



Native-plant amendments and topsoil addition enhance soil function in post-mining arid grasslands

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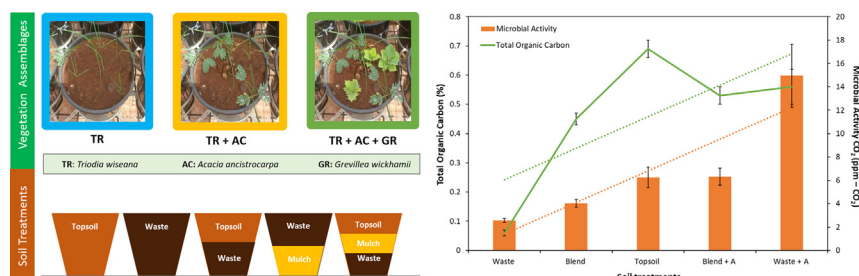
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HIGHLIGHTS

- A native-plant based amendment increased C and N contents of reconstructed soils.
- High microbial activity and C mineralisation were found in the amended mine waste.
- Low N mineralisation in amended soils suggests N immobilisation by soil microbes.
- The amendment did not increase emergence, survival or growth of native plants.
- Adding topsoil to reconstructed soils increase native plants' survival and growth.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 4 October 2017

Received in revised form 19 November 2017

Accepted 19 November 2017

Available online xxxx

Editor: D. Barcelo

Keywords:

Arid zone restoration
Triodia (Spinifex)
 Land degradation
 Organic amendment
 Soil organic carbon
 Microbial activity
 Pilbara

ABSTRACT

One of the most critical challenges faced in restoration of disturbed arid lands is the limited availability of topsoil. In post-mining restoration, alternative soil substrates such as mine waste could be an adequate growth media to alleviate the topsoil deficit, but these materials often lack appropriate soil characteristics to support the development and survival of seedlings. Thus, addition of exogenous organic matter may be essential to enhance plant survival and soil function. Here, we present a case study in the arid Pilbara region (north-west Western Australia), a resource-rich area subject to intensive mining activities. The main objective of our study was to assess the effects of different restoration techniques such as soil reconstruction by blending available soil materials, sowing different compositions of plant species, and addition of a locally abundant native soil organic amendment (*Triodia pungens* biomass) on: (i) seedling recruitment and growth of *Triodia wiseana*, a dominant grass in Australian arid ecosystems, and (ii) soil chemical, physical, and biological characteristics of reconstructed soils, including microbial activity, total organic C, total N, and C and N mineralisation. The study was conducted in a 12-month multifactorial microcosms setting in a controlled environment. Our results showed that the amendment increased C and N contents of re-made soils, but these values were still lower than those obtained in the topsoil. High microbial activity and C mineralisation rates were found in the amended waste that contrasted the low N mineralisation but this did not translate into improved emergence or survival of *T. wiseana*. These results suggest a short- or medium-term soil N immobilisation caused by negative priming effect of fresh un-composted amendment on microbial communities. We found similar growth and survival rates of *T. wiseana* in topsoil and a blend of topsoil and waste (50:50) which highlights the importance of topsoil, even in a reduced amount, for plant establishment in arid land restoration.

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1. Introduction

Dryland regions, including arid and semi-arid areas, occupy over 40% of the global land surface, store 45% of the active global carbon, and support 40% of the global population (Millenium Ecosystem Assessment, 2005). Around 20% of these regions are currently degraded and will continue to degrade at a rate of 12 million ha per year (Kildisheva et al., 2016). Intensive land uses, including extractive activities such as mining, and natural disturbances in extreme environmental conditions (Keesstra et al., 2017), have largely contributed to degradation of these arid and semi-arid lands worldwide, leading to a substantial decrease in the services provided by soils at a global scale (Anaya-Romero et al., 2016; Pereira et al., 2015, 2017). Restoring these disturbed areas has become a pressing international priority in order to maintain ecosystem function, conserve biodiversity, and improve ecosystem resilience to climate change (Menz et al., 2013; Keesstra et al., 2016, 2018).

One of the most critical challenges faced in post-disturbance restoration of arid lands, particularly in post-mining, is the limited availability of topsoil, i.e. the upper 5–10 cm of the soil profile prior to extraction operations (Golos and Dixon, 2014; Muñoz-Rojas et al., 2016a). Stripping, stockpiling and re-spreading of topsoil are common practices in mining operations. During these processes topsoil may be buried, eroded and/or degraded, leading to a topsoil deficit for use in restoration (Luna et al., 2016; Merino-Martín et al., 2017). A possible approach to overcome this deficit is the reconstruction of soil profiles using alternative substrates, including mine waste generated during the mining process and, where topsoil is available, a blend of topsoil and waste material (Machado et al., 2013; Brevik and Lazari, 2014; Muñoz-Rojas et al., 2016a; Merino-Martín et al., 2017).

Given the absence of topsoil that contain valuable nutrients and seedbank, direct seeding is often needed for reinstating biodiverse vegetation communities (Bateman et al., 2016; Kildisheva et al., 2016; Erickson et al., 2017). However, seedling emergence is a critical stage of the plant life-cycle in dryland ecosystems, with rates of seed mortality frequently exceeding 90% (James and Carrick, 2016). Reconstructed soils using alternative mine substrates often lack the necessary soil characteristics to support the development and survival of seedlings. Issues include poor soil structure, low water retention, inadequate levels of organic matter, nutrients and microbial activity (Merino-Martín et al., 2017). Thus, investigation into restoration strategies that re-establish both plant communities and soil health after mining has gained momentum in recent years (Maisto et al., 2010; Luna et al., 2016, 2017; Muñoz-Rojas et al., 2016b; Wubs et al., 2016). Plant diversity can improve soil carbon (C) and nitrogen (N) levels, increase microbial biomass and activity, as well as recover overall soil fertility (Breulmann et al., 2012; Lange et al., 2015). Therefore, promoting plant diversity in soil restoration and reconstruction practices could enhance below-ground ecosystem recovery and consequently ecosystem functioning (Klopf et al., 2017).

