended (18-20 μg/g dry soil) for pasture soils (www.scu.edu/schools/esm/eal, last accessed May, 2014), but differences among the textural classes were not significant. Total soil C and labile soil carbon were similarly variable, and did not differ significantly among soil textural classes. The average total C across sites was 2.81% ± 0.20 and ranged from 1.2% to 6.3%. Labile carbon ranged from 0.25% to 1.89% with an average of 0.50 ± 0.06% across all of the pasture sites (Fig 1b). Soil CEC (Table 2) was similar in loam and clay loam soils, with an average of 8.37 ± 1.63 $cmol^+$/kg and 8.08 ± 0.90 $cmol^+$/kg respectively, while CEC on average was lower in clay soils 6.28 ± 0.50 $cmol^+$/kg, although not significantly different. The EC of all of the soils was generally low, indicating that salinity was not an issue in this study area.

The potentially mineralizable nitrogen (PMN) differed significantly (P=0.0005) among textural classes, with PMN significantly higher in the loam soils (20.0 ± 1.8 μg/g dry soil), compared to the clay (12.1 ± 2.6 μg/g dry soil) and clay loam soils (10.0 ± 1.3 μg/g dry soil) (Fig 2a). Mycorrhizal colonization of roots was generally high (57.0 ± 2.0%) and did not differ significantly among soil textural classes (P>0.05) (Fig 2b). Microbial biomass carbon (MBC) did not differ significantly among soil textural classes (Fig 2c).

Drivers of changes in soil properties among textural classes (Aim 2)

To further explore patterns in the measured indicators of soil health, simple linear regressions were performed for each textural class separately. We found positive correlations between PMN and CEC, total C, and total N in the clay loam soils (Table 4). We also found a positive correlation between PMN and CEC in the clay soils. Microbial biomass carbon was positively correlated with CEC in both clay loam and loam soils but not in the clay-textured soils. Microbial biomass carbon was also positively correlated with NH_4^+-N and plant biomass in loam soils, and with pH in the clay soils. Interestingly, whereas MBC was positively correlated with MBN in clay loam soils, that same was not true of the other soil textural classes. For MBN a significant positive correlation was also found with mineralisable N, and with mycorrhizal colonization in the clay soils (Table 5).

For all soils, total C was positively correlated with total N and labile carbon. In addition, total C was associated with high mineral nitrogen (NH_4^+-N and NO_3^--N) in loam soils (Table 3), high CEC in clay loam soils and high plant available (Colwell) P in the clay soils. Total C was also negatively associated with bulk density in clay soils.

Regression tree analysis was conducted to further explore the relationship between soil biological properties (PMN, MBC, MBN, mycorrhizal colonization) and multiple soil physicochemical properties. This analysis was undertaken in an effort to identify more easily measured proxies for these soil biological properties. A CART was also constructed for soil C in order to compare the predictability of soil biological properties with a more routinely measured physicochemical variable of relevance to soil biology. Whereas total soil C was well explained in the CART analysis by other properties measured here, the same was not true for the soil biological properties analyzed in this way. Specifically, for mycorrhizal colonization, PMN, MBC and MBN the CART analysis only explained 32%, 39%, 20%, 32% of the variation within our dataset, respectively (see also Table 6). By contrast, the best CART model could explain 69 % of the variation in soil C among the soils in this study. Soil CEC explained the greatest proportion of variation (in the CART analysis) in soil C, with higher total C associated with soils exhibiting CEC values greater than

7.8 mmol kg^{-1} (Fig 3). The CART also highlighted associations between total soil C and levels of soil extractable copper, manganese and silicon. However, it must be noted that cross-validation of this model indicated a reduction in the model fit (explaining only 46% of the variation in soil C), hence their use as broad indicators of soil C needs further validation in other soil types.

Remnant-pasture comparison- soil physicochemical properties (Aim 3)

Both soil physicochemical and soil biological properties differed between patches of remnant vegetation and their adjacent pastures. Total soil C, CEC and total soil N were significantly ($P<0.05$) higher in remnant than pasture soils (Fig 4a, c, f). The higher soil C in the remnant sites coincided with slightly (albeit not significantly) higher total plant above-ground biomass in the remnants (Fig 4d) but the same was not true for labile carbon (Fig 4a). In contrast, mycorrhizal colonization was significantly lower in the roots of grasses collected from the remnant sites, than the pasture soils (Fig. 4b). There was, however, no difference in MBC and MBN between land-use (Fig 4g, h). When pasture and remnant soils were compared, there were no significant differences ($P>0.05$) in terms of plant available (Colwell) P (pasture = 26.6 ± 7.2 μg/g dry soil, remnant = 29.3 ± 7.5 μg/g dry soil); root biomass (pasture = 12.9 ± 2.6 g/dry soil, remnant = 8.0 ± 1.0 g/ dry soil); bulk density (pasture = 1.1 ± 0.1 g/cm^3, remnant = 1.0 ± 0.1 g/cm^3); and. NO_3^--N (pasture = 1.9 ± 1.0 μg/g dry soil, remnant = 2.8 ± 1.4 μg/g dry soil); NH_4^+-N (pasture = 4.2 ± 1.2 μg/g dry soil, remnant = 4.2 ± 1.1 μg/g dry soil); and PMN (pasture = 10.0 ± 2.5 μg/g dry soil, remnant = 14.0 ± 5.5 μg/g dry soil).

