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Soil carbon and litter development along a reconstructed biodiverse forest chronosequence of South-Western Australia

S. J. George · R. N. Kelly · P. F. Greenwood · M. Tibbett

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Abstract Soil organic matter (SOM) increases with time as landscape is restored. Studying SOM development along restored forest chronosequences would be useful in clarifying some of the uncertainties in quantifying C turnover rates with respect to forest clearance and ensuing restoration. The development of soil organic matter in the mineral soils was studied at four depths in a 16-year-old restored jarrah forest chronosequence. The size-separated SOM fractionation along with $\delta^{13}C$ isotopic shift was utilised to resolve the soil C temporal and spatial changes with developing vegetation. The restored forest chronosequence revealed several important insights into how soil C is developing with age. Litter accumulation outpaced the native forest levels in 12 years after restoration. The surface soils, in general, showed increase in total C with age, but this trend was not clearly observed at lower depths. C accumulation was observed with increasing restoration age in all three SOM size-fractions in the surface 0–2 cm depth. These biodiverse forests show a trend towards accumulating C in recalcitrant stable forms, but only in the surface 0–2 cm mineral soil. A significant reverse trend was observed for the moderately labile SOM fraction for lower depths with increasing restoration age. Correlating the soil $\delta^{13}C$ with total C concentration revealed the re-establishment of the isotopically depleted labile to enriched refractory C continuum with soil depth for the older restored sites. This implied that from a pedogenic perspective, the restored soils are developing towards the original native soil carbon profile.

Keywords Jarrah forest · Stable isotope · Nitrogen · Bauxite mining · Rehabilitation · Fractionation

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Introduction

Globally, soils contain approximately 1500 Pg of C, making it the largest terrestrial C pool (Post et al. 1990; Eswaran et al. 1993; Lal 2004) and account for two or three times more carbon than the atmospheric CO_2 pool (Davidson et al. 2000). Of the estimated 1,500 Gt of organic C in soils, 250–530 Gt are in C fractions with turnover times of decades or less (Trumbore 1997). Furthermore, SOM has been gaining importance for its carbon sequestration potential in mitigation of climate change. Since soils are the

largest carbon reservoir in terrestrial ecosystems, even a five per cent increase in the size of this pool with modified land management techniques has the potential to decrease the amount of atmospheric C by up to 16% (Baldock 2007). The effects of reforestation/change of land-use on soil C pools has potential to either release or sequester soil C (Guo and Gifford 2002; Silver et al. 2004; Lo′pez-Ulloa et al. 2005). The balance between deposition and decomposition of organic material primarily governs the accumulation or loss of SOC. It has been suggested that improved land management could result in sequestration of a substantial amount of soil C within a few decades and can be an option to reduce atmospheric CO_2 concentration (Pastian et al. 2000; Metting et al. 1999; Post et al. 1990). With increasing global carbon losses following disturbance/deforestation, old-growth forests have a key role in maintaining and accumulating quantities of carbon for centuries (Zhou et al. 2006; Luyssaert et al. 2008). However, large uncertainties remain in quantifying the rates of turnover of soil organic matter to the current terrestrial carbon sink (Wang and Hsieh 2002; Pacala et al. 2001). Studying SOM development along a reconstructed or newly formed land-use chronosequence would be extremely informative in clarifying some of these uncertainties, particularly with respect to the rate at which SOM accumulates/depreciates when factors such as site preparation, stocking, fertilisation, fire management are considered as part of post-mining restoration (Paul et al. 2002).

The increased loss of SOM in initial post-surface mined areas following restoration is probably similar to mechanical disturbance following whole-tree harvesting where 6% of C was lost (Johnson and Curtis 2001). Restored forest soils in Australia have shown a general trend of soil carbon accumulation over time that can at least match the natural undisturbed forests in terms of total carbon concentration (Schwenke et al. 2000a, b; Koch and Samsa 2007; Tibbett 2010). SOM is a biochemical continuum ranging from fresh plant detritus which readily undergoes microbial degradation to the highly stabilised portions formed through interactions with soil mineral particles and aggregates (Kögel-Knabner et al. 2008). However, the majority of measurements were based on various forms of total soil carbon (Guo and Gifford 2002) measured using either dry combustion (Van Moort and De Vries 1970) or organic carbon (wet oxidation; Walkley and Black 1934). These methods provide generally useful information; but provide little discrimination of SOM (Gartzia-Bengoetxea et al. 2009). A more informative approach is to partition SOM having mean residence time varying from months to centuries into conceptual pools with different levels of stability (Christensen 2001; von Lutzow et al. 2007). These pools usually grouped as active, slow or intermediate and passive provide more advanced information especially when monitoring the SOM changes in a recovering ecosystem. Emerging evidence show a slower rate of carbon sequestration (compared to biomass and litter accumulation) due to rapid soil carbon turnover rates despite high carbon inputs to the mineral soil, especially in soils with coarse texture and low-activity clay mineralogy following afforestation (Richter et al. 1999). Soil aggregation increases in less disturbed systems and that organic materials within soil aggregates (microaggregates) have lower decomposition rates than those located outside of aggregates (Oades 1984; Six et al. 2000). But the time-scale of this aggregation process is not well understood.