Several soil characteristics have been proposed as indicators of soil fertility, quality, health or function in restored soils (Anaya-Romero et al., 2015; Costantini et al., 2016; Muñoz-Rojas et al., 2016b, 2016c). Specifically, soil organic C has been extensively used to assess soil quality in amended soils as it reflects the nutrients and water reservoir of soils and strongly influences plant growth (Luna et al., 2016; Francaviglia et al., 2017). In recent years, biochemical processes and biological indicators such as microbial activity and C and N decomposition have been the focus of numerous studies due to the immediate and precise information that these bio-markers provide on soil and ecosystem recovery following management practices (Maisto et al., 2010; Bastida et al., 2015; Wang et al., 2015).

Organic amendments from different sources, including compost, organic wastes and mulches have been broadly used to promote plant growth, improve soil fertility and structure, reduce erosion and improve the overall hydrological condition of degraded and/or disturbed soils (Maisto et al., 2010; Luna et al., 2016; Yazdanpanah et al., 2016). Several

studies have focused on the use of organic soil amendments in restoration (including compost or mulches) to enhance plant performance and soil functions (Bastida et al., 2008; Jordán et al., 2011; Benigno et al., 2013; Parras-Alcántara et al., 2016; Gebhardt et al., 2017). Adding external sources of organic C has shown to improve water availability for plants, recover soil structure and enhance the biochemical and biological status via the exogenous input of plant litter (You et al., 2016; Yanardağ et al., 2017). But, despite this extensive research on the general use of organic amendments, the effects of native-plant amendments combined with alternative soil materials on the quality and functionality of reconstructed soils is largely unknown.

Triodia spp., also referred to as 'Spinifex', are key restoration targets in the Australian arid zone due to their inability to successfully re-establish following land rehabilitation operations (Erickson et al., 2016a; Lewandowski et al., 2017). These dominant plant species of the Pilbara region cover one-third of the Australian continent, making them a readily available resource for multiple uses (Gamage et al., 2012). Yet, their use as a plant-based amendment to increase soil quality in dryland restoration has not been explored.

Visual indicators such as above-ground diversity and coverage have been used as primary indicators for successful land rehabilitation (Shackelford et al., 2017). However, soil chemical, physical and biological parameters, including nutrient contents and microbial activity, can provide immediate and precise indication of ecosystem responses to stress and recovery (Costantini et al., 2016). Some of these parameters have been extensively used to assess soil quality and health and are strongly associated with ecosystem functions, e.g. biological productivity, nutrient cycling, or physical stability and support for plant growth (Doran and Zeiss, 2000; Muñoz-Rojas et al., 2016c; Griffiths et al., 2016; Francaviglia et al., 2017). Understanding the effects of soil modifications on native species recruitment, growth and soil quality indicators, is essential to improve current restoration practices to overcome critical challenges in mine rehabilitation.

Here, we present a case study in the arid Pilbara region (north-west Western Australia), an animal and flora biodiversity hotspot subject to intensive mining activities (Department of Environment and Heritage, 2003; Brueckner et al., 2013). The main objective of our study was to assess the effects of different restoration techniques on (i) seedling recruitment and growth parameters of *Triodia wiseana*, a dominant grass in Australian arid ecosystems, and (ii) soil chemical and biological characteristics (e.g. microbial activity, total organic C, total N, and C and N mineralisation) of reconstructed soils. The combined strategies included: (i) the creation of soil blends from available soil materials in mining operations, including topsoil and mine waste; (ii) sowing of different compositions of native plant species used in restoration (e.g. monoculture of *T. wiseana*, a mixture of *T. wiseana* and *Acacia ancistrocarpa*, and a combination of the former species with *Grevillea wickhamii*), and (iii) the addition of *T. pungens* dry biomass as a locally abundant native soil organic amendment.

2. Materials and methods

2.1. Study area

This study was conducted over 12 months, from December 2015 to December 2016, in Kings Park and Botanic Garden's controlled glasshouse facilities, (Perth, Western Australia). Glasshouse environmental conditions mimicked those from the Pilbara biogeographical region (north-west Western Australia, 22°03'S, 118°07'E to 23°19'S 119°43'E), characterised by a semi-arid climate with a mean annual precipitation ranging from 250 to 400 mm. In the Pilbara, most rainfall occurs during the wet-season (December–March) when extreme temperatures, commonly over 40 °C, are reached (Bureau of Meteorology, 2016). The interim Pilbara biogeographical region covers 178,060 km² and soils in this area are red shallow stony soils on hills and ranges and sands on plains comprising Red Kandosols, Red Ferrosols and Leptic Rudosols

Table 1

Determination of amendment dose for waste and mixture treatments dependent on pre-determined topsoil organic carbon and organic matter levels (Muñoz-Rojas et al., 2016a). Top up is the amount of organic matter required to increase organic matter levels in waste and mixture soil materials to the equivalent of topsoil.

Soil material	Organic carbon (g)	Organic matter (g) ^a	Top up (g)	Organic matter in 2 kg	Plant-based amendment in 2 kg
Topsoil	0.7	1.4			
Blend	0.4	0.8	0.6	12.0	15.0
Waste	0.1	0.2	1.2	24.0	30.0

^a 50% of the organic matter is considered as organic carbon (Balara and Johnson, 2013).

(Isbell, 2016). Vegetation is predominantly composed of hummock grasslands, tussock grasslands and sclerophyll shrublands. The most common plant genera are *Acacia*, *Aristida*, *Ptilotus*, *Senna*, and *Triodia*.

2.2. Experimental design

Soil treatments included: topsoil (T) as a control, mine waste (W), a 50:50 blend of the former soil materials (TW), amended waste (W + A) and amended blend (TW + A) (Tables 1 and 2). In addition to *Triodia wiseana* (C₄ perennial grass), two plant species native to the Pilbara, with highly adaptable ecophysiological traits to dryland infertile landscapes, were selected for this study to form different vegetation compositions. These species were *Acacia ancistrocarpa* (C₃ N₂-fixing legume shrub), and *Grevillea wickhamii* (C₃ tree-like), both widely used for mine restoration programs in the Australian arid zone (Bateman et al., 2016; Muñoz-Rojas et al., 2016a). The different compositions created were: (i) *T. wiseana* in monoculture (TR), (ii) a mixture of *T. wiseana* and *A. ancistrocarpa* (TRAC) and (iii) a combination of the former species with *G. wickhamii* (TRACGR) (Table 3). In total, the multifactorial experiment included five soil treatments and three vegetation compositions that were replicated (n = 10) in a randomised microcosm setting (total n = 150).