Discussion

Here we present results of a broad-scale survey of soil biological and chemical properties commonly associated with soil health. Both soil physicochemical and biological properties were found to be variable among sites, with only clear differences in PMN observed between soil textural classes. Although the selected soil biological properties (MBC, PMN and mycorrhizal colonization) were difficult to predict using more-easily measured physico-chemical variables, we were able to predict total soil C with a reasonably high degree of confidence using other (albeit no more easily measured) soil physicochemical properties (especially CEC). Further, in a comparison of soil and vegetation properties between grazed pastures and adjacent patches of remnant vegetation, we found total soil C and N, and CEC to be consistently lower in the grazed sites, but mycorrhizal colonization of roots to be higher. The results presented here provide previously lacking baseline information on a number of biological indicators of soil health for grazed pasture soils (Aim 1), and allow us to explore relationships between these variables and soil physicochemical properties (Aim 2). They also allow us to explore the impact of land-use on these same soil biological and physicochemical indicators of soil health (Aim 3). These results, which are now discussed in the context of sustainable pasture-based grazing systems in south-eastern Australia, will be useful in informing future efforts seeking to monitor soil health in this, and other regions.

Indicators of soil health (Aim 1)

Variation in the productivity and resilience of grazed systems reflects differences in soil properties, climate conditions, locations, plant communities and management practices (Milchunas and Lauenroth 1993; He *et al.* 2011). Soil biological properties play a critically important role in maintaining the capacity of soils to cycle and retain nutrients and energy. However, these properties are highly variable between soil types and indicators are often context specific (Cavagnaro and Martin 2011; Ross and Hart 1990). Nevertheless, we still detected trends in biological properties between soil textural classes, with PMN being greatest in the loam soils. This is in line with previous studies showing that soils with higher clay contents have lower net mineralization as a result of greater protection of carbon (i.e. in aggregates), when compared to loam soils (Verberne *et al.* 1990). These findings have implications for N cycling rates, and hence the potential for plant assimilation, as well as N losses from farms, which can be substantial in grazed systems (Hatch *et al.* 2002).

Soil chemical properties provide important information on the capacity of soils to deliver nutrients to plants, and so, are considered important indicators of soil health (Bennett *et al.* 2010; Cardoso *et al.* 2013; Doran *et al.* 1996; Doran and Zeiss 2000). Soil pH, CEC and organic matter levels are often associated with assessing the health of soils because of their role in regulating the availability of nutrients and toxicants (Kelly et al 2009). Irrespective of soil type, plant available (Colwell) P, mineral N and soil C varied widely in the present study. The amount of plant available P in the majority of the soils surveyed here were found to be well above recommended levels (Target 10, 2005); this was especially true for the loam soils. Taken together, the generally high values for phosphorus and mineral N observed here are strongly suggestive of fertilizer application. Additional variability in these soil properties no doubt also reflects differences in management practices between the monitored sites, including stocking rates, grazing rotations and frequency of fertilizer usage. However, we were unable to explore this issue further due to limited access to past farm management histories. Although fertilizer inputs are important from a productivity perspective, excessive soil nutrient levels can also impact upon the health of soils and water bodies adjacent to grazing systems (Brooks & Lake 2007; Gregory et al 1991; Palmer *et* al. 2005), and must be managed accordingly. More efficient management of nutrient inputs will also provide economic benefits to farmers.

Soil biological properties are variable and difficult to predict (Aim 2)

Soil biological properties are important indicators of change in the soil environment, but their quantification can be difficult and time consuming. Therefore, we sought to identify potential proxies for key soil biological properties that are more easily quantified, as has been done for other soil properties, such as soil C (see Smith *et al.* 2012). First, we undertook to identify simple linear correlations among soil biological properties and a range of soil physicochemical, and basic vegetation properties. Using this approach we found the greatest number of significant correlations between properties in the loam soils. In particular, the linkages between plant (root and shoot) biomass, microbial biomass, CEC, total and mineral N suggest a stronger inter-reliance between productivity and N fertility in loam soils than in the heavier-textured clay loam and clay soils. In contrast, correlations were strongest between measures of soil organic matter (total C, total N), bulk density and P availability in heavier-textured clay soils, possibly reflecting the capacity of clay minerals to adsorb and stabilize previous inputs of organic matter and P (Six *et al.* 2002; Tinker and Nye, 2000). These results suggest that although texture may not explain the absolute values of soil health indicators, it is an important consideration for understanding the relationships between particular indicators. That relationships between soil properties were not consistent across all soil textural classes, highlights the important of taking soil texture into account when making generalizations about indicators of soil health. We therefore, strongly recommend that soil texture be taken into consideration in further studies of soil health.

Moving beyond simple correlations, we used CART analysis to explore these relationships, as have been done in other farming systems (Smuckler *et al.* 2008; Davey and Koen, 2012). For example, Davy and Koen (2012) used CART analysis to predict soil C across the SW slopes and plains regions of NSW. Their model, using physico-chemical variables as predictors, explained between 31-61% of variability, with higher organic C stocks associated with high exchangeable K and Ca in the plains region, and high exchangeable Al and high CEC in the slopes region. This is in general agreed with our model ($r^2 = 0.69$), which identified a clear association between CEC total soil C within our study sites. While CEC does not provide a more easily measured surrogate for total soil C, this information may be useful in making inferences about soil C in other studies where only CEC data are available. This, however, should be done with due caution, and requires further validation. In comparison to total soil C, CARTs based on physico-chemical properties were not particularly useful in explaining the large-scale field variation found within our selected biological indicators. Thus, while we consider this approach useful, there is clearly more work needed in identifying the factors influencing soil biological properties and how they can be integrated into measures of soil health.

