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Robin Hale, Paul Reicha, Tom Daniel, Philip S. Lake, Timothy R. Cavagnaro Scales that matter: guiding effective monitoring of soil properties in restored riparian zones

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1	Scales that matter:	guiding effective	e monitoring of soil	properties in	restored riparian
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2 zones

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#### 1 Abstract

2 Considerable effort has been directed at restoring riparian zones to ensure they continue to 3 provide ecosystem services and one of the most common aims of these activities is to reduce nutrients 4 (in either water or soil) entering waterways. Vegetation plays a major role in nutrient interception, but 5 nutrients in terrestrial ecosystems are strongly influenced by edaphic factors. Therefore understanding 6 the effectiveness of riparian restoration efforts is dependent on knowledge of the complex and highly 7 dynamic nature of nutrient cycling processes in riparian soils and their adjacent landscapes.

8 Our primary aim was to assess the potential utility of a range of common soil indicators for 9 monitoring responses to riparian restoration, and to use this information to provide guidance for more 10 effective monitoring. A range of soil physiochemical properties in riparian zones and adjacent 11 paddocks as a comparison were measured, incorporating both structural (e.g. bulk density) and 12 functional (e.g. nitrogen) variables likely to differ in terms of both their responsiveness to restoration, 13 and degree of natural spatial and temporal variation. Soil properties across the three spatial scales 14 considered here (among creeks, among sites and within sites) varied considerably, particularly levels 15 of phosphorus, ammonium and nitrate. Total organic carbon and total nitrogen were less variable and 16 more uniform across all scales. Potential explanations for these patterns were explored by examining 17 relationships between soil properties and vegetation measures, and between a subset of the most 18 promising indicators (carbon, total nitrogen and bulk density, based on inherently low spatial 19 variability) and adjacent land-use. Potential explanations for these patterns were explored by 20 examining relationships between soil properties and vegetation measures, and a subset of the most promising indicators based on inherently low spatial variability (carbon, nitrogen and bulk density) 21 22 and adjacent land use. Fertilizer inputs appear to be a strong determinant of soil phosphorus but 23 otherwise soil properties were not strongly related to vegetation or adjacent land-use. For mineral N 24 this is likely a reflection of the highly spatiotemporally dynamic nature of nutrient cycling in riparian 25 zone soils. Improving our

- A better understanding of <u>natural spatial</u>-variability in soil properties before restoration-will greatly aid in <u>developing</u> more effective monitoring <u>programs to assess potential changes in riparian</u> soil properties. Management of riparian systems to recover soil ecosystem services will depend upon identifying effective ecological indicators that can be used as measures of progress towards restoration goals. This study represents a necessary first step towards guiding meaningful monitoring of soil properties at riparian zones subject to restoration efforts.
- 7 Key words: riparian restoration, spatial scale, spatial variability, ecological indicators.

#### 1 1 Introduction

2 Riparian zones represent the interface between terrestrial and aquatic environments, and act as 3 critical transition zones between river catchments and channels (Naiman and Décamps, 1997; Ewel et 4 al., 2001; Lake, 2005). They are often areas of high biodiversity, and mediate the flow of energy, 5 biota, sediments and nutrients between the two environments (Lake, 2005; Naiman et al., 2005). Riparian zones therefore provide the final point in the landscape to intercept nutrients before they 6 7 enter waterways (Burger et al., 2010). However, in many regions of the world, riparian zones exist 8 within the context of highly disturbed landscapes, and are themselves often severely degraded. One 9 major consequence of this degradation is the loss of key ecosystem services, including nutrient 10 interception and retention (Jones et al., 2010).

11 To restore the ecosystem services riparian zones provide, considerable effort has been 12 directed towards their restoration, typically involving the replanting of vegetation and exclusion of 13 livestock (Bernhardt et al., 2005; Brooks and Lake, 2007, Jones et al., 2010). The aim of these 14 restoration activities (where defined) is generally to improve the condition and functioning of these 15 important zones and the benefits they confer to aquatic systems. Reducing the risk of nutrients, whether dissolved in water or bound to soil particles, from entering waterways is one of the most 16 17 commonly cited reasons for undertaking riparian management (Sudduth et al., 2007). More recently, 18 the restoration of riparian zones has been considered an effective way to mitigate the impacts of 19 climate change, with benefits including carbon sequestration (both above- and below-ground) and the 20 buffering of stream water temperatures (e.g. Seavy et al., 2009; Thomson et al., 2012, Capon et al., 21 2013). How best to achieve any or all of these goals relies upon restoration efforts being based on a 22 sound understanding of riparian ecosystem functioning, as well as the major sources of environmental 23 heterogeneity in these systems.