2.3. Experimental set up and methods

To maximise germination success, seeds were processed to remove non-viable seed and pre-treated following recommendations by Erickson et al. (2016a, 2016b, 2016c) to overcome physical (i.e. *A. ancistrocarpa*) and physiological dormancy (i.e. *T. wiseana* and *G. wickhamii*). *T. wiseana* seeds were cleaned from the covering florets structures by carefully rubbing florets on a ribbed rubber mat and separating the seed from the floret debris using vacuum separation ("Zig Zag") Selecta, Machinefabriek BV, Enkhuizen, the Netherlands). Seeds were then examined under a binocular microscope, to ensure that embryos were not damaged, and along with seeds of *G. wickhamii*, were then soaked for 24 h in karrikinolide (KAR₁; 3-methyl- 2H-furo[2,3-c]pyran-2-one; synthesised following Flematti et al., 2005). After soaking in KAR₁ seeds were re-dried at 15 °C/15% relative humidity for 2–3 days prior to sowing. Seeds of *A. ancistrocarpa* were treated for two minutes in hot water (90 °C) (Erickson et al., 2016a).

Soil materials were collected in September 2015 from an active mine site located in the southern region of the Pilbara, and consisted of previously stockpiled topsoil (upper 10 cm of the soil profile) and mine

Table 2

Composition of the soil treatments derived from topsoil and waste material. Amendment doses varied dependent on initial total organic carbon composition of soil.

Soil treatment	Treatment composition
Topsoil	2 kg of topsoil material per pot
Waste	2 kg of waste material per pot
Blend	1:1 kg blend of topsoil and waste
Waste + A	30 g of dried <i>T. pungens</i> 2 kg of waste material
Blend + A	15 g of dried <i>T. pungens</i> to 1:1 kg of blend (topsoil: waste)

Table 3

Seeding ratios of vegetation assemblages. Mixed vegetation assemblages were introduced to determine whether neighbouring plant species would facilitate *Triodia* recruitment, long-term survival and growth.

Vegetation assemblages	Plant species	Seeding ratio (%)
TR	<i>Triodia wiseana</i>	100
TRAC	<i>T. wiseana</i> and <i>Acacia ancistrocarpa</i>	80:20
TRACGR	<i>T. wiseana</i> , <i>A. ancistrocarpa</i> , <i>Grevillea wickhamii</i>	60:20:20

waste that had previously extracted from within the mine pit and is commonly used in landform reconstruction (Bateman et al., 2016; Muñoz-Rojas et al., 2016a, 2016b). Immediately prior to use, the substrates were analysed for a range of soil properties (Table 4). To form the blend, topsoil and waste material (TW), were mixed thoroughly into the proportion required (50:50).

The native organic amendment consisted of the biomass (leaves and shoots) of the grass *Triodia pungens* collected from the southern Pilbara (near Newman town) and oven-dried at 75 °C for 72 h. The amount of applied amendment was calculated according to the organic matter content of the plant material (45% OM) in order to increase the initial organic matter in the amended waste and blend to 1.4%, approximating the organic matter contents of topsoil (Muñoz-Rojas et al., 2016a, 2016b) (Table 2).

The experimental microcosms setting consisted of a total of 150 pots (12 × 12 × 21 cm and 2.16 L volume) each filled with 2 kg of substrate (n = 10 for each vegetation assemblage and soil treatment combination) (Table 2). Each pot was sown with 40 seeds according to the plant species proportion (Table 3). Three additional "dummy" pots per soil treatment that were not sown, were also included. Pots were maintained under controlled conditions (30 °C, relative humidity of 50%) and watered every other day to maintain field capacity (14% v/v) over 30 days. Pots were then placed under reticulation (watered every second day in two intervals of 5 min) and regularly randomised to mitigate water bias for the remainder of the experiment. Twelve months after sowing, plants were harvested.

From each of the initially collected soil materials (topsoil and waste) three composited soil samples of 500 g were taken. Samples were air-dried, sieved (2 mm mesh) and divided into two sub-samples, one was used for physical and chemical analysis, and the other was stored at 4 °C for one week before microbial analysis. Quality control information is provided in the supplementary material (Supplementary file 1). Particle size was analysed by laser diffraction using a Mastersizer 2000 (Malvern Instruments, Malvern, England) after removing the organic matter with H₂O₂. Soil pH and electrical conductivity (EC) were measured using an AD8000 microprocessor based pH meter. Total

Table 4

Soil characteristics of initially collected soil materials (Topsoil, Waste). Mean values ± standard error (n = 3). Different superscript letters in the same row indicate significant difference between soil substrates (Tukey's comparisons, p < 0.05). Each chemical component represents the total amount present in each soil type). EC: electrical conductivity, TOC: total organic carbon, TN: total nitrogen, C/N: carbon to nitrogen ratio, MA: microbial activity CO₂, qM: carbon mineralisation quotient (%), NM: nitrogen mineralisation (mg/kg NH₃-N).