Soil properties differ between land-use types (Aim 3)

Soil health is a complex issue, partly because the term is context specific. For example, what might be considered ‘healthy’ for one land-use or component of the landscape, may not be for another. Consequently, any measure of soil health must be considered in the appropriate context. To further explore the sensitivity of indicators of soil health, we made a direct comparison between two strongly contrasted land-use types, that is, remnant vegetation and adjacent grazed pastures. These sites provided a strong contrast in land-use in which we expected to detect differences in different measures of soil health. For example, while working in northern Victoria, Cunningham *et al*. (2012) found soil C increased under greater canopy coverage with increased litter input in vegetated sites. Here, remnant vegetation sites represent minimally disturbed soils and were found to have significantly higher CEC, total C and total N, when compared with adjacent soils subject to grazing and pasture management. Our results are consistent with earlier work where higher levels of soil C under remnant vegetation, compared to adjacent farms lands, have been reported (e.g. Burger *et al.* 2010; Murphy *et al.* 2002; Tighe *et al.* 2009; Wilson *et al.* 2011) and suggests grazing exclusion can help to minimize the impacts of agriculture on these soils. The higher total soil C in the patches of remnant vegetation is likely associated with higher vegetative cover, greater litter inputs, and the absence of grazing activities (Taylor *et al*. 1993). This in turn is likely to result in greater C sequestration and nutrient cycling (Reeder and Schuman, 2002), although this was not reflected in a higher PMN here, as in our earlier work in re-vegetated riparian systems (Smith *et al*., 2012).

Higher levels of total N, but not NO_3^--N and NH_4^+-N, were observed in the soils collected from the remnant sites. This higher total N may be due to a number of factors. It may indicate that N is readily returned to the soil with leaf litter and made available for mineralization in the remnant sites. This is interesting given that the pasture sites typically receive significant N-fertilizer inputs, and include legumes in the pasture swards. Conversely, grazing can stimulate N uptake by plant roots under high soil nutrient regimes, but not under low soil nutrient regimes (Chapin and McNaughton 1989). Thus, less N is often found in grazed pasture soils, as much of the N is taken up by the plants, much of which is removed during grazing, with a smaller fraction returned to the soil through animal waste. Such patterns have been proposed to explain decreases in soil N in pasture soils (Chapin and McNaughton 1989).

The exclusion of grazing animals in remnant sites may help to explain higher levels of CEC in the remnant sites as CEC can be strongly affected by physical disturbance. The CEC of a given soil plays an important role in the capacity of the soil to retain nutrients (Hazelton and Murphy 2010; Metson 1961). Although heavy textured clay soils generally have high CEC by virtue of their mineralogy, lighter textured soils may be limited in their fertility through lower CEC (Hazelton and Murphy 2010; Metson 1961). Our results support the role for building soil C so as to improve CEC in these soils. This was not unexpected given that soil C is an important determinant of CEC (Hazelton and Murphy 2010; Metson 1961).

Root biomass was greater in the grazed sites than that of the remnant sites, consistent with earlier studies. For example, Cornish (1987) and Greenwood and Hutchinson (1998) suggested that more mature grasses in established pastures are more likely to have roots near the soil surface, allowing the plants to compensate for poorer soil physical conditions induced by grazing animals. Interestingly, we also found that colonization of roots by arbuscular mycorrhizal fungi was higher in the pasture soils than in adjacent remnant sites. Although it might be predicted that in the more disturbed and higher nutrient input pasture sites, colonization would be lower (Baon *et al.* 1992; Bolan *et al.* 1984; Smith, Read 2008), there are some studies showing higher levels of AM formation under grazing conditions (Hartley and Amos, 1999; Hokka *et al*., 2004). Taken together, we found that a range of soil biological properties commonly used as measures of soil health did change in response to a strong shift in land-use (remnant vegetation versus pasture), and support their use as indicators of change in land-use. How sensitive they are to changes specific farming practices is an important point that needs further investigation.

Conclusions

Soils are a valuable asset, and healthy soils are essential to meet the increasing demands of animal based products, and indeed all agricultural products, globally. The results presented here provide useful baseline information on soil biological properties commonly used to assess soil health, for the pasture grazing systems for three regions in southeastern Victoria, Australia. Further, simple linear regressions and CART analysis helped to explore patterns in these properties. While this approach did not allow us to identify easily measurable proxies for soil biological properties, we did find a strong relationship between total soil C and CEC, which is of interest given the intense interest in maximizing soil C levels. The relationships between key biological and physico-chemical indicators of soil health were found to vary between the soil textural classes studied here. Furthermore, comparisons between pasture and remnant soils in the region highlight differences in soil properties associated with soil health between these land-use types. Moreover, these comparisons demonstrated the suitability and sensitivity of these measures to detect changes in soils with a shift in management. In future studies it would be interesting to relate changes in these properties to changes in specific land management practices. Taken together we conclude that while soil biological properties are useful indicators of changes in soil condition, any assessment of soil health must be based on region, soil textural class, and land-use specific and relevant information.