The $\delta^{13}C$ of the SOM and its size separated fractions can also help resolve the temporal and spatial changes in vegetation (Garten 2006; Balesdent and Mariotti 1996; Ehleringer et al. 2000). Stable isotope values can reflect vegetation and soil management changes over short- (50–100 years) and longer-term (100 s of years) time scales (Boutton et al. 1998; Krull et al. 2005). Well drained, relatively undisturbed soils often show increasing $\delta^{13}C$ and $\delta^{15}N$ with soil depth (Mariotti et al. 1980; Nadelhoffer and Fry 1988; Powers and Schlesinger 2002).

Based on the time-frame of the post-disturbance restored chronosequence studied, we predicted to see a progression in C accumulation where coarser labile SOM fractions approached the levels in natural forest more quickly (chronosequence time) than for the finer, more recalcitrant fractions. The $\delta^{13}C$ gradient with soil depth will be progressively re-establish with increasing restoration age. In order to test our predictions, we tracked the post-disturbance developmental trajectories followed by the restored south west Australian jarrah forest chronosequence based on: (i) the quantitative development of SOM dynamics with time, for both whole soil (total C) and size-fractionated SOM; (ii) monitoring temporal and spatial (depth) changes in $\delta^{13}C$ stable isotope.

Materials and methods

Description of study location

Bauxite mining in the Mt Saddleback Timber Reserve (32°50′S, 116°30′E) near Boddington, south-west Western Australia (123 km southeast of Perth) commenced in the early 1980s. Mt Saddleback lies within the low rainfall region (731 mm yr^{-1}) of Western Australia's northern jarrah (*Eucalyptus marginata*) forest (Loneragan et al. 2007). It has a Mediterranean climate with hot, dry summers and mild, wet winters. Rainfall is strongly seasonal, most falling during the winter months of June–August.

The mining operation typically clears 180 ha of forest every year and the mine operators have a long-term policy of post-mining restoration of the native forest. The mining process and the regolith material disturbed are eco-toxicologically benign and the landscape can be restored to a high standard after what is primarily a physical disturbance (Tibbett 2010). This mine site has experienced a consistent rehabilitation program for the last 16 years, providing a unique opportunity to study the development of SOM along the restored chronosequence over this time. Soil and litter samples from 'space for time' substituted sites restored at different periods up to 16 years were analysed and compared.

Studies of restoration regions of the South-west of Australia have shown the vegetation which develops to be distinctly different from the native forest (Koch 2007). For about the first 7 years there is a predicable dominance of acacias that are short-lived and produce copious seed. After approximately 10 years, the acacias decline in their relative basal area, contributing much litter upon senescence. The overstorey eucalypts begin to dominate, and the restored forest begins to exhibit similar stand silvics to native Jarrah forest (Koch and Samsa 2007). The overstorey vegetation is typically dominated by just a few standard species. In the understorey, where much of the floristic diversity occurs, mean species similarities between restored mine sites and unmined native forest are still about half the value of original forests (Koch 2007; Standish et al. 2008). Average above-ground biomass and litterfall for a mature jarrah forest is 266 and 11 Mg ha^{-1} (Hingston et al. 1981) compared to 27 and 9 Mg ha^{-1} (Ward and Pickersgill 1985) for a 3.3 yr old restored forest and 62 and 41 Mg ha^{-1} (Ward and Koch 1996) for a 15.5 yr old restored forest.

The Mount Saddleback area is currently mined for bauxite ore. These forest have been classified as a global hotspot (www.hotspot.com) based on the highly biodiverse forest and the mine operators have a long-term policy of restoring the native jarrah forest after mining has ceased. The mining process typically involves removal of all vegetation, burning of residue, removal of 10–15 cm of topsoil for later use in rehabilitation and removal of overburden (approximately 40 cm depth) before the ore is mined (Koch 2007; Tibbett 2010). Pit floors are ripped and reshaped to blend in with the surrounding landscape, overburden and topsoil are returned, and the areas are deep-ripped to about 1.2 m (Ward 2000). All the sites sampled for this study had top soil replaced from other currently mined sites ('direct-return'). Seed of both tree and understorey species are sown, followed by fertilisation. The site has undergone consistent restoration programme over the past 17 years, thus providing a unique opportunity to study the progression of ecosystem development over this time frame.