Many of the functions of riparian zones depend on intact vegetation, which is one of the reasons why restoration typically focuses on vegetation recovery (Jones et al., 2010). Vegetation plays a major a role in intercepting nutrients (e.g. Asghari et al., 2005; Asghari and Cavagnaro, 2011;

1 Asghari and Cavagnaro, 2012) but the fate of nutrients in terrestrial ecosystems is strongly governed 2 by edaphic factors (Jackson et al., 2008). Nutrients in the soil environment can undergo complex 3 transformations which can greatly alter their mobility. For example, mineralization of organic matter 4 can release ammonium, which if not immobilised by plants or microbes, can be rapidly transformed 5 into the highly mobile form-nitrateN-containing compound nitrate (Tinkler and Nye, 2000). To assess 6 the degree to which restored riparian zones can effectively intercept nutrients, we need to To ensure 7 that restored riparian zones effectively intercept nutrients before they enter waterways, we need to 8 understand the complex and highly spatiotemporally dynamic nature of nutrient cycling processes in 9 riparian soils (Smukler et al., 2010; Smith et al., 2012). It is also important to take into account the 10 landscape and adjacent land-use context in which the riparian zone of interest exists. For example, the 11 stocks of nutrients, especially phosphorus, in riparian zones soils are often strongly correlated with 12 those in adjacent farmlands (Burger et al., 2010). If we are to design effective monitoring programs, 13 we need to have a good understanding of underlying variation of soil properties at the commencement 14 of the restoration process. Until now, such data are lacking.

15 We contend that to manage riparian systems to recover soil ecosystem services, it will be 16 necessary to identify soil properties that can be used as ecological indicators to measure the progress of riparian management activities. Such indicators must be ecologically relevant. Further for them to 17 18 be used by land managers, such indicators must be able to be measured with sufficient precision and 19 accuracy, within the confines of a field-based monitoring program. Many ecological restoration 20 projects are not effectively monitoring, and one of the main reasons that monitoring programs fail is 21 that the selection of indicators is not clearly justified (Lindenmayer and Likens, 2010). There is likely 22 to be highest uncertainty about the potential usefulness of indicators at the beginning of projects, 23 when less is known about the study system and likely responses to management, and the best 24 approach may be to begin with a larger pool of potential variables and make informed simplications by testing their applicability and sensitivity Dale and Belever, 2001, Doren et al., 2009). 25

1 There is a substantial body of literature that outlines guidelines to help select ecological 2 indicators (e.g. Cairns et al., 1993, Jackson et al., 2000, Niemi and McDonald, 2004) and one of the 3 key criteria is that effective indicators need to be conceptually relevant, both in relation to ecological 4 function and the goals of the project. While restoration activities are often monitored poorly or not at 5 all (Brooks and Lake 2007), it is easy to predict conceptually many of the changes that are likely, and 6 their likely influence on soil properties. For example, soil bulk density is often higher in degraded 7 riparian zones (NRCS 2007) and is likely to decrease shortly after restoration as the effects of 8 livestock trampling and heavy vehicle traffic are removed. After livestock exclusion and replanting, 9 increases in vegetation cover and the accumulation of organic matter (e.g. leaves and twigs) and 10 decreases in bare ground are likely (e.g. Robertson and Rowling 2008), leading to increased soil 11 carbon and improved soil structural stability (Bronick and Lal 2005). However, it may take several 12 years, or even longer, for changes in vegetation to lead to changes in soil properties (Burger et al. 13 2010). In contrast, there is likely to be greater uncertainty around how some other soil properties 14 respond to restoration. For example, despite clear relevance as a measure of restoration success and 15 change in ecosystem functioning, -mineral nitrogen pools may respond less predictably based on their 16 extremely rapid rates of cycling and high degree of spatiotemporal variation (Burger and Jackson, 17 2004; Burger et al., 2005). It may also be the case that other soil properties are more strongly related 18 to adjacent land-use than processes occurring in the riparian zone, for example the likely links 19 between soil phosphorus and fertiliser inputs (e.g. Burger et al. 2010), and restoring the riparian zone 20 per se may be less important for these variables that the management of surrounding paddocks.

In general, the spatial and temporal scales over which most soil properties might be expected to change after restoration are relatively uncertain but need to be understood if effective postrestoration monitoring protocols are to be developed. A key element in the design of environmental monitoring programs is to identify variables to monitor that allow the effects of interest to be distinguished from the "noise" generated by natural variability (Osenberg et al. 1994, Leunda et al. 2009). Distinguishing possible responses to restoration is likely to be more difficult and require increased replication for indicators that are inherently highly variable. Therefore characterising the

1	degree to which various potential soil indicators vary in the absence of restoration, and the scales at
2	which this variability occurs, will help identify which indicators are likely to be most useful in
3	detecting future responses. For example, it may be possible to decrease sampling costs by not
4	including indicators that exhibit a high degree of natural variability, given it is likely to be more
5	difficult to detect future responses. Characterising natural variability has proved to be a useful tool in
6	evaluating ecological indicators and guiding the design of monitoring programs in other contexts (e.g.
7	Johnson 1998, Leunda et al. 2009). Some insights can be gained from work in freshwater ecosystems
8	where monitoring protocols are much better established. For example, Johnson (1998) analysed
9	variability in invertebrate indicators within lakes as a way of assessing the likelihood of detecting
10	environmental impacts, and found that taxon richness and diversity were likely to be the most robust
11	indicators to detect changes. Johnson (1998) also highlighted that spatial variability was lowest in one
12	of three different habitats within lakes, and recommended that this is the most appropriate habitat for
13	monitoring responses to acidification. We are unaware of similar approaches that have been
14	undertaken in relation to assessing likely changes in soil properties following altered management.
15	However, knowing the spatial extents at which indicators vary can help guide the selection of the
16	most appropriate ecological indicators, and also where monitoring should be undertaken.
17	Here we present results from a study established in 2005 using an MBACI (Multiple Before-
18	After Control-Impact) design to examine ecological responses to riparian restoration in lowland
19	streams of southern Australia. Based on previous work in the study region (Burger et al. 2010), we
20	predicted that significant changes in soil properties (e.g. increased C. decreased N and P) were
21	unlikely to occur for several years after restoration, potentially longer. The overarching goal of this
22	study was to assess the utility of potential soil indicators based on their conceptual relevance and
22	degree of natural variability. Our aim was to help guide future monitoring by identify conceptually
23	relevant indicators that are likely to be most useful for assessing notential responses to restoration
27	based on a low degree of natural variability. We focus on a range of soil physicochemical properties
25	incorporating both structural (a.g. bulk density) and functional (a.g. nitrogen) responses likely to
20	lifes in terms of hoth their responses in the state of th
27	differ in terms of both their responsiveness to restoration, and degree of natural spatial and temporal