Soil properties	Topsoil	Waste
EC (ms/m)	41.7 ± 5.8 ^a	55.7 ± 10.7 ^a
pH	8.0 ^a ± 0.2 ^a	7.3 ± 0.0 ^b
Clay (%)	4.6 ± 0.1 ^a	2.1 ± 0.3 ^b
Silt (%)	24.9 ± 0.7 ^a	11.8 ± 1.0
Sand (%)	70.5 ± 0.9	86.1 ± 1.0 ^a
TOC (%)	0.7 ± 0.1 ^a	0.1 ± 0.0 ^c
TN (%)	0.1 ± 0.0 ^a	0.0 ± 0.0 ^c
C/N	9.2 ± 3.8 ^a	10.4 ± 1.3 ^a
MA (ppm-CO ₂)	5.4 ± 0.3 ^b	1.5 ± 0.1 ^b
qM (%)	7.8 ± 0.0 ^b	12.1 ± 1.3 ^b
NM (mg/kg NH ₃ -N)	35.0 ± 1.5 ^a	0.0 ± 0.0 ^b

organic carbon (TOC) was determined by dichromate oxidation using the Walkley–Black method, and total nitrogen (TN) was determined using the Kjeldhal method. Soil microbial activity (ppm-CO₂) was measured using the 1-day CO₂ Solvita test which determines soil microbial respiration rate based on the measurement of the CO₂ burst produced after moistening dry soil and incubation at 25 °C for 24 h (Muñoz-Rojas et al., 2016b, 2016c). Nitrogen mineralisation (mg/kg NH₃-N) was measured with the 1-day Solvita-LAN test that calculates the labile fraction of amino-groups (Khan et al., 2001). The carbon mineralisation quotient (qM) was calculated as described by Francaviglia et al. (2017):

$$qM = CO_2 / TOC \quad (1)$$

where:

qM is C mineralisation quotient (%)
CO₂ is microbial activity (CO₂-C ppm)
TOC is total organic carbon (%)

Twelve months after sowing, soil samples were collected and analysed for physical, chemical and biological properties. From each soil treatment, three soil samples of 500 g were collected from three randomly selected individual pots and analysed following the methods described above. Seedling emergence (%) and survival (%) were determined as the average seedlings per pot after 16 days and 12 months respectively, divided by the number of seeds per pot (Muñoz-Rojas et al., 2016a). To determine plant growth parameters (shoot and root length, shoot:root ratio, and plant biomass), plant materials (one plant per pot, n = 4) were harvested after 12 months and shoot length and biomass were assessed as in Bateman et al. (2016). Root length was measured using a flatbed scanner (WinRHIZO, Regent Instruments, Sainte Foy, Canada). Subsequently plants were dried at 75 °C for 72 h, and weighed using a five-point balance PB403-S/FACT to determine total biomass.

2.4. Statistical analysis

All variables analysed, i.e. seedling growth parameters, and soil characteristics, were tested for normality and homogeneity of variance using the Kolmogorov–Smirnov and Levene's test. Data were log transformed as necessary for analysis but all presented data are non-transformed for ease of interpretation. Differences across treatments for each studied variable were then tested using a two-way ANOVA. Comparisons between means (where significance was found) was performed with the Tukey's HSD (honestly significant difference) test. Principle component analysis (PCA) was used to assess differences and clusters in soil properties across soil treatments. All analyses were performed with R statistical software version 3.1.2 (R Core Team, 2017). The R package FactomineR, which auto scales and centers the variables, was used for the PCA analysis.

3. Results

3.1. Soil quality indicators

Soil quality parameters were generally significantly different ($p < 0.05$) across soil and vegetation treatments (Fig. 1). Soil microbial activity (ppm-CO₂) of reconstructed soils ranged between 2.3 and 25.5 ppm-CO₂ with the highest values found under *T. wiseana* monoculture. Generally, the combination of species did not increase soil microbial activity, instead, high values were found in *T. wiseana* monoculture in the amended waste (Fig. 1A).

N-mineralisation was highest in the topsoil but the plant-amendment added to the waste and blend significantly increased the amount of labile ammonia compared to the non-amended soils ($p < 0.05$; Fig. 1B). The C mineralisation quotient was significantly higher ($p < 0.05$) in the waste and amended waste, with the highest values obtained in the *T. wiseana* monoculture (Fig. 1C). Furthermore, the C mineralisation quotient was