Soil and litter sampling regime

Sampling took place over 2 days in late May 2007. Geo-referenced study sites, comprising two sets of 2, 5, 8, 11, and 16 year old restored sites were sampled, resulting in a total of 10 sample sites. We further analysed the mineral content of both the native forest and restored forest soils by XRF to ensure that they are of similar parent material so that any changes in SOM may be attributed to time since restoration and not confounded by difference in pedology (data not shown). Previous studies have predicted initial reduction in residual SOC following pre-cleared land-use, especially after a severe disturbance event (Paul et al. 2002); we used 2 years as our initial sample to better observe SOM accumulation trends following restoration. Three undisturbed native forest sites were separately sampled for comparative purposes and to set a benchmark for the trajectory of the restored sites. A total of three 'pseudo' replicates were taken from each site along a transect to account for local spatial heterogeneity. Soil and litter were sampled at a distance of 5 m for pre-existing floristic monitoring plots, close enough to infer floristic diversity from surveys conducted within these

monitoring plots. At each sampling location, entire litter above the mineral soil was collected from 1 × 1 m quadrat and stored in a paper bag. Litter was sampled over a ridge–furrow sequence formed by ripping of the landscape surface as part of the restoration process, so that results were not biased by differences in accumulation patterns. After the litter layer was removed, soil was sampled by cutting steps at intervals of 0–2, 2–5, 5–10 and 10–20 cm using a trowel to investigate SOM changes with depth. For consistency, soil samples were taken in furrows and not ridges (George et al. 2006; Banning et al. 2008). The soil was stored in plastic zip lock bags, sealed for transport and re-opened within 24 h. Samples were air-dried (in a drying room maintained at a constant temperature of 40°C) and sieved to 2 mm prior to further analysis. A separate set of undisturbed cores were used for bulk density determination.

Litter samples were dried at 70°C until a constant weight was achieved and dry mass then determined. Subsequently, the litter was sub-sampled, sorted and weighed into *E. marginata* leaves, other leaves, small woody debris (≤2.5 cm diameter) and comminuted litter.

Whole soil elemental analysis for total carbon and nitrogen was determined on sieved and air-dried soil samples ground to a homogenous powder using a ball mill grinder. Ground samples were then weighed (~200 mg) and analysed for total C and N by the dry combustion gas method using a Vario Macro Elementar analyser (Germany) and expressed as dry weight equivalent. All soil samples were tested with HCl and found to be carbonate-free, hence the measured total C was inferred to be of organic origin. Description of related basic soil chemical changes with increasing restoration age and depth is detailed in Ward (2000).

Soil carbon fractionation procedure

Soil carbon fractionation was conducted on the same set of soil samples used for the total carbon and nitrogen analysis. Whole soils (10 g) were separated into three size fractions (as described by Krull and Bray (2005) and Cambardella and Elliott (1992)) relating to carbon pools that differ in soil residence times and bioavailability. The whole soil was first dispersed on an end-over-end shaker with 80 ml of 25 g l^{-1} sodium hexametaphosphate for a period of 16 h to disaggregate. This disaggregated soil was then passed through a series of sieves; initially through a 200 μm pore size followed by a 53 μm pore size. The sediment, consisting of sand and particulate organic matter, was gently worked with a spatula to ensure that no aggregates were retained in the particulate fractions. This procedure divided the soil into three size classes: (1) >200 μm; 2) 200–53 μm; and (3) <53 μm fractions. The >200 μm (highly labile carbon pool) and 200–53 μm (moderately labile carbon pool) fractions represent particulate organic carbon (POC) and were dried at 90°C then ground to a fine homogenous powder using a ball mill grinder. The <53 μm fraction (representing the recalcitrant fractions of the soil—formed after various SOM conversions and/or structurally unaltered SOM stabilised by various processes) was collected in a measuring cylinder (1 l) after passing through both sieves in de-ionised water. The samples were flocculated with addition of a saturated aluminium sulphate solution and subsequently freeze-dried.

The fractions were then weighed and analysed for total C and N by the dry combustion gas method using a Vario Macro Elementar analyser (Germany).

Stable Carbon (δ^{13}C) Isotope Analysis

The soil δ^{13}C ratio was analysed using a Micromass IsoPrime isotope ratio mass spectrometer interfaced to a EuroVector Euro-EA3000 elemental analyser. A sample mass yielding between 300 and 800 μg carbon was placed into ultraclean tin capsules and sealed. All samples were standardised against a secondary reference of radish collegate (3.167% N, δ^{15} N 5.71; 41.51% C, δ^{13}C −28.61) that was in turn standardised against primary analytical standards (IAEA, Vienna).

Floristic sampling

The area described in this study comes under a global mega-diversity hotspot (www.biodiversityhotspots.org). The native vegetation (northern jarrah forest) and adjoining vegetation has been extensively described elsewhere (Hopper and Gioia 2004; Loneragan et al. 2007; Wardell-Johnson and Horwitz 1996; Norman et al. 2006). The vegetation analysis conducted for this study was based on the floristic survey within the restored and native forest sites. Species densities were assessed within a 20 × 20 m plot, based on twenty 2 × 2 m quadrats.

Statistical analysis

One way ANOVA (without blocking) for each soil depth was carried out on the C, C:N for each year since restoration and a native forest site using Genstat Ver 10 (www.vsni.co.uk) statistical software. The carbon concentration and $\delta^{13}C$ of the three SOM fractions (>200, 200–53 and <53 μm) were similarly processed.