1	variation. We also measured the same suite of potential indicators in agricultural lands adjacent to the
2	riparian zones sampled here, as a point of reference. Our specific aims were to:
3	1. Test whether there are pre-restoration differences in soil properties between ten sites located
4	on five creeks, and also between samples from the riparian zone and paddocks at these sites;
5	2. Characterise spatial variability in soil properties across three spatial scales (i.e. between
6	creeks and sites and within sites); and
7	3. Examine relationships between three commonly used soil indicators (carbon, nitrogen and
8	phosphorus) and four common vegetation indicators (bare ground, plant (groundcover) cover,
9	canopy cover, and dead organic matter) and also two descriptors of land-use
10	intensity/fertiliser input. The third aim here was undertaken as a method to examine the
11	support for our predictions about the likely links between soil properties and vegetation/land
12	use.
13	Of the soil properties considered here, we predict soil bulk density likely to be least variable across all
14	scales, and soil mineral nitrogen pools (nitrate and ammonium) to be most variable. Furthermore, we
15	anticipate that soil properties will be more spatially homogeneous in paddock zones across all scales
16	compared to riparian zones, given the dynamism (e.g. hydrology, heterogeneity of vegetation) of
17	riparian zones.

#### 1 2 Materials and Methods

#### 2 2.1 Study sites

3 This study focussed on 10 sites located on five small, lowland streams in the southern 4 Murray-Darling Basin, south-eastern Australia. These lowland streams have largely intermittent flow 5 regimes with cease-to-flow periods occurring generally in the austral summer. Periods of high 6 discharge lead to floodplain inundation at all study sites with the probability of flows exceeding 7 bankfull height in any year ranging from between 0.2 and 0.8 (P. Reich and T. Daniel unpublished 8 data). Historically, the riparian vegetation at these sites would have been dominated by river red gum 9 (Eucalyptus camaldulensis Dehnh); however, they are now degraded due to disturbances over the past 10 century including land clearance, stock grazing, fertiliser application and the introduction of exotic 11 species (Lake 2005). Basic site characteristics are outlined in Table 1, with further descriptions of the 12 general study region and the specific sites in Reich et al., (2009).

13 The sites used in this study form the basis of a larger project examining ecological responses 14 to riparian restoration, in the form of livestock exclusion and replanting of native tubestock (including 15 a mixture of native grasses, shrubs and trees: for further details see Reich et al., 2009). This larger 16 project has been established as an MBACI (Multiple Before-After Control Impact) experiment, with 17 the ten sites used here designated as either treatment (i.e. to be restored) or control sites, with a pair of 18 each located on each of the five creeks (with the control site upstream). Soil sampling was undertaken 19 in the austral winter during the early stages of the experiment, at four of the five creeks in 2007, with 20 the fifth sampled in 2008 (Little Billabong Creek). The initiation of restoration (for further details, see 21 Reich et al., 2009) was staggered across the five sites between 2005 and 2008, with all sites sampled 22 before restoration had occurred, except Faithful Creek which was sampled two years following 23 replanting and livestock removal.

24

#### 1 2.3 Field methods

2 We used a hierarchical sampling approach, with soils sampled from the 10 sites nested within 3 the five creeks. At each site, cores were collected from six randomly selected cross-sections (along the 4 length of the site, separated by at least ~75 m). A number of physical and ecological variables have 5 been (and continue to be) sampled at these locations as part of the wider experiment described above. 6 At each location, soil was collected at two different distances from the stream channel onto the 7 floodplain: from ~0.5 m above bank-full onto the floodplain for 3 m (hereafter "riparian") and another 8 3 m section located 50 m onto the floodplain from bank-full (hereafter "paddock"). 9 At all locations, 10 soil cores were taken from the 0-100 mm soil layer, using a hand auger, at 10 randomly located positions along each transect. The soil cores from within each transect were

11 combined, thoroughly mixed and a 2 kg sub-sample stored at 4° C until returned to the laboratory for 12 further analysis (following Cavagnaro et al., 2006). Thus, for each site, 12 soil samples in total were 13 collected i.e. six from the riparian zone and six from adjacent paddock. Our level of replication is 14 similar to a recent study demonstrating that soil properties differ across a gradient of impacted-15 remnant sites (Burger et al., 2010). Samples to estimate soil bulk density were taken by gently tapping 16 a metal core of known volume into the soil centred on a depth of 50 mm (i.e. the mid-point of the 0-17 100 mm soil layer; following Minoshima et al., 2007).