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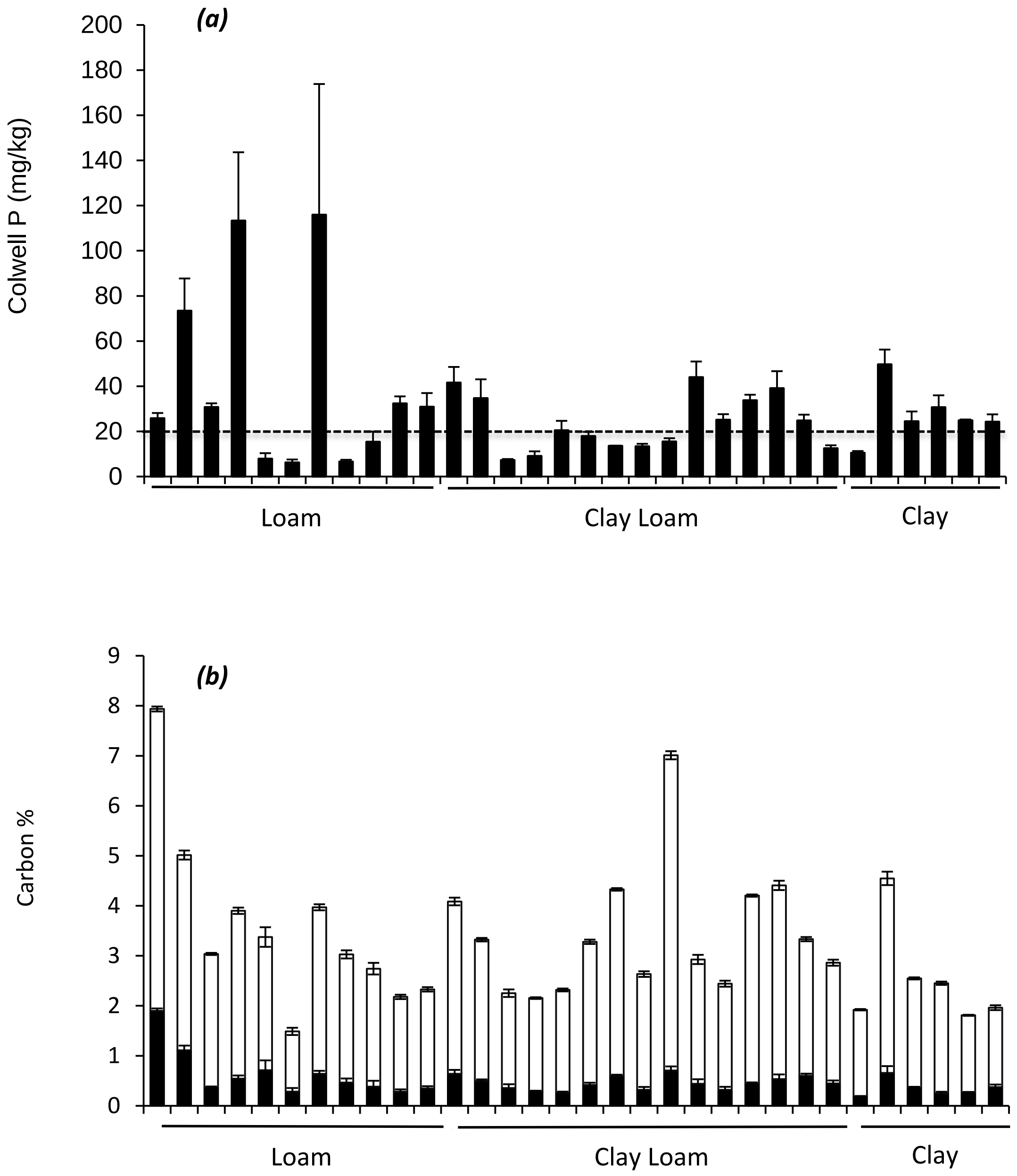

Fig. 1. Change in soil plant available phosphorus (Colwell P) across textural classes in samples collected from actively grazed pasture sites (1a) Line of recommended levels is indicated by orange line. Percentage total C (dark grey bars) and labile carbon (light grey bars) across loam (L), clay loam (CL) and clay (C) soils (1b). Values are means ± SE.