Results

Litter mass development along the restored chronosequence

Litter mass showed a strong incremental trend ($P < 0.01$) along the restored 16-year-old chronosequence (Fig. 1). Interpolated litter mass exceeded the native forest benchmark value approximately 12 years after restoration. A break up into component litter categories reveals that the leaf mass of the jarrah forest keystone species *E. marginata*, approached benchmark native forest levels only by year 16 since restoration, after continually being low. The combined leaf litter mass for all other species peaks by year 8 since restoration followed by continuous decline to match the unmined forest site at 16 years since restoration. The two major litter mass components—comminuted litter and small woody debris—showed a consistent increase along the chronosequence and final levels were in excess of the native forest level.

Whole soil total C and associated C:N ratio

The soil texture is predominantly loamy sand. Soil total C in surface soils showed a slight (0–2 cm) to moderate (2–5 cm) increasing trend (non-significant $P \leq 0.05$)) with restoration age (Fig. 2). At these shallow depths, the 16-year-old restored soils had lower C than the native forest soils. No clear trends could be elucidated from the deeper samples (5–10 and 10–20 cm) with chronosequence age. The deeper layers also showed a higher level of variability with restoration age. The 2 year restored soils had higher C than the native soils at all depths except for 0–2 cm depth.

The total soil C:N ratio showed a significant downward trend with restoration age at all depths (Fig. 3) except the top 0–2 cm depth. The C:N ratio of the top 0–2 cm fluctuated with restoration age (Fig. 3). The lower soil depths (5–10 and 10–20 cm) also showed a more pronounced decrease in C:N than the 2–5 cm depth. The C:N ratio at 16-years restoration were significantly lower than corresponding native forest value, particularly for the two lower depths (5–10 and 10–20 cm).

Total soil C dynamics of SOM size-fractions with increasing restoration age

All three soil size fractions from largest (>200 μm) to the smallest (<53 μm) showed an increasing trend for total soil C in the top 0–2 cm depth with restoration age (Fig. 4) with the >200 and 200–53 μm size

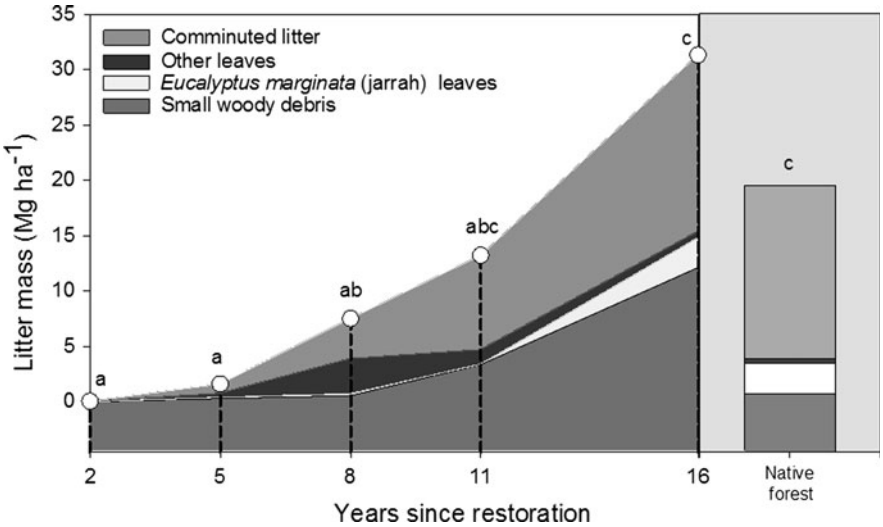

Fig. 1 Total litter mass changes with restoration age compared to benchmarked native forest (*grey panel*) along a reconstructed jarrah forest chronosequence. Relative proportions of jarrah leaves, other leaves, small woody debris and communited litter are shown with different grey-scale shading. Same letter denotes lack of significant difference at $P \leq 0.05$ level