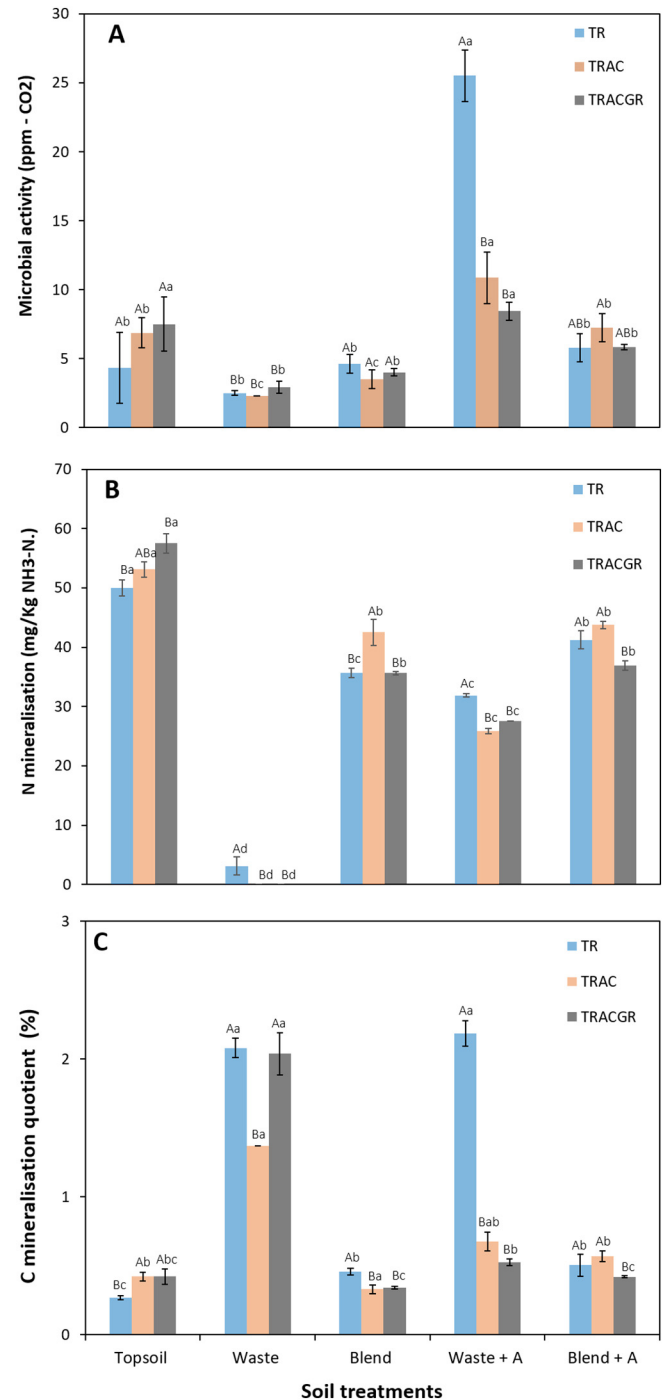


Fig. 1. Soil microbial activity (ppm-CO₂) (A), N mineralisation (mg/kg NH₃-N) (B) and C mineralisation quotient (%) (C) (Mean ± SE) (n = 4) in five soil treatments (topsoil, waste, blend, amended waste (Waste + A) and amended blend (Blend + A)) and vegetation compositions (*Triodia wiseana* in monoculture) (TR), a biodiverse mixture of *Triodia wiseana* and *Acacia ancistrocarpa* (TRAC) and a combination of the former species with *Grevillea wickhamii* (TRACGR). Different lower case letters indicate significant differences within each vegetation composition among soil treatments. Capital letters indicate significant differences among vegetation compositions within soil treatments (Tukey's comparisons, $p < 0.05$).

significantly greater in the waste treatment, in particular the TRACGR vegetation composition, however was not significantly different to the TR monoculture ($p < 0.05$; Fig. 1C).

The addition of *Triodia pungens* biomass as an organic amendment had a strong positive effect ($p < 0.05$) on total organic C (TOC), with values increasing from 0.1% in the non-amended waste to 0.6% on

average with amendment addition, similar to organic carbon content in the topsoil (Fig. 2A). Total N (TN) was low in all studied treatments but there was a significant increase in TN in the amended waste (Fig. 2B). The C/N ratio was significantly ($p < 0.05$) higher in the amended waste compared to other treatments (Fig. 2C).

The first two axes of the PCA analyses explained 85.4% of the total variation of the soil samples and indicated a clear distinction between

the waste and amended waste from each other and from the other treatments which were strongly grouped (Fig. 3). Total organic C and N correlated positively with N mineralisation rates and negatively with C mineralisation. The C/N ratio was significantly higher in the amended waste compared to other treatments ($p < 0.05$). The amendments and blend of materials did not significantly ($p > 0.05$) affect the texture and the pH and EC values (results not shown) of the initially collected topsoil and waste (Table 4).

3.2. Seedling emergence, survival, and growth of *Triodia wiseana*

Emergence of *T. wiseana* seedlings ranged between 1 and 53.4% across all treatments, and significantly ($p < 0.05$) higher values were obtained in the topsoil followed by the blended substrate, and the amended blend (Fig. 4A). Differences in seedling emergence among vegetation compositions were not significant in topsoil, but in the waste and the blend soils emergence of *T. wiseana* seedlings was significantly higher in the *T. wiseana*, *Acacia* and *Grevillea* combination.

About 18–20% of sowed seeds of *T. wiseana* (on average) survived in the topsoil treatment after 12 months (approximately 50% of emerged seedlings). Rates of survival were higher in both the topsoil and the blended soil (20–30% of the emerged seedlings), particularly in the TRAC and TRACGR treatments (Fig. 4B). Despite significantly higher ($p > 0.05$) seedling emergence in the topsoil, survival rates in both topsoil and blend were similar and not significantly different ($p > 0.05$). Adding amendments to the waste and blend did not result in higher emergence or survival. Seedling biomass was generally higher in the topsoil (0.6–1.2 g), but the shoot length in the topsoil (234.9 ± 59.6 mm) was not significantly ($p > 0.05$) different to that in the blend treatment (267.4 ± 55.1 mm) in the *Triodia* monoculture (Table 5). The shoot to root ratio were higher in the topsoil for the TRACGR treatment and the blend for the TRAC treatment, respectively.

4. Discussion

4.1. Effects of plant-based amendments and alternative soil materials on quality and function of reconstructed soils

Our results showed that soil chemical and biochemical properties differed substantially across soil treatments. Although the amendments had a positive effect on total C and N contents, these values were still lower than those found in the topsoil. The addition of the plant amendment strongly affected microbial activity, particularly when it was incorporated into the waste soil substrate. This effect could be partly explained by the combined litter inputs from dead plants and added plant amendment into soil waste, which stimulated microbial communities (Breulmann et al., 2012; Lange et al., 2015; Luna et al., 2016). This result may also be attributed to the fact that twice the amount of organic amendment was added to the waste substrate compared to the blend in order to achieve similar levels of organic C in the soil as is present in the natural topsoil.

Furthermore, the high levels of microbial activity in the amended waste correlated with high values of the C/N ratio, that were three-fold higher in the amended waste compared to the topsoil. The high C mineralisation values found in the amended waste contrasted the low N mineralisation rates suggesting a short- or medium-term soil N immobilisation as a response to the negative priming effect of fresh or un-composted amendments on soil microbial communities (Bastida et al., 2008). Due to the low levels of N in arid and semi-arid soils under grasslands, a strong microbial competition for N is likely to result in a high proliferation of microbial communities (Chen et al., 2015). This effect has been previously reported by Wang et al. (2015) who observed that adding un-composted organic amendments stimulated microbial activity in excess, restricting N availability and increasing the mineralisation rate of soil organic