18

# 19 2.4 Laboratory methods

In the laboratory, the soil samples were sieved (2 mm) to remove rocks, coarse roots and other debris prior to physicochemical analysis as follows. Gravimetric moisture was determined after drying approximately 50 g moist soil samples at 105°C for 48 h. Triplicate soil samples (30 g moist soil) were taken, extracted with 2M KCl, and inorganic N content determined colorimetrically using a modification of the methods of Miranda *et al.* (2001) for nitrate (plus nitrogen dioxide) and Forster (1995) for ammonium. A soil sub-sample was air-dried and pH and electrical conductivity (EC) measured on a 1:5 soil-water suspension using a TPS WP-81 pH, TDS, Temperature & Conductivity

Meter (EnviroEquip Biolab, Australia). Total carbon and nitrogen were also determined on air dried
 samples, which had been ground to a fine powder in a mortar and pestle, by dry combustion (CHN 2000 analyser, Leco). Plant available phosphorus was determined using the Mehlich 3 extraction
 method (Carter and Gregorich, 2008).

5

#### 6 2.5 Vegetation and land-use

7 Vegetation communities at these sites have been sampled as part of the wider project at each 8 of the riparian locations sampled here. As a proxy for canopy cover, site openness (a measure of light 9 availability) was measured, with three hemispherical digital images taken 1.3 m above the ground, 10 analysed using Gap Light Analyses © v.2 software (Frazer et al., 1999) and combined to derive an 11 average. Canopy cover in the paddock sampling locations was zero at all sites. At each riparian location, five randomly located 1m<sup>2</sup> quadrats were sampled, with percentage cover of bare ground, 12 13 dead organic matter (including dead plants with attached roots, leaf litter, twigs and fruiting material) 14 and total plant cover visually estimated. Vegetation surveys were conducted at sites in the austral 15 summer preceding soil sampling (i.e. several months before soil sampling was undertaken).

16 Contextual land management data were collected annually by conducting landholders surveys 17 (see Reich et al., 2009 for details) which provided qualitative and semi-quantitative information about 18 the timing and location of livestock grazing, cropping and chemical application (e.g. fertilisers, 19 herbicides and pesticides). From these surveys, we calculated two indices of land-use intensity – dry 20 sheep equivalents (DSE), which is a standard unit frequently used in Australia to compare the feed 21 requirements of livestock or to assess the carrying capacity/potential productivity of grazed lands 22 (Griffiths, 1998), and fertiliser inputs (expressed as kg/ha of phosphorus and nitrogen).

23

#### 24 2.6 Statistical analysis

1 We used partially-nested analysis of variance (ANOVA) models to test for initial differences 2 in soil properties. These models included Creek as a Fixed factor, and Sites nested within Creeks as a 3 random factor. For the purposes of this analysis, we were interested simply in examining whether 4 there were differences between creeks and sites, rather than specifically comparing Control and 5 Treatment sites (this is the aim of the larger experiment). We conducted preliminary examinations of 6 the data to examine whether treating sites as random is appropriate given that restoration had 7 commenced at Faithfuls Creek; however, there was no evidence to suggest that any soil properties had 8 responded to restoration over the short-term (see for example Figure 1 and 2), and any differences 9 between the two Faithful Creeks sites were well within the range of variability observed between sites 10 on the same creek across the dataset. The assumptions of these analyses were examined, and where 11 necessary variables were log<sub>10</sub> transformed (Quinn and Keough, 2002).

12 To estimate variability at three spatial scales (i.e. between creeks, between sites nested within 13 creeks, within sites), we used various calculations of the coefficient of variation (CV), following a 14 similar methodology to Johnson (1998) and Trigal et al., (2006). These values were classified into the 15 following categories: Low (<0.15), Moderate (0.15-0.35), High (0.35-0.75) and Very High (>0.75), 16 similar to Zhang et al., (2011). Coefficient of variation values represent the standard deviation 17 expressed as a percentage of the mean (i.e. CV = standard deviation/mean) and provide a measures of 18 the variability within a population that is independent of the units of measurement (Sokal and Rohlf, 19 1995; Quinn and Keough, 2002). They therefore provide a better comparative measure of variability 20 than sample variance alone (Schneider, 1994).

Linear regression analyses were used to examine potential relationships between riparian soil properties and vegetation metrics, and also between soil properties and land-use. We excluded the site on Faithfuls Creek where restoration had previously commenced from these analyses – while there was no evidence of any short-term responses (see results); this was deemed the most conservative way to eliminate the potential confounding effects of restoration. The majority of these analyses were conducted as simple regressions, although multiple regression models were used to examine

- 1 relationships between soil nitrogen/phosphorus and the two land-use metrics (DSE and fertiliser
- 2 inputs). Boxplots and residuals were examined to check the common assumptions (e.g. homogeneity
- 3 of variances, normality for all models, potential collinearity for multiple regression models) of all
- 4 analyses, and variables were  $log_{10}$  transformed where necessary. All analyses were conducted using R
- 5 version 2.9.0 (R Development Core Team, 2009).
- 6