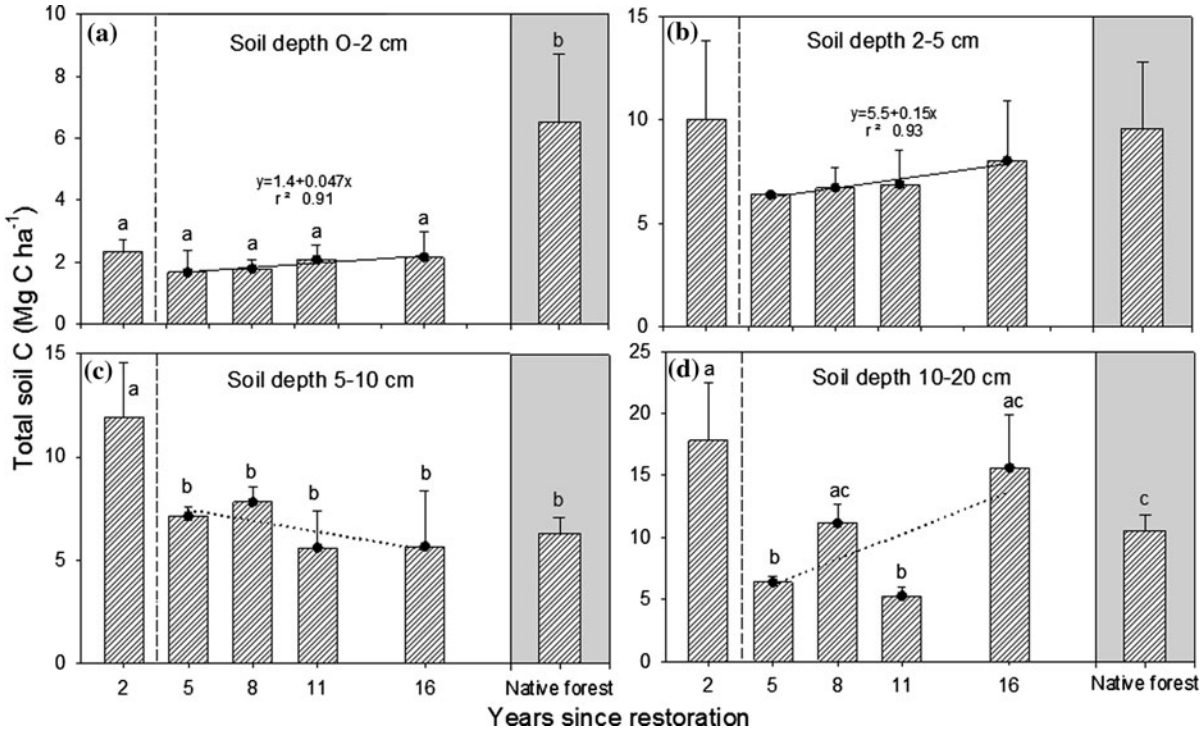

Fig. 2 Total soil C development with restoration age at various depths: (**a**) 0–2 cm (**b**) 2–5 cm, (**c**) 5–10 cm and (**d**) 10–20 cm along a reconstructed jarrah forest chronosequence compared to benchmarked native forest (*grey panel*). Linear fitted continuous line depicts significant ($P \leq 0.05$) and dotted line depicts a non-significant trend for total soil C with increasing restoration age. The fitted liner models excluded initial 2-year-old restored sites since they still showed evidence of higher level of residual carbon of native forest origin. Error bars ± SE. Same letter denotes lack of significant difference at $P \leq 0.05$ level

fraction values of the 16 year restored soils close to native forest levels. This trend continued only in the 2–5 cm layer for the coarser >200 μm size fraction. At this depth, the C levels of the 200–53 and <53 μm size fractions showed a decrease with restoration age. At the lower depths a similar inverse trend was evident only in the 200–53 μm size fraction. This progressive decline in C for the 200–53 μm size fraction at the lower depths led to significantly ($P \leq 0.05$) lower levels than in the corresponding fractions of the native forest soils. The >200 and <53 μm size fractions of the 5–10 and 10–20 cm depths showed no clear trend.

Soil δ^{13}C and δ^{15}N isotopic signature

The δ^{13}C values (Fig. 5) of the 0–2 cm soil layer showed a decreasing trend with forest development. A similar but less subtle trend was evident in the 2–5 cm soils. At 2-years restoration, the δ^{13}C of these shallow soils was 1 (2–5 cm) to 3 (0–2 cm) ‰ more enriched than the equivalent native soils. At deeper depths, the δ^{13}C value of the native soils and the soils at all stages of restoration were similar.

In contrast with the stable C isotopic signature dynamics, δ^{15}N isotopic values for the 10–20 cm depth for all restored soils were enriched in δ^{15}N relative to the native soil (Fig. 6).

The older restored sites (11 and 16 yr), like the native forest showed a strong linear correlation between δ^{13}C (‰) and log-transformed C concentrations with soil depth (Fig. 7). However, a similar relationship was not evident in the less restored soils (≤8 yrs).

Vegetation development

After the initial pulse of N-fixing dominated pioneer species, the plant and legume density fell markedly