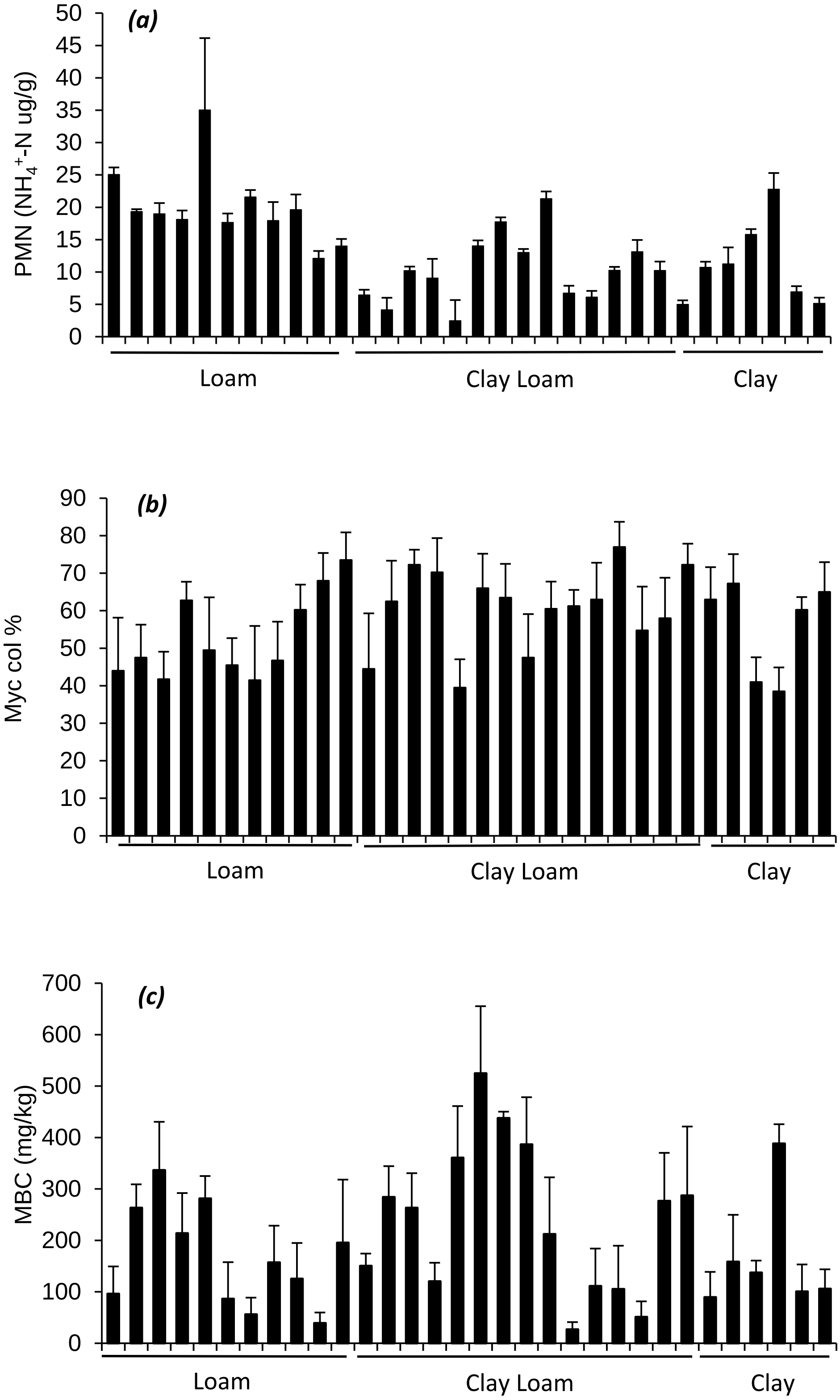

Fig. 2. Changes in biological indicators of soil acitivity across textural classes in pasture soils. Mineralisable N measured from samples collected from loam (L), clay loam (CL) and clay (C) soils (2a). Mycorrhizal colonisation (percent root length colonised) of roots (2b) and changes in soil microbial biomass C of field soils (2c) collected from 32 pasture sites. Values are means ± SE.

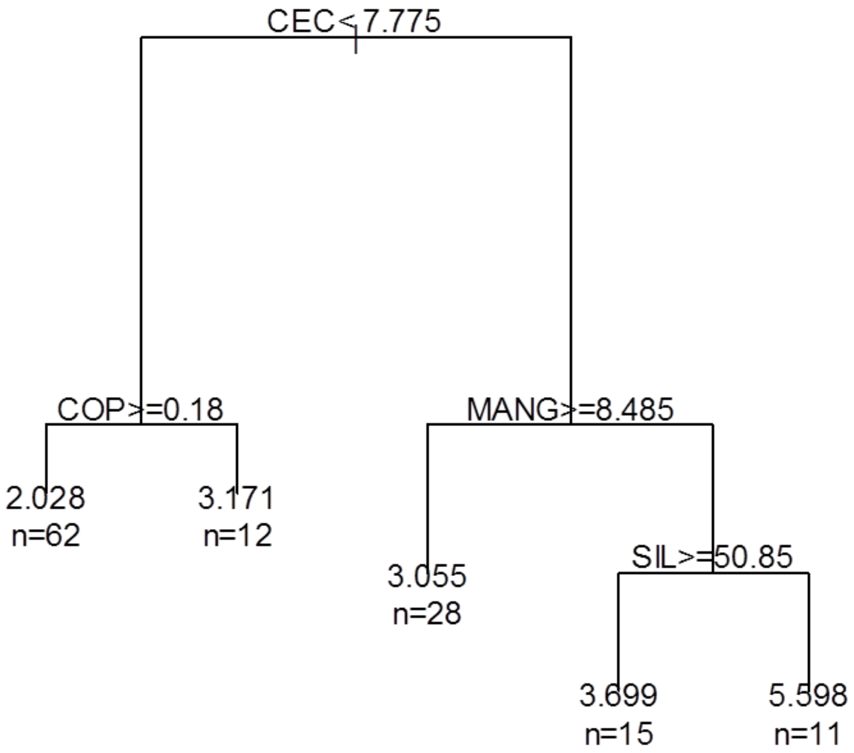

Fig 3: CART pruned by 4, predicting total soil C across 128 plots using 30 soil physicochemical properties, showing the largest amount of variation is explained by CEC.