#### 1 3 Results

2 3.1 Differences in soil properties between and within sites

3 Overall, there was considerable variability in soil properties both between and within sites, as 4 well as across riparian and paddock samples. Soil carbon differed significantly between creeks but not 5 within sites in the riparian samples (Figure 1, for full statistical results, see Supplementary Material 6 Table S1), and there was also some evidence (albeit not statistically significant) of differences at both 7 scales in the paddock samples. The rank order of creeks in terms of carbon also differed between 8 riparian and paddock samples (highest and lowest values of carbon at Faithfuls and Joyces Creek, 9 respectively, in comparison with Middle and Narrallen Creeks for paddock samples where carbon was 10 highest and lowest, respectively). There was also evidence (although not statistically significant in 11 some cases) that nitrogen, phosphorus, ammonia and nitrate in paddock samples differed both within 12 and between sites (Figure 1 and 2, Table S1). While we detected similar results in some cases for 13 riparian soil properties (e.g. between site differences in ammonium, nitrate, phosphorus [also between 14 creeks]), there were also instances where differences observed in paddock samples were not 15 consistent (e.g. no differences between sites/creeks for riparian total nitrogen, ammonium and nitrate). Soil bulk density (Figure 1) in paddocks differed significantly between both creeks and sites (ranging 16 17 from 1.21-1.55), but was relatively uniform across creeks and sites in the riparian samples (ranging 18 from 1.23-1.33) (Figure 1).

19

20 3.2 Variability in soil properties between and within sites

21 Concentrations of phosphorus, ammonia and nitrate in the soil (Figure 3) were all highly 22 variable (coefficient of variation CV > 0.5) across the three spatial scales examined here (i.e. among 23 creeks, among sites and within sites) for both riparian and paddock samples. Total carbon, nitrogen 24 and bulk density (Figure 3) were both comparatively less variable (CV values <  $\sim 0.3$ ).

1 3.3 Relationships between soil properties and vegetation (ground and canopy cover)

2	In general, there were poor relationships between soil properties and the four vegetation
3	metrics (Table 2, supplementary material Figures S1-4), indicating that the vegetation properties
4	measured here are not good indicators of soil properties. However, we did detect some weak evidence
5	of a positive relationship between soil carbon and canopy cover, negative relationships between bulk
6	density and bare ground, plant cover and canopy cover, and a negative relationship between total
7	phosphorus and bare ground.
8	
9	3.4 Relationships between soil properties and adjacent land-use
10	Soil carbon, soil nitrogen and bulk density were not strongly related to adjacent land-use (p-
11	values for overall model fit, and for each land-use variable all $p > 0.1$ , Supplementary material S5-
12	S6). In comparison, soil phosphorus was strongly correlated with inputs of phosphorus from
13	fertilisers, and there was some evidence of a weaker relationship with land-use intensity (Table 3,
14	Supplementary material S5). These results illustrate that levels of soil phosphorus in the riparian zone
15	are litely to be influenced by inputs from adjacent noddocks

#### 1 4 Discussion

2 We detected considerable variation in soil properties across all spatial scales, with 3 phosphorus, ammonium and nitrate in particular varying within and between sites, and also between 4 creeks. However, soil carbon and nitrogen were comparatively less variable, and were also more 5 uniform across all scales in the paddock samples. Characterising this initial variation and its sources will be key to designing longer-term monitoring programs. In general, there were poor relationships 6 7 between soil properties and vegetation measurements for all variables, and also between three soil 8 indicators (soil carbon, nitrogen and bulk density) and adjacent land-use. However, fertilizer inputs 9 appear to be a strong determinant of soil phosphorus, and explain very high soil phosphorus levels in 10 some samples.

11

#### 12 4.1 Differences and spatial variability in soil properties

13 As expected there was considerable variation in the soil properties measured here, at all scales 14 considered. Importantly, the magnitude of this variation, and the scale at which it was most prevalent 15 differed between the different soil properties. Moreover, there were differences between in some soil 16 properties between creeks, but not others. For example, the variation in mineral nitrogen pools (i.e. 17 ammonia and nitrate) and total soil carbon between creeks is likely due to differences in nutrient 18 inputs between sites, endogenous rates of nutrient cycling, which can be extremely dynamic in these 19 systems (Smith et al., 2012), and patterns of vegetation (for carbon) between sites. Similarly, there 20 were also differences in soil properties between paddock and riparian soils (see below), as seen in 21 previous work in these systems (Burger et al., 2010).

Importantly, the degree of variation in soil properties was not consistent across the spatial scales sampled here. For example, the concentrations of phosphorus, ammonium and nitrate were highly variable across all scales. For the mineral nitrogen pools this was expected given the extremely dynamic nature of nitrogen cycling in soils at all scales (Burger and Jackson, 2004; Burger et al., 1 2005). While we recognize that temporal variation in mineral N pools in the soil will exist, our 2 emphasis here is on the degree of <u>spatial</u> variation. Thus, as pool sizes may change through time, we 3 expect patterns in variation, if not absolute values, to remain relatively constant. This is speculative 4 but there is some evidence from the literature to support this notion. For example, it has been found 5 that rates of denitrification, a major process determining soil ammonia levels, which differed among 6 landscape positions (in a riparian zone), did so uniformly between wet and dry seasons (Wang et al. 7 2013). Nevertheless, we do caution that there is clearly a need for further studies of the seasonal 8 dynamics of mineral N pools across spatial scales. It is this uncertainty that underpins our conclusion