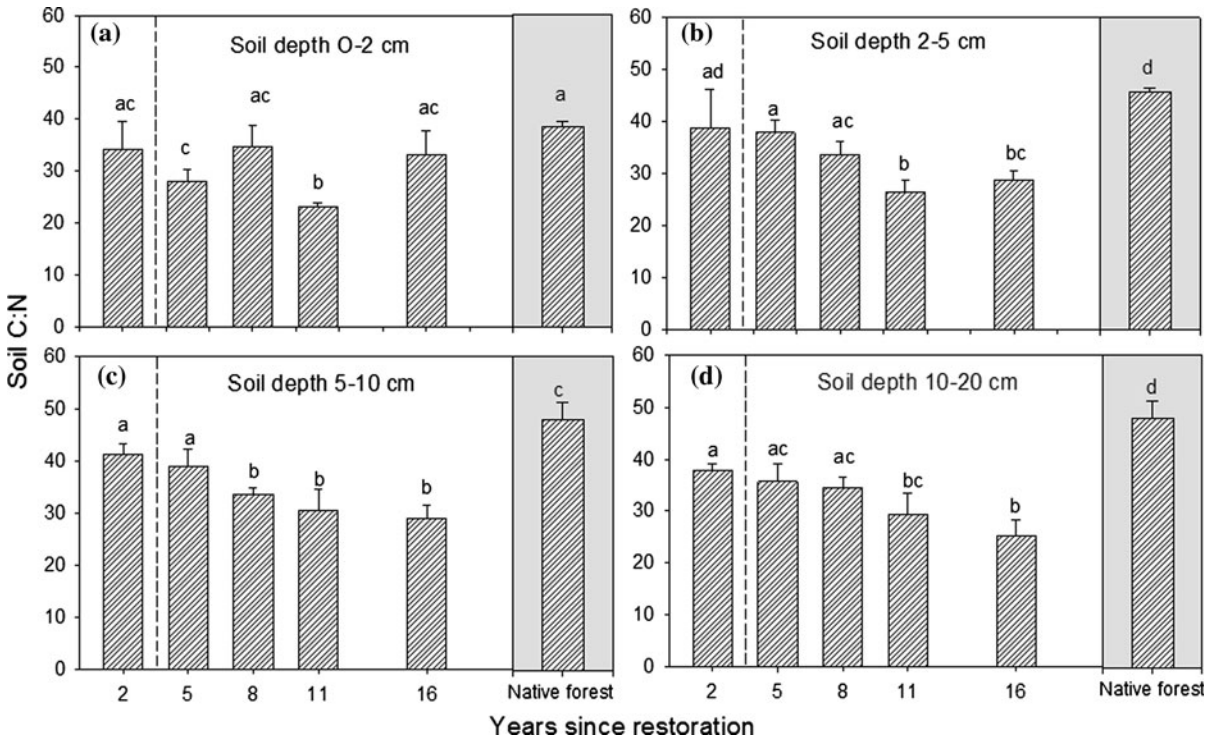

Fig. 3 Soil C:N development with restoration age at various depths: (**a**) 0–2 cm, (**b**) 2–5 cm, (**c**) 5–10 cm and (**d**) 10–20 cm along a reconstructed jarrah forest chronosequence compared to benchmarked native forest (*grey panel*). The initial 2-year-old restored sites still showed evidence of higher level of residual carbon of native forest origin. Error bars ± SE. Same letter denotes lack of significant difference at $P \leq 0.05$ level

with increasing restoration age (Fig. 8). This decline was well fitted by an exponential decay model.

Discussion

Soil C development with increasing restoration age: SOM size-fractionation approach

The generally higher level of soil C for the 2-year-old restored site of the chronosequence is mainly from the 'direct-return' soil (as part of the site preparation for post-disturbance land restoration) from the native forest cleared for mining (Tibbett 2010; Koch 2007). This is followed by a distinct loss of soil organic C, which has been previously observed and is typical of restored soils after major ecosystem disturbance (Schwenke et al. 2000a, b; Banning et al. 2008). Initial mixing of surface soil with deeper soil layers, during the post-mined land preparation, resulted in a dilution of soil C concentrations at the surface and increase in SOC in the lower depths (Ward 2000). The subsequent loss of organic C may be attributed to higher mineralisation of SOM by soil microorganisms but not offset by the organic matter input (by litterfall) due to low contribution from the establishing plant community. There were increasing amounts of litter with increased restoration age, reflecting the increased organic inputs that occur as the forest develops and its net primary productivity increases (Tibbett 2010). However, the C:N ratios of soils from lower depths that have undergone longer periods of forest growth following restoration were significantly lower than non-mined forest soil, and is most likely due to additional N inputs by the high-density leguminous understorey in restoration (Ward 2000). Only with a reduction in the dominance of the N-fixing species would the C:N ratio return to pre-mining levels (Fig. 8). The lack of a clear trend for the C:N ratio in the surface (0–2 cm) soils may be attributed to the extremely mobile nature of the nitrate anions.