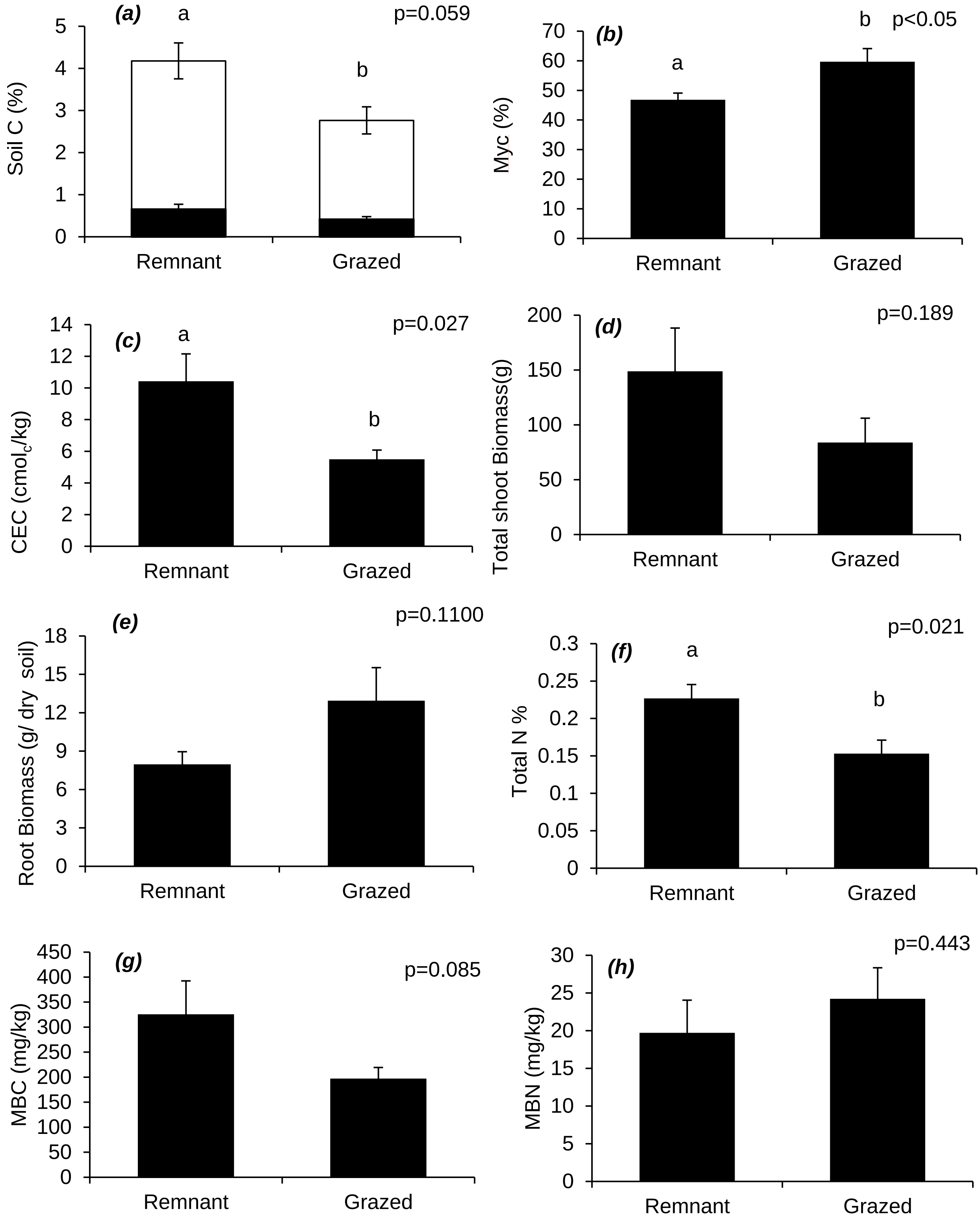

Fig. 4. Soil properties in remnant vegetation patches with greater tree density and adjacent open pasture sites. The percentage of soil C (total and labile) (a), cation exchange capacity (c), total soil N (f) and the percentage of mycorrhizal colonisation (b) across sites were significantly ($p<0.05$) different between land-uses. Total shoot biomass (d), root biomass (e) and microbial biomass C and N (g,h) were not significantly different between landuse. Values are means ± SE.

Table 1: Details of soil types and locations sampled in Victoria's south-east. Soil textures are denoted as follows (C-clay, CL-clay loam, L-loam).

Site	Location	Soil Texture
1	-38.43, 145.50	L
2	-38.43, 145.50	L
3	-38.51, 145.71	L
4	-38.51, 145.71	L
5	-38.51, 145.30	L
6	-38.51, 145.30	L
7*	-38.04, 145.90	L
8	-38.04, 145.90	L
9	-38.06, 145.83	L
10	-38.06, 145.66	L
11*	-38.06, 145.66	L
12	-38.30, 145.73	CL
13	-38.30, 145.73	CL
14*	-38.40, 145.31	CL
15	-38.40, 145.31	CL
16	-38.40, 145.66	CL
17	-38.40, 145.66	CL
18	-38.30, 145.90	CL
19	-38.30, 145.90	CL
20	-37.99, 145.53	CL
21*	-38.03, 145.77	CL
22	-38.03, 145.77	CL
23	-38.10, 145.73	CL
24	-38.07, 145.76	CL
25	-38.14, 145.73	CL
26	-38.14, 145.73	CL
27	-38.10, 145.73	C
28	-38.07, 145.76	C
29	-38.53, 145.65	C
30	-38.53, 145.65	C
31*	-38.32, 145.59	C
32	-38.32, 145.59	C

***Locations where both pasture and remnant sites were sampled**

Table 2: Soil physicochemical characteristics across 32 grazed pasture sites in the Western Port catchment, Victoria.