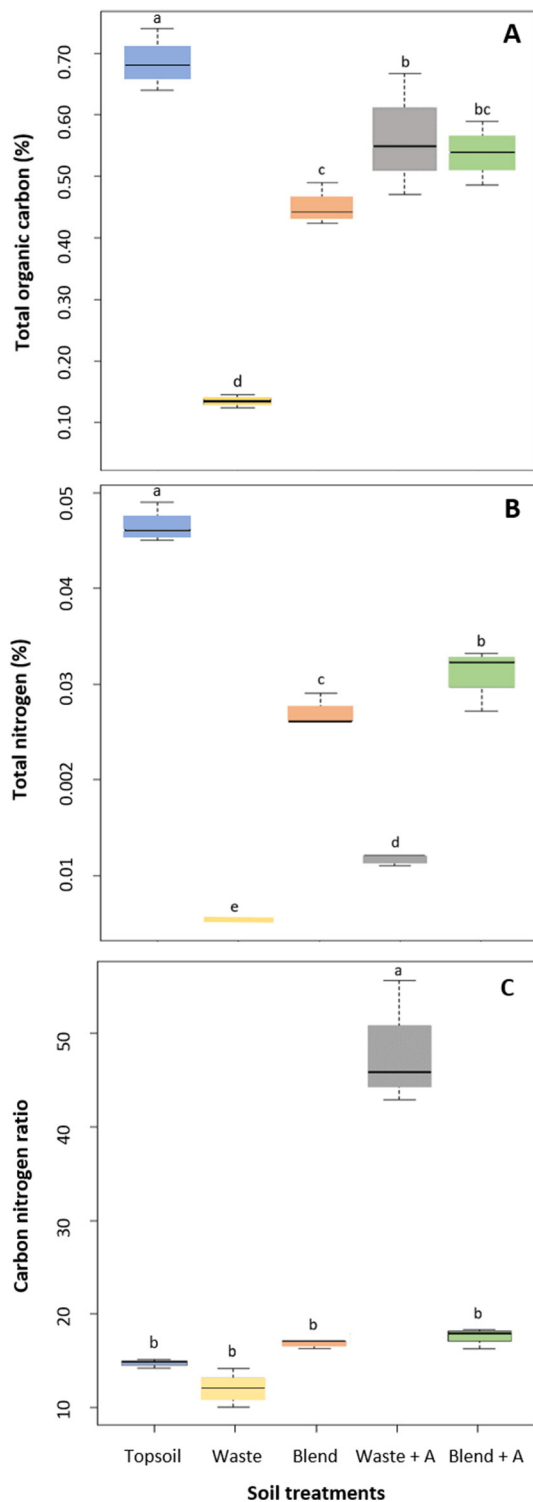


Fig. 2. Boxplots ($n = 4$) of total organic carbon (TOC, %) (A), total nitrogen (N, %) (B), and carbon to nitrogen ratio (C/N) across the five soil treatments (topsoil, waste, blend, amended waste (Waste + A) and amended blend (Blend + A)). Different lower case letters indicate significant differences between soil treatments (Tukey's comparisons, $p < 0.05$).

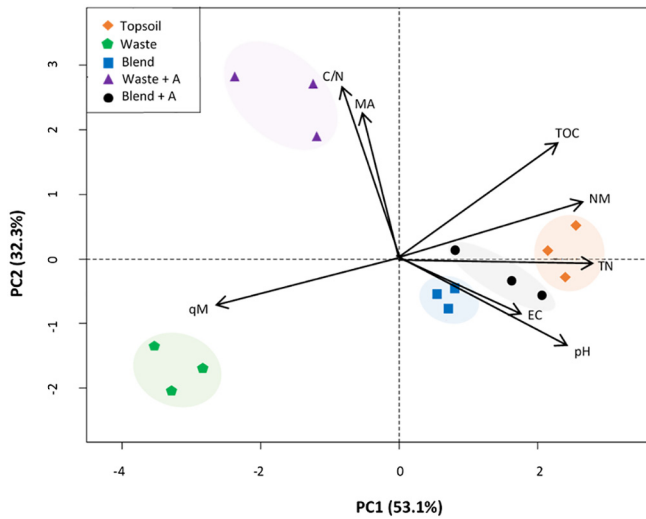


Fig. 3. Biplot of the first and second axes obtained from the principle component analysis of soil chemical and biological properties showing dependencies between soil quality indicators. Points represent samples ($n = 3$) in five soil treatments (topsoil, waste, blend, amended waste (Waste + A) and amended blend (Blend + A)). Components include EC: electrical conductivity, TOC: total organic carbon (%), TN: total nitrogen (%), C/N: carbon to nitrogen ratio, MA: microbial activity CO_2 (ppm- CO_2), qM: carbon mineralisation quotient (%), NM: nitrogen mineralisation (mg/kg $\text{NH}_3\text{-N}$).

matter. Although C contents were low in the waste substrate, C mineralisation was relatively high, which evidence the rapid access of microbial organisms to the available carbon also in non-amended soils. Contrary to other studies (e.g. You et al., 2016), we generally did not find significant differences in soil characteristics across plant compositions with the exception of the amended waste, where higher levels of microbial activity were observed in the *Triodia* monoculture. This effect can be explained by the high mortality in this treatment (Fig. 4B) that resulted in soil surface modification by in situ litter return. Although previous studies showed a negative effect of pH and EC on seedlings of native plants that resulted in higher mortality rates (Luna et al., 2017), we did not find differences in these variables for the different treatments.

4.2. Effects of plant-based amendments and alternative materials on seedling recruitment and growth of *Triodia wiseana*

Seedling emergence in arid and semi-arid conditions is primarily driven by water availability (Benigno et al., 2013; Lewandowski et al., 2017), which is dependent on the soil type and edaphic conditions (Muñoz-Rojas et al., 2016a; Mayence et al., 2017). Our results showed that emergence and survival of *T. wiseana* tended to increase when seedling in combination with other species including *A. ancistrocarpa* and *G. wickhamii*. However, this effect did not demonstrate a clear trend, and the recruitment of these species was mostly dependent on the soil material used as growth media. Higher rates of seedling emergence were found in the topsoil growth media compared to the other substrates (53.4%), which is not surprising given the higher levels of organic C in these original soils that may hold larger water amounts for plants (Muñoz-Rojas et al., 2016a; Merino-Martín et al., 2017). Nevertheless, we found similar growth and survival rates (12 months after sowing) in the blend and the topsoil (16.4% survival on average in the blend and 18.9% on average in the topsoil), which highlights the importance of this soil material (even in a reduced amount) for plant establishment over the long term. Moreover, these results evidence how the critical early plant growth stages e.g. germination and emergence are extremely sensitive to edaphic constraints (Muñoz-Rojas et al., 2016a).