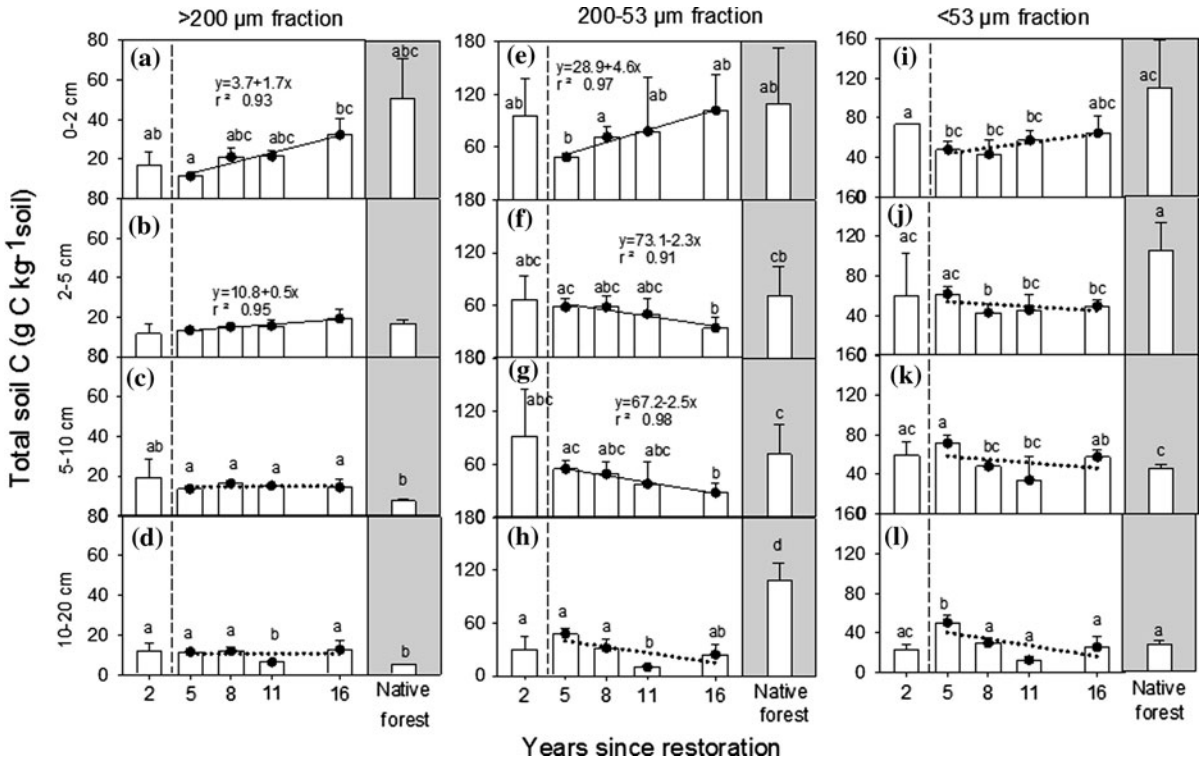

Fig. 4 Total soil C concentrations in >200, 200–53 and <53 µm fractions for various depth intervals: (i) 0–2 cm (**a**, **e**, **i**) (ii) 2–5 cm (**b**, **f**, **j**) (iii) 5–10 cm (**c**, **g**, **k**) and (iv) 10–20 cm (**d**, **h**, **i**) along a reconstructed jarrah forest chronosequence compared to benchmarked native forest (*grey panel*). Linear fitted continuous lines depict significant ($P \leq 0.05$) and *dotted lines* depict a non-significant trend for total soil C with increasing restoration age. The fitted liner models excluded initial 2-year-old restored sites since they still showed evidence of higher level of residual carbon of native forest origin. Error bars ± SE. *Same letter* denotes lack of significant difference at $P \leq 0.05$ level

As predicted, after 16 years of restoration, the labile >200 µm SOM size-fraction (with an annual turnover rate) and the passive <53 µm (with millennial turnover rates) had the fastest and slowest recovery rate respectively, although this was only evident in the surface horizon. Even with a fast turnover rate, >200 µm SOM size-fraction showed gains in the top two depths with increasing restoration age which can be attributed to the high litter input. Upon contact with soil, plant litter undergoes microbial aided transformation and ensuing compounds may adhere to clay particles (Jones and Donnelly 2004). As microbes selectively degrade the less recalcitrant compounds, the more recalcitrant compounds become selectively enriched. Thus <53 µm SOM size-fraction represent SOM that is physically or biochemically recalcitrant with a turnover time of hundreds to thousands of years (Jenkinson 1990; Post and Kwon 2000). These biodiverse forests show a trend towards accumulation of C in recalcitrant stable forms, but only in the surface 0–2 cm mineral soil. In an ecosystem undergoing restoration after disturbance, the inception of spatial isolation and selective preservation of recalcitrant compound may initially happen in the very top surface. Hence soil sampling resolution of 0–2 cm may be paramount in highlighting these initial changes. In addition to this, the biodiverse nature of the litter accumulating on the surface 0–2 cm depth may be the reason for the observed increase in SOM C for all three size-fractions studied.