Site	N-NO$_3^-$ (mg/kg)	N-NH$_4^+$ (mg/kg)	pH	EC (dS/m)	CEC (cmol$_c$/Kg)	Total N (%)	C:N	Bulk density (g/cm^3)
1	8.6±1.3	8.5±0.4	5.6±0.2	0.07±0.0	10.4±0.9	0.3±0.0	18.0±0.3	1.12±0.1
2	10.7±2.6	8.4±2.6	6.8±0.2	0.29±0.1	14.2±3.0	0.3 ±0.0	14.5±0.6	1.00±0.1
3	6.7±0.5	7.5±0.6	6.2±0.1	0.08±0.0	13.9±0.7	0.3 ±0.0	10.5±0.1	1.07±0.0
4	8.9±1.5	7.6±0.9	6.0±0.1	0.09±0.0	19.4±0.7	0.3 ±0.0	10.5±0.1	1.02±0.0
5	2.8±0.5	4.1±0.4	5.2±0.1	0.06±0.0	5.3±0.8	0.1 ±0.0	21.6±2.6	1.53±0.0
6	2.3±0.5	3.8±0.7	5.5±0.2	0.04±0.0	3.0±0.4	0.1 ±0.0	17.0±0.7	1.28±0.0
7	7.8±0.6	3.2±0.1	5.9±0.2	0.09±0.0	8.7±0.8	0.3 ±0.0	12.9±0.2	1.07±0.0
8	2.7±0.0	3.3±0.4	4.9±0.1	0.05±0.0	3.9±0.3	0.1 ±0.0	25.0±1.0	1.27±0.0
9	7.7±3.2	3.6±1.4	4.9±0.1	0.08±0.0	4.5±0.6	0.1 ±0.0	22.1±1.8	1.01±0.0
10	4.3±0.6	3.0±0.2	6.0±0.0	0.05±0.0	4.6±0.4	0.1 ±0.0	15.1±0.3	1.42±0.0
11	5.3±0.4	2.9±0.2	5.8±0.1	0.05±0.0	4.2±0.2	0.1 ±0.0	14.4 ±0.6	1.42±0.0
12	3.4±0.1	4.6±0.3	5.3±0.1	0.05±0.0	7.7±0.3	0.2±0.0	15.6±0.6	1.08±0.1
13	5.1±1.0	8.8±1.4	5.4±0.1	0.06±0.0	8.5±0.3	0.2±0.0	14.7±0.5	1.11±0.1
14	2.1±0.9	6.8±2.1	5.9±0.1	0.09±0.0	5.7±0.4	0.1 ±0.0	14.5±0.4	1.09±0.0
15	6.1±2.0	25.0±20.7	6.0±0.2	0.09±0.0	4.8±0.3	0.1 ±0.0	13.8±0.6	1.20±0.0
16	5.9±1.3	9.5±0.9	5.3±0.0	0.06±0.0	7.4±0.2	0.2 ±0.0	11.5±0.4	0.90±0.0
17	5.5±1.4	8.6±0.6	5.3±0.1	0.06±0.0	12.6±0.6	0.3 ±0.0	10.2±0.2	0.98±0.0
18	2.8±0.6	11.8±0.7	5.3±0.0	0.06±0.0	17.0±0.3	0.4 ±0.0	10.2±0.1	0.94±0.0
19	3.5±0.3	8.0±0.6	5.0±0.0	0.06±0.0	7.5±0.5	0.2 ±0.0	10.5±0.3	1.01±0.1
20	3.9±0.9	9.8±0.5	5.4±0.1	0.06±0.0	9.5±0.2	0.4 ±0.0	17.9±0.7	0.97±0.0
21	4.7±0.8	8.4±3.8	5.5±0.3	0.06±0.0	4.6±0.2	0.2 ±0.0	16.1±0.8	1.04±0.0
22	3.1±0.4	3.3±0.5	5.2±0.0	0.06±0.0	4.2±0.4	0.1 ±0.0	14.4±0.5	0.93±0.0
23	5.6±0.9	8.8±0.5	5.1±0.0	0.07±0.0	10.6±0.1	0.3 ±0.0	11.6±0.2	1.12±0.0
24	6.1±1.0	7.0±0.7	5.4±0.1	0.19±0.0	10.0±1.3	0.3 ±0.0	12.8± 0.3	0.99±0.0
25	4.5±0.2	5.6±1.4	5.3±0.0	0.07±0.0	5.1±0.3	0.2 ±0.0	14.2±0.1	1.12±0.1
26	3.0±0.3	4.5±0.4	5.1±0.0	0.07±0.0	6.0±0.3	0.2 ±0.0	14.0±0.4	0.95±0.1
27	3.8±0.3	5.4±0.4	5.4±0.0	0.05±0.0	5.3±0.3	0.1 ±0.0	12.1±0.5	1.34±0.0
28	4.8±1.1	6.7±1.1	4.9±0.0	0.10±0.0	6.3±0.7	0.3 ±0.0	14.6±0.5	0.92±0.0
29	4.6±1.1	11.2±1.7	5.6±0.0	0.07±0.0	6.5±0.2	0.2 ±0.0	10.7±0.2	1.20±0.0
30	8.9±1.8	8.5±1.0	5.2±0.1	0.09±0.0	8.5±0.4	0.2 ±0.0	11.1±0.3	1.06±0.0
31	6.4±0.6	5.7±0.6	5.9±0.0	0.08±0.0	5.1±0.3	0.1 ±0.0	10.9±0.1	1.35±0.1
32	2.0±0.6	4.4 ± 1.0	6.3 ± 0.2	0.06±0.0	6.1 ± 0.9	0.1 ± 0.0	14.0 ± 0.6	1.22±0.1

Table 3: Correlations between key physicochemical and biological measures of soil health: loam textured soils. Values shown are R^2values, with significant (P<0.05) correlations shown in bold.

	Colwell								**Labile**			**Myc**	**Shoot**		**Root**			
	P	**N-NO_3^-**	**N-NH_4^+**	**pH**	**EC**	**CEC**	**Total C**	**Total N**	**C%**	**BD**	**PMN**	**Col**	**BM**	**C:N**	**BM**	**MBC**	**MBN**	**MBCN**
Colwell P	1.00																	
NO_3^--N	**0.67**	1.00																
NH_4^+-N	0.30	**0.69**	1.00															
pH	**0.61**	**0.61**	0.57	1.00														
EC	0.42	**0.69**	0.58	**0.71**	1.00													
CEC	**0.70**	**0.75**	**0.84**	**0.68**	0.53	1.00												
Total C	0.31	**0.66**	**0.72**	0.23	0.36	0.53	1.00											
Total N	**0.68**	**0.83**	**0.82**	**0.60**	0.45	**0.88**	**0.82**	1.00										
Labile C	0.11	0.51	**0.65**	0.17	0.37	0.33	**0.94**	**0.65**	1.00									
BD	-0.54	**-0.81**	-0.59	-0.37	-0.51	**-0.66**	-0.45	**-0.64**	-0.27	1.00								
PMN	-0.13	-0.05	0.18	-0.29	0.03	0.04	0.39	0.13	0.46	0.15	1.00							
Myc Col	0.00	-0.05	-0.34	-0.03	-0.22	-0.16	-0.37	-0.26	-0.39	0.33	-0.49	1.00						
Shoot BM	0.56	0.51	**0.69**	0.58	0.40	**0.90**	0.23	**0.65**	0.01	-0.59	-0.01	-0.24	1.00					
C:N	**-0.67**	-0.50	-0.41	**-0.81**	-0.26	**-0.66**	-0.08	-0.60	0.06	0.34	0.33	-0.10	**-0.62**	1.00				
Root BM	0.09	0.52	0.25	0.19	0.47	0.21	-0.07	0.04	-0.09	**-0.62**	-0.36	0.23	0.18	-0.10	1.00			
MBC	0.06	0.06	**0.30**	0.19	0.18	**0.36**	0.00	0.06	0.01	0.12	0.02	0.03	**0.45**	0.17	0.14	1.00		
MBN	0.02	0.01	0.00	0.09	0.04	0.00	0.13	0.00	0.17	0.03	0.19	0.05	0.04	0.24	0.00	0.01	1.00	
MBC:N	0.09	0.10	0.20	0.02	0.19	0.25	0.04	0.07	0.04	0.06	0.19	0.00	0.15	0.00	0.10	**0.55**	**0.35**	1.00

Table 4: Correlations between key physicochemical and biological measures of soil health: clay loam textured soils. Values shown are R^2-values, with significant ($P<0.05$) correlations shown in bold.