Addition of a plant amendment, although positively affected soil functionality, did not improve the recruitment of *T. wiseana*. This suggests that the slow decomposition of the plant amendment did not influence plant responses at early developmental stages, supporting the idea that soil microbes are strong short-term competitors for soil N, and plants are long-term competitors (Chen et al., 2015; Liu et al., 2016). Similarly, previous studies have reported a poor response or even lower emergence and survival rates of native species in arid and semi-arid systems following the addition of C-rich amendments (Hueso-González et al., 2016, 2018; Gebhardt et al., 2017). Contrasting our results, Benigno et al. (2013), found an improvement in seedling survival of *Banksia* plants (native to Western Australia) with the addition of a native-sourced mulch. They used rather different soil materials than the substrates in this study (over 94% sand and <1% clay) and found higher survival rates after 2 years since sowing in their field trial.

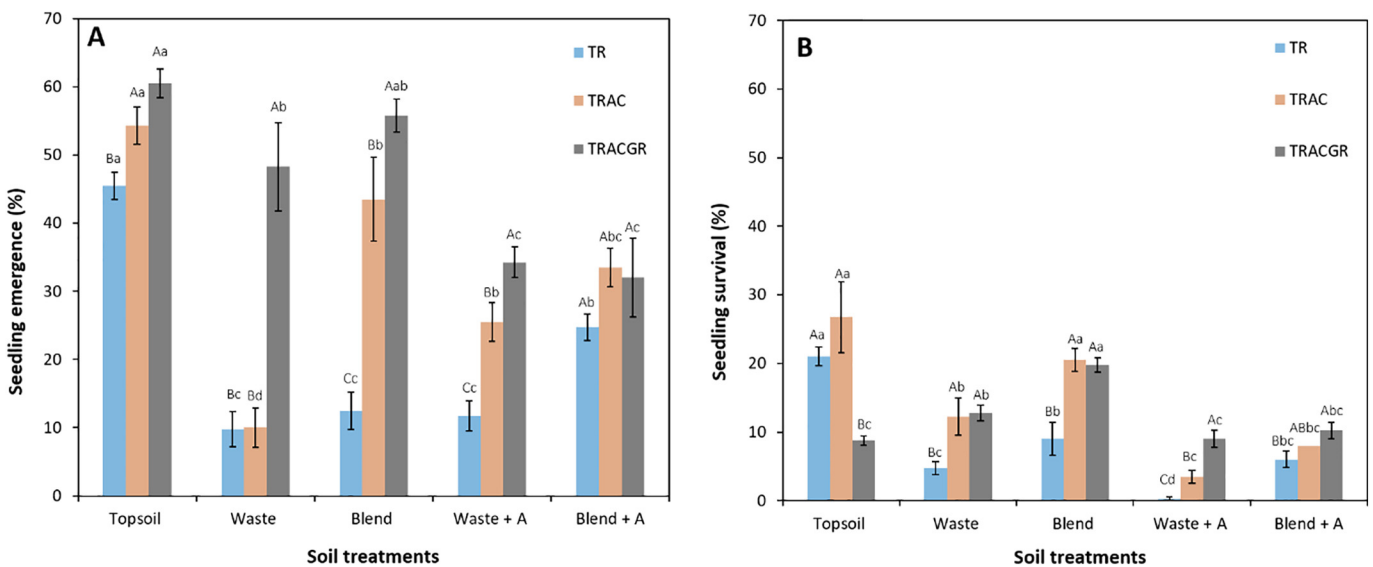


Fig. 4. Seedling emergence (%) (A) and survival (%) (B) (mean \pm SE) ($n = 10$) of *Triodia wiseana* in the five soil treatments (topsoil, waste, blend, amended waste and amended blend) and vegetation compositions (*T. wiseana* in monoculture) (TR), a biodiverse mixture of *T. wiseana* and *Acacia ancistrocarpa* (TRAC) and a combination of the former species with *Grevillea wickhamii* (TRACGR). Different lower case letters indicate significant differences within each vegetation composition among soil treatments. Capital letters indicate significant differences among vegetation compositions within soil treatments (Tukey's comparisons, $p < 0.05$).

Table 5

Seedling growth parameters, e.g. shoot and root length (mm), shoot:root ratio and seedling biomass (g) (mean \pm SE) ($n = 10$) of *Triodia wiseana* in the five soil treatments (topsoil, waste, blend, amended waste and amended blend) and vegetation compositions (*T. wiseana* in monoculture) (TR), a biodiverse mixture of *T. wiseana* and *Acacia ancistrocarpa* (TRAC) and a combination of the former species with *Grevillea wickhamii* (TRACGR). Different lower case letters indicate significant differences within each vegetation composition among soil treatments. Capital letters indicate significant differences among vegetation compositions within soil treatments (Tukey's comparisons, $p < 0.05$).