Contrary to our prediction, SOM in the 200–53 µm (intermediate turnover pool) had a significant downward trend for all depths apart from the top 0–2 cm. The release of the intra-aggregate particulate organic matter (with an average size of 200–53 µm) from the physical protection of macro-aggregates, following soil disturbance event, may

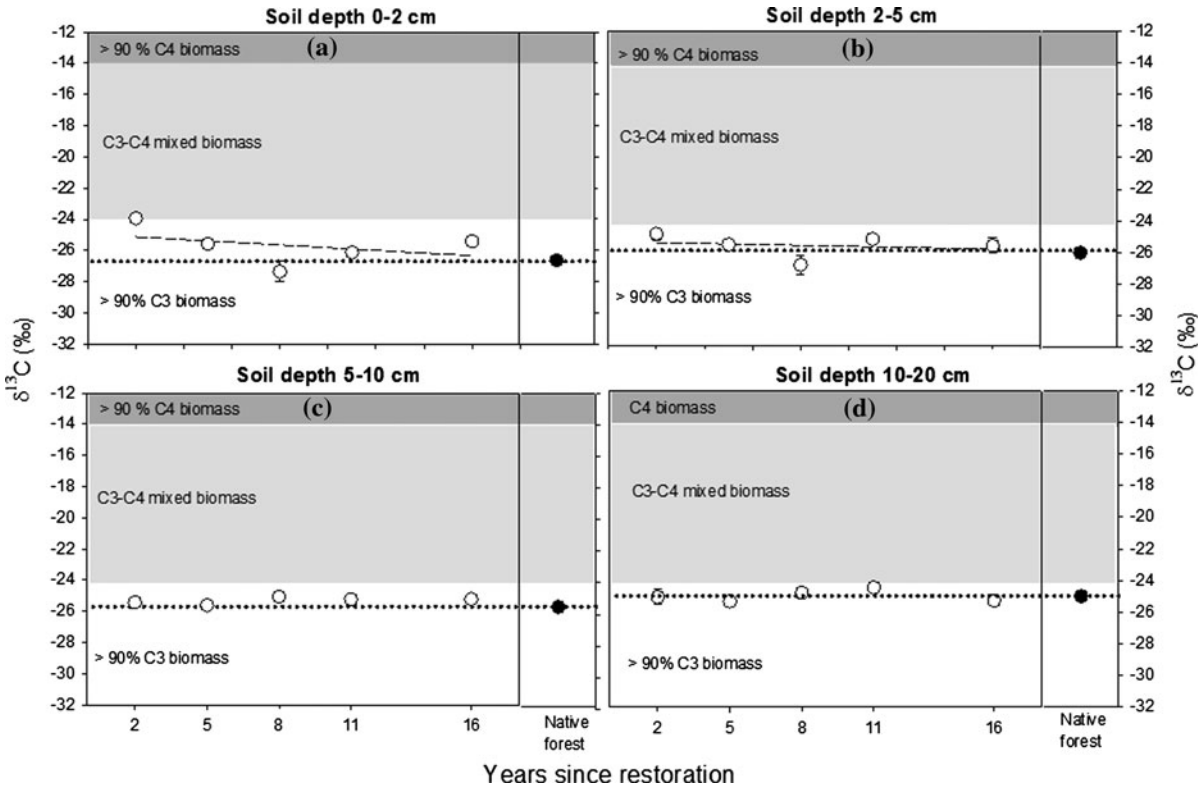

Fig. 5 Soil $\delta^{13}C$ changes with rehabilitation age at various depth: (**a**) 0–2 cm, (**b**) 2–5 cm, (**c**) 5–10 cm and (**d**) 10–20 cm along a reconstructed jarrah forest chronosequence compared to benchmarked native forest. *Grey areas* show typical mean and standard deviation of C3 and C4 biomass (from compilation of Cerling et al. 1998). *Closed symbols*: native forest $\delta^{13}C$; *open symbols*: Restored sites $\delta^{13}C$. The *dotted line* shows the benchmarked native $\delta^{13}C$ level. *Error bars* ± SE

result in rapid oxidation of this particulate organic matter (Grandya and Neff 2008). Further complications due to slower re-establishment of fungal networks and recolonisation of the microbiota in the deeper soil layer may hinder the incorporation of this SOM into larger aggregates. Whilst this pool is probably the least understood, it's composition and dynamics may be more important to the function of the soil than the other two pools (Elliott et al. 1996).

Influence of developing vegetation on SOM: stable isotopic signature evaluation

After 16 years of vegetation development the litter accumulation exceeded the native forest level, plausibly due to the high primary productivity of the pioneer N-fixing, r-strategist dominated the initial phase of vegetation development (Brown and Lugo 1990). As evident from the negative decay function explaining the decline in N-fixing and total frequency of individual species with increasing restoration age (Fig. 8), a substantial reduction in primary productivity can be expected following canopy closure at the restored sites. In-depth analysis of a similar post-mined restored chronosequence also attributed the higher litter accumulation of restored sites to disrupted microbial functional development (Spain et al. 2006). A parallel study of the same suite of geo-referenced chronosequence sites revealed a reasonable re-establishment of community-level microbial function (unpublished data Honours Thesis, University of Western Australia). Furthermore, a slower rate of re-colonisation by some key microbiota, primarily responsible for the initial breakdown of the detritus, may have a rate-limiting effect as revealed by restored sites studied in the same region (Folgarait 1998).

Stable isotopes can delineate the carry over effect of vegetation development on whole soil C (O'Leary