	Colwell								**Labile**			**Myc**	**Shoot**		**Root**			
	P	**N-NO_3^-**	**N-NH_4^+**	**pH**	**EC**	**CEC**	**Total C**	**Total N**	**C%**	**BD**	**PMN**	**Col**	**BM**	**C:N**	**BM**	**MBC**	**MBN**	**MBCN**
Colwell P	1.00																	
NO_3^--N	0.35	1.00																
NH_4^+-N	-0.59	0.44	1.00															
pH	-0.29	0.05	**0.59**	1.00														
EC	0.13	0.37	0.10	0.27	1.00													
CEC	-0.06	0.04	0.07	-0.25	0.04	1.00												
Total C	0.17	0.01	-0.08	-0.21	0.06	**0.51**	1.00											
Total N	0.08	0.10	-0.02	-0.38	0.12	**0.86**	**0.83**	1.00										
Labile C	0.32	-0.19	-0.26	-0.18	0.01	0.40	**0.80**	**0.61**	1.00									
BD	0.12	0.23	0.43	0.49	0.07	-0.31	-0.17	-0.28	0.02	1.00								
PMN	-0.32	-0.15	0.17	0.02	0.13	**0.59**	**0.70**	**0.76**	0.50	-0.13	1.00							
Myc Col	-0.27	-0.10	0.21	0.31	0.06	0.04	-0.02	0.01	-0.05	0.35	0.15	1.00						
Shoot BM	**-0.73**	-0.06	**0.54**	0.31	0.16	0.15	-0.30	-0.09	-0.41	-0.20	0.12	0.08	1.00					
C:N	0.26	-0.20	-0.12	0.31	-0.08	**-0.54**	0.31	-0.26	0.41	0.23	-0.09	0.05	-0.43	1.00				
Root BM	0.07	-0.19	-0.35	**-0.52**	-0.46	-0.17	-0.13	-0.19	0.01	-0.29	-0.40	**0.55**	-0.22	0.03	1.00			
MBC	0.21	0.01	0.01	0.01	0.06	**0.36**	0.00	0.11	0.01	0.13	0.16	0.09	0.16	**0.41**	0.00	1.00		
MBN	0.00	0.24	0.00	0.05	0.01	0.20	0.00	0.11	0.00	0.10	0.01	0.06	0.00	0.26	0.00	**0.30**	1.00	
MBC:N	0.17	**0.34**	0.00	0.04	0.04	0.00	0.03	0.03	0.03	0.01	0.03	0.04	0.07	0.01	0.00	0.17	0.19	1.00

Table 5: Correlations between key physicochemical and biological measures of soil health: clay textured soils. Values shown are R^2-values, with significant ($P<0.05$) correlations shown in bold.

	Colwell P	**N-NO$_3^-$**	**N-NH$_4^+$**	**pH**	**EC**	**CEC**	**Total C**	**Total N**	**Labile C%**	**BD**	**PMN**	**Myc Col**	**Shoot BM**	**C:N**	**Root BM**	**MBC**	**MBN**	**MBCN**
Colwell P	1.00																	
NO$_3^-$-N	0.23	1.00																
NH$_4^+$-N	0.15	0.41	1.00															
pH	-0.51	-0.47	-0.33	1.00														
EC	**0.87**	0.64	0.27	-0.49	1.00													
CEC	0.37	0.60	0.52	-0.39	0.47	1.00												
Total C	**0.87**	0.11	0.23	-0.78	0.65	0.28	1.00											
Total N	0.80	0.34	0.53	**-0.84**	0.70	0.41	**0.94**	1.00										
Labile C	**0.88**	-0.24	0.05	-0.35	0.56	0.05	**0.86**	0.70	1.00									
BD	**-0.90**	-0.26	-0.30	0.66	-0.73	-0.64	**-0.89**	**-0.85**	-0.77	1.00								
PMN	0.16	0.74	0.74	-0.60	0.36	**0.85**	0.24	0.50	-0.16	-0.45	1.00							
Myc Col	0.10	-0.62	**-0.84**	0.14	-0.18	-0.71	0.14	-0.19	0.36	0.11	**-0.85**	1.00						
Shoot BM	-0.16	-0.15	0.62	0.49	-0.15	0.20	-0.29	-0.12	-0.07	0.11	0.15	-0.58	1.00					
C:N	0.51	-0.55	-0.55	-0.10	0.09	-0.11	0.53	0.20	0.71	-0.49	-0.45	0.73	-0.38	1.00				
Root BM	-0.11	-0.40	-0.30	-0.48	-0.38	-0.39	0.33	0.18	0.14	-0.01	-0.19	0.53	-0.61	0.42	1.00			
MBC	0.07	0.32	0.03	**0.68**	0.11	0.43	0.21	0.25	0.00	0.30	0.53	0.08	0.26	0.00	0.11	1.00		
MBN	0.00	0.56	0.61	0.23	0.09	0.65	0.01	0.15	0.08	0.09	**0.97**	**0.85**	0.07	0.36	0.08	0.38	1.00	
MBC:N	0.08	0.24	0.50	0.04	0.01	0.04	0.17	0.01	0.22	0.10	0.19	0.64	0.42	**0.85**	0.41	0.05	0.34	1.00

Table 6. CART model fits for prediction of soil C and soil biological properties, using leave-one-out cross validation.

Soil characteristic	Relative Error	Cross-validation error
Total C	0.31	0.48
Mycorrhizal colonization	0.68	0.94
Potentially mineralisable N	0.61	>1
Microbial biomass C	0.80	>1
Microbial biomass N	0.68	0.86