9 <u>that mineral N pools may not be a readily measured indicator of restoration success.</u>

10 It is well recognised that the distribution of phosphorus in the soil can be extremely 11 heterogeneous due in large part to the relatively low mobility of phosphorus in the soil environment 12 (Tinkler and Nye, 2000). Interestingly, total soil carbon and nitrogen were relatively less variable 13 across scales. In the case of total nitrogen this is likely due to the fact that most of the nitrogen 14 measured is from geologic sources (e.g. Hollaway and Dahlgren 1999), compared to the relatively 15 small contribution from the much more variable and dynamic mineral nitrogen pools. While we have 16 observed much variation in mineral nitrogen in these soils, total nitrogen remains relatively consistent. 17 The main source of soil carbon in these systems is expected to be plant derived (Lal, 2007); the 18 relatively homogeneous distribution of vegetation in the carbon depleted soil in large part explains the 19 relatively low variability in total carbon in these soils. Consequently, total soil C may be a useful 20 indicator of change following restoration, although we note that it may take some time for a strong 21 signal to be detected (Cunningham et al. 2012, Hoogmoed et al. 2012). 22 Interestingly, data collected from the site (Faithfuls Creek treatment), two years after

replanting and livestock exclusion was similar to that collected from other sites. These observations suggest that there have been no short-term changes in soil properties as a result of riparian management, indicating that any responses are likely to occur over longer time periods. Conversely, it is also important to consider that in the transition from a relatively homogeneous pasture system to a

1 restored riparian zone, spatial heterogeneity at the site level may be expected to increase, potentially 2 making it progressively more difficult to detect significant changes using mean values of soil 3 properties. There is also the possibility that increases in heterogeneity could represent an indication of 4 restoration effectiveness in its own right. While most studies focus on mean values, there is growing 5 recognition that variance can be an important ecological attribute (e.g. Benedetti and Cecchi 2003, 6 Fraterrigo and Rusak 2008). Changes to environmental conditions have been demonstrated to affect 7 the spatial heterogeneity of vegetation communities (e.g. Houseman et al. 2008). If, for example, 8 restoration results in increased within-site patchiness of plant cover and organic matter, there is the 9 potential that this could translate into increased heterogeneity of soil properties (e.g. carbon, nitrogen, 10 phosphorus) following restoration. Understanding potential changes in variability in response to 11 restoration depends on characterising initial variability before any potential responses have occurred, 12 as we have done in this study.

13 We did not detect any clear relationships between riparian and adjacent paddock soil carbon, 14 bulk density nor mineral nitrogen pools, highlighting the fact that variation at the local scale is likely 15 to be most important for these factors. In comparison, our results illustrate that there are strong links 16 between riparian soil phosphorus and adjacent paddocks. Given that much of the phosphorus entering 17 riparian zones will do so bound to soil particles (i.e. via erosive processes), this was not unexpected, 18 and is consistent with our earlier work in these systems (Burger et al., 2010). However, it does 19 highlight two important considerations to more effectively manage the systems studied here if the 20 goal is to reduce riparian phosphorus. Firstly, it will be important to consider the surrounding 21 landscape, especially processes occurring on adjacent floodplain paddocks. For example, it may be 22 the case that managing fertiliser inputs is more important than restoration of the riparian zone. 23 Secondly, most restoration sites in the study region have riparian buffers that are 10-15 m wide at 24 best, and therefore consideration may need to given to setting minimum widths and lengths of riparian 25 zones (Burger et al. 2010). Collectively, these observations illustrate the importance of considering 26 the potential influence of soils in adjacent paddocks on restored riparian zones as suggested by Burger et al., (2010). 27

- 1 We did not observe any relationships between soil properties and vegetation, and this is most 2 likely due to the highly degraded nature of the study sites before restoration commenced. Our sites 3 exhibited many of the characteristics that would be expected from anthropogenic disturbances over 4 the past century, for example low soil carbon (generally <3%) and low plant cover and organic matter. 5 We anticipate that relations between soil properties and vegetation are likely to develop as restoration 6 proceeds and the environmental conditions at sites improves, as has been documented in other studies 7 in these systems (Burger et al. 2010). If so, vegetation properties may prove to be a useful, 8 inexpensive proxy for monitoring soils.
- 9
- 10 4.2 Assessing the utility of soil indicators

11 Three soil indicators appear to be most promising based on our estimates of spatial variability 12 across different scales - soil bulk density, total nitrogen and total carbon. For these indicators, we 13 observed relatively low variability across all three spatial scales, and relatively low (CV values < 0.3) 14 at the site-scale. These results suggest that it is appropriate to monitor potential responses by these 15 variables to restoration at the site scale (as we have defined it here), and also that significant effects in 16 the future are likely to be detected with the level of replication used in this study. In comparison, 17 phosphorus, ammonium and nitrate were relatively more variable across all three scales. For these 18 three properties, high variability at the between-site scale means that we are likely to have a poor 19 ability to detect any future responses to restoration, unless changes are large enough to be detected over the effects of this background noise. Therefore, phosphorus, ammonium and nitrate will require 20 21 significantly increased replication to characterise within and between site variability.