Treatment	Shoot length			Root length		
	TR	TRAC	TRACGR	TR	TRAC	TRACGR
Topsoil	234.9 \pm 59.6 ^{Aa}	230.8 \pm 33.8 ^{Aa}	244.5 \pm 22.6 ^{Aa}	267.8 \pm 97.7 ^{Aa}	52.6 \pm 8.0 ^{Ba}	41.1 \pm 10.4 ^{Bb}
Waste	8.2 \pm 0.3 ^{Ab}	12.8 \pm 2.5 ^{Ac}	10.5 \pm 2.3 ^{Ac}	5.4 \pm 1.7 ^{Bb}	11.4 \pm 1.2 ^{Ac}	4.5 \pm 0.90 ^{Ba}
Blend	267.4 \pm 55.1 ^{Aa}	144.6 \pm 17.6 ^{Bb}	184.3 \pm 12.9 ^{ABb}	89.6 \pm 22.3 ^{ABb}	26.4 \pm 3.9 ^{Bb}	108.4 \pm 34.13 ^{Aa}
Waste + A	0.0 \pm 0.0 ^{Bc}	9.9 \pm 1.8 ^{Ac}	4.84 \pm 1.7 ^{Ac}	0.0 \pm 0.0 ^{Bb}	3.9 \pm 0.3 ^{Ac}	8.5 \pm 5.88 ^{Ab}
Blend + A	65.1 \pm 7.2 ^{Ab}	21.7 \pm 7.5 ^{Bc}	31.27 \pm 11.9 ^{Bc}	46.7 \pm 8.8 ^{Ab}	5.1 \pm 1.5 ^{Bc}	22.6 \pm 11.34 ^{ABb}
Treatment	Shoot:root			Seedling biomass		
	TR	TRAC	TR	TRAC	TR	TRAC
Topsoil	1.5 \pm 0.7 ^{Ba}	4.7 \pm 1.1 ^{Bab}	1.5 \pm 0.7 ^{Ba}	4.7 \pm 1.1 ^{Bab}	1.5 \pm 0.7 ^{Ba}	4.7 \pm 1.1 ^{Bab}
Waste	2.0 \pm 0.6 ^{Aa}	1.2 \pm 0.3 ^{Ab}	2.0 \pm 0.6 ^{Aa}	1.2 \pm 0.3 ^{Ab}	2.0 \pm 0.6 ^{Aa}	1.2 \pm 0.3 ^{Ab}
Blend	3.2 \pm 0.9 ^{Aa}	5.6 \pm 0.6 ^{Aa}	3.2 \pm 0.9 ^{Aa}	5.6 \pm 0.6 ^{Aa}	3.2 \pm 0.9 ^{Aa}	5.6 \pm 0.6 ^{Aa}
Waste + A	0.0 \pm 0.0 ^{Ba}	2.7 \pm 0.7 ^{Aab}	0.0 \pm 0.0 ^{Ba}	2.7 \pm 0.7 ^{Aab}	0.0 \pm 0.0 ^{Ba}	2.7 \pm 0.7 ^{Aab}
Blend + A	1.5 \pm 0.1 ^{Aa}	4.9 \pm 2.2 ^{Aa}	1.5 \pm 0.1 ^{Aa}	4.9 \pm 2.2 ^{Aa}	1.5 \pm 0.1 ^{Aa}	4.9 \pm 2.2 ^{Aa}

4.3. Implications for drylands restoration

Alternative soil materials such as mine waste can be an adequate alternative growth media in arid land restoration contributing to alleviate the topsoil deficit found in mine rehabilitation (Arnold et al., 2015; Bateman et al., 2016). Despite previous research showing similar levels of soil fertility in these re-made soils under vegetation patches (Muñoz-Rojas et al., 2016a), our results showed that seedling recruitment and soil function of reconstructed soils can be challenged in the absence of external sources of organic matter, e.g. topsoil or organic amendments. Blending topsoil with waste in a 50:50 proportion has proved here to be an adequate replacement to topsoil in order to support plant establishment of native arid species such as *Triodia* species. Even though early seedling emergence of *T. wiseana* was higher in the topsoil compared to other soil treatments, survival rates after 12 months in the blended soil were not significantly different to those found in the topsoil. The use of appropriate and adjusted doses of topsoil can therefore maximise seedling establishment and, simultaneously, extend the availability of this valuable resource (Bateman et al., 2016; Wubs et al., 2016).

In the complete absence of topsoil, external sources of organic matter might be key to increase the levels of organic matter in reconstructed soils, which in turn will result in development of soil microorganisms and long-term soil fertility (Bastida et al., 2008, 2015; Luna et al., 2016). *Triodia* (Spinifex) grasslands have gained attention in recent years for their potential application as sources of biomaterials (Gamage et al., 2012). However, harvesting *Triodia* plants for application as organic amendments in broad scale restoration has not yet been explored.

Our results showed that *Triodia pungens* biomass used as organic amendments can be beneficial to kick-start microbial activity and activate C and N mineralisation processes in reconstructed soils, despite the poor response on seedling performance. Nevertheless, there are additional factors that would influence these results in the field in a longer time frame. For example, the time frame for microorganisms to break down the fresh compost may be longer than the one considered in this study. Also, the effects of other organisms (e.g. worms) that are highly relevant in soil structure processes, may influence soil characteristics of amended soils.

Composting *Triodia* plant material before application into the soil could potentially stabilise the plant substrates into high quality organic matter sources and increase N mineralisation preventing microbial competition (Bastida et al., 2008; Yanardağ et al., 2017), and is worthy of further investigation. However, this process might

be challenging at the large scale required for restoration. The focalised use of topsoil and organic amendments to create patches of soil fertility and promote autogenic recovery could be an effective practice at broad-scale restoration of arid and semi-arid lands (Wubs et al., 2016; Bulot et al., 2017). Nature-based solutions such as the proposed use of available mine soil materials and *Triodia* plants as organic amendments, may be critical to achieve the goals of the 2030 Agenda of the UN, which underpin the restoration of degraded land and soil as one of the global priorities within the Sustainable Development Goals (SDGs) (Keesstra et al., 2016, 2018). Further research is needed to explore the full potential of using native plants in restoration programs and it would be useful to transfer the experiments reported here to larger-scale field trials.

5. Conclusions

Seedling growth and survival rates were similar in topsoil and topsoil/waste blend highlighting the importance of topsoil addition to reconstructed soils to ensure plant recruitment of key native species. Seeding *Triodia wiseana* in combination with other native species (*Acacia* and *Grevillea*) tended to increase their recruitment but did not influence soil parameters compared to *T. wiseana* monoculture. Adding native-plant amendments (*Triodia pungens* leaf and shoot material) to waste material used in mine restoration can increase the levels of organic C, total N and microbial activity. The higher rates of C mineralisation and low levels of N found in the amended soil suggest a soil N immobilisation process as a response to the negative priming effect of fresh or un-composted amendments on soil microbial communities. Despite the positive effect of the amendments in enhancing soil quality and function of reconstructed soils, their addition did not increase the emergence or survival of *T. wiseana* plants. The poor recruitment in the amended soils suggest that the C-rich plant material was decomposed at a rate too slow to positively influence seedling growth. Further research is needed to improve the outcomes resulting from the use of native-plant amendments.

Acknowledgements

This research project was supported by BHP Billiton Iron Ore Community Development Project (contract no. 8600048550) under the auspices of the Restoration Seedbank Initiative (2013–2018), a partnership between BHP Billiton Iron Ore, The University of Western Australia, and the Botanic Gardens and Parks Authority

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2017.11.219>.

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