Describing variability is only one step in assessing indicators, as they must also be examined in light of their conceptual relevance (i.e. to the goals of the project and ecological function), feasibility of implementation (e.g. costs/logistics associated with sampling), and interpretation and utility (i.e. to the goal of the project and management actions (Cairns et al., 1993; Jackson et al.,

2000). As outlined in the introduction, all of the soil indicators we have considered in this study could
conceptually be expected to respond to riparian restoration and thus be relevant for potential inclusion
in a monitoring program. In terms of the three indicators that had low response variability, soil bulk
density can be sampled inexpensively and relatively rapidly with basic equipment. In comparison,
while soil carbon and nitrogen can be quantified with a high degree of accuracy, sampling these
indicators is time and labor intensive and also requires specialized analyses (Carter and Gregorich,
2008).

8 While soil nitrogen and phosphorus appear to be less promising indicators on the basis of 9 response variability than soil carbon and bulk density, reducing concentrations of these nutrients is a 10 common goal in stream restoration around the world (e.g. Craig et al., 2008, Jones et al 2010). It is 11 important that indicators are also assessed in terms of their interpretation and utility (Jackson et al., 12 2000) - this may mean that indicators that are highly variable and logistically challenging or 13 expensive should be considered based on the goals of the project. For example, if the goals of 14 restoration works are to reduce nitrogen and phosphorus, these indicators will need to be monitored, 15 even if they are more variable than other alternatives. In addition, here we have only considered the 16 levels of variability present for these indicators prior to restoration, and there is the possibility that 17 post-restoration responses will be significantly large to override this high pre-treatment variability, as 18 is the case of any measure of change in these systems.

#### 19 5 Conclusion

One of the key elements in managing riparian zones is to select ecological indicators that can be used to effectively monitor responses to restoration. In particular, it is important that variables that are likely to be most informative are selected, and that these are monitored effectively to track progress. Based on their likely conceptual relevance and our assessments of their degree of natural variability, carbon, nitrogen and bulk density appear to have the most promise as ecological indicators. All three of these variables have been illustrated in other studies to be responsive to

26 <u>changes in land-use or soil management (e.g. carbon: Post-Kwon 2000, Yong-Zong 2009, bulk</u>

1	density and nitrogen: Kauffmann et al. 2004), which supports our assumptions around their likely
2	conceptual relevance. These assumptions will be explicitly tested as hypotheses during future
3	monitoring at these sites.
4	Describing and understanding natural variability in ecological indicators, and examining
5	potential causes of this variability are important initial steps that can improve our understanding of
6	what responses are likely after restoration and to guide effective monitoring to detect these. This
7	approach has been demonstrated to provide important information about the likely usefulness of
8	different ecological indicators to assess changes in environmental conditions in other studies (e.g.
9	Johnson 1998, Leunda et al. 2009). As this study demonstrates, this approach is also a useful way of
10	assessing the potential utility of different soil properties as ecological indicators and can help guide
11	the development of more effective monitoring programs.
12	
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23	
2 <del>4</del> 25	
20	

Creek	Catchment	Latitude/	Elevation	Average	Average	Bank substrate	Soil type	Soil EC		Soil	Soil		
	and area (km²)	longitude	( <b>m</b> )	rainfall (mm)	riparian canopy width (m)			Mean (SE)		Mean (SE) N		Mean PH (SE) Mean (SE)	
Faithful	Goulburn-	-36.619,	148	647	5	Silt/clay	Hard neutral-	Т	134.50	5.83	0.28		
	River 151	145.523					alkaline yellow mottled soils, occasional gilgai microassociations of gley cracking clays	C	(17.97) 121.45 (15.06)	(0.06) 5.99 (0.12)	(0.01) 0.29 (0.01)		
Joyces	Loddon	-37.127,	223	558	2.1	Silt/clay with gravel/sand	Gilgai plains of cracking grey	Т	245.13 (64.66)	6.35 (0.2)	0.21 (0.02)		

Table 1 Summary of characteristics of study sites (From Reich et al., 2009). T refers to Treatment site where restoration is planned, and C to the control.

	River – 195	143.962				deposits	clays and hard	C	93.07	6.10	0.17
							alkaline yellow		(13.37)	(0.05)	(0.01)
Middle	Loddon	-37.139,	238	500-600	0.2	Silt and clay	mottled soils;	Т	165.93	6.65	0.29
	River – 171	143.913				with large	basalt boulders		(16.78)	(0.14)	(0.02)
						boulders/bedrock		С	145.68	6.03	0.29
						intrusions			(22.55)	(0.13)	(0.02)
Narrallen	Boorowa	-34.231,	481	610	5.4	Silt and clay	Hard neutral red	Т	99.67	6.57	0.18
	River – 52	148.678					soils with rocky		(33.98)	(0.12)	(0.01)
							outcrops	С	70.27	6.63	0.21
									(22.82)	(0.08)	(0.01)
Little	Billabong	-35.629,	268	700	10.2	Mainly sand and	Hard acidic red	Т	165.93	6.65	0.25
Billabong	Creek 327	147.459				silt	soils and shallow		(9.25)	(0.06)	(0.01)
Creek							loamy soils with	С	145.68	6.03	0.27
							rock outcrops		(3.33)	(0.14)	(0.01)

	Bare ground		Dead organic matter			Р	lant cov	er	Canopy cover			
	<b>F</b> <sub>1,52</sub>	р	R <sup>2</sup>	<b>F</b> <sub>1,52</sub>	р	R <sup>2</sup>	<b>F</b> <sub>1,52</sub>	р	R <sup>2</sup>	<b>F</b> <sub>1,52</sub>	р	R <sup>2</sup>
Carbon	1.37	0.25	0.03	0.02	0.88	0.01	0.95	0.34	0.02	2.52	0.12	0.03
										y = 0	0.007 <i>x</i> +	2.07
Total Nitrogen	0.57	0.45	< 0.01	0.21	0.65	< 0.01	0.001	0.93	< 0.01	0.001	0.96	< 0.001
Total Phosphorus	1.93	0.17	0.02	0.15	0.70	<0.01	0.76	0.38	<0.01	0.67	0.41	< 0.01
	<i>y</i> = -	0.25 x +	49.67									
Bulk density	1.73	0.19	0.02	0.28	0.60	<0.01	4.22	0.04	0.06	2.37	0.12	0.03
y = 0.00		0.0008 <i>x</i> -	+ 1.27				y = -0	0.0013 x ·	+ 1.33	<i>y</i> = -0	.0013 x -	+ 1.34

Table 2 Linear regression analyses examining the relationship between soil properties and four metrics summarising vegetation communities.

Table 3 Multiple regression analysis examining the relationship between phosphorus and adjacent land-use. Overall model  $F_{2,51} = 15.99$ , p < 0.0001,  $R^2 = 0.39$ 

	Estimate	Standard Error	Τ	р
Intercept	19.65	7.31		
Dry sheep equivalents	0.003	0.002	1.39	0.17
Phosphorus inputs	5.46	1.07	5.10	<0.01

Figure 1 Summary plots of total carbon, total phosphorus and soil bulk density in riparian (left panels: a, c, e) and paddock (right panels: b, d, f) samples. Along the a-axis, the first letter designates Creek (i.e. F - Faithfuls, J - Joyces, L - Little Billabong, M - Middle and N - Narrallen) and the second letter designates whether the site is to be restored or left as a control as part of an on-going restoration experiment (T – treatment, C - control).

















Figure 3 Estimates of spatial variability in soil chemistry indicators for (a.) Riparian and (b.) Paddock samples



(a.)

(b.)



# **Supplementary material**

Table S1 Results of partly-nested ANOVA model to test for initial differences in soil chemistry properties between creeks (Creek = a fixed factor, df = 4) and sites nested within creeks (a random factor, df = 5). Denominator for (a.) Creek term MS = Creek/Site(Creek) and (b.) Site (Creek) = Site(Creek)/Error.

		Riparian			Paddock		
		MS	F	Pr	MS	F	Pr
Bulk density	Creek	0.005	0.42	0.73	0.14	4.67	0.06
	Site (Creek)	0.011	0.79	0.56	0.03	2.70	0.03
	Error	0.015					
Carbon (log transformed)	Creek	0.09	9.00	0.01	2.11	3.63	0.09
	Site (Creek)	0.01	0.42	0.83	0.58	2.04	0.09
	Error	0.38			0.28		
Nitrogen	Creek	0.003	1.50	0.33	0.022	3.67	0.09
	Site (Creek)	0.002	0.54	0.74	0.006	1.85	0.12
	Error				0.003		
Phosphorus	Creek	7395	3.93	0.08	7916	4.84	0.06
	Site (Creek)	1879	4.67	0.001	1633	3.19	0.01
	Error	402			512		
Ammonium	Creek	0.60	0.51	0.73	2.06	3.10	0.12
	Site (Creek)	1.16	3.02	0.02	0.67	1.24	0.31
	Error	0.38			0.54		
Nitrate	Creek	926.5	1.59	0.31	7.33	5.23	0.05
	Site (Creek)	582.6	3.07	0.02	1.39	2.80	0.03
	Error	189.6					

Figure S1 Relationship between soil carbon and bare ground, dead organic matter, plant cover and canopy cover across nine sites (excluded Faithfuls Treatment). Site numbering is as follows: 1 = Faithful Control (C), 2 = Joyces C, 3 = Joyces Treatment (T), 4 = Little Billabong C, 5 = Little Billabong T, 6 = Middle C, 7 = Middle T, 8 = Narrallen C, 9 = Narrallen T.



Figure S2 Relationship between soil nitrogen and bare ground, dead organic matter, plant cover and canopy cover across nine sites (excluded Faithfuls Treatment). Site numbering follows Figure S1.





Figure S3 Relationship between soil phosphorus and bare ground, dead organic matter, plant cover and canopy cover across nine sites (excluded Faithfuls Treatment). Site numbering follows Figure S1.



Figure S4 Relationship between soil bulk density and bare ground, dead organic matter, plant cover and canopy cover across nine sites (excluded Faithfuls Treatment). Site numbering follows Figure S1.

Figure S5 relationships between soil phosphorus/soil nitrogen and land-use across nine sites (excluded Faithfuls Treatment). Site numbering follows Figure S1.



Figure S6 relationships between soil carbon/bulk density and DSE across nine sites (excluded Faithfuls Treatment). Site numbering follows Figure S1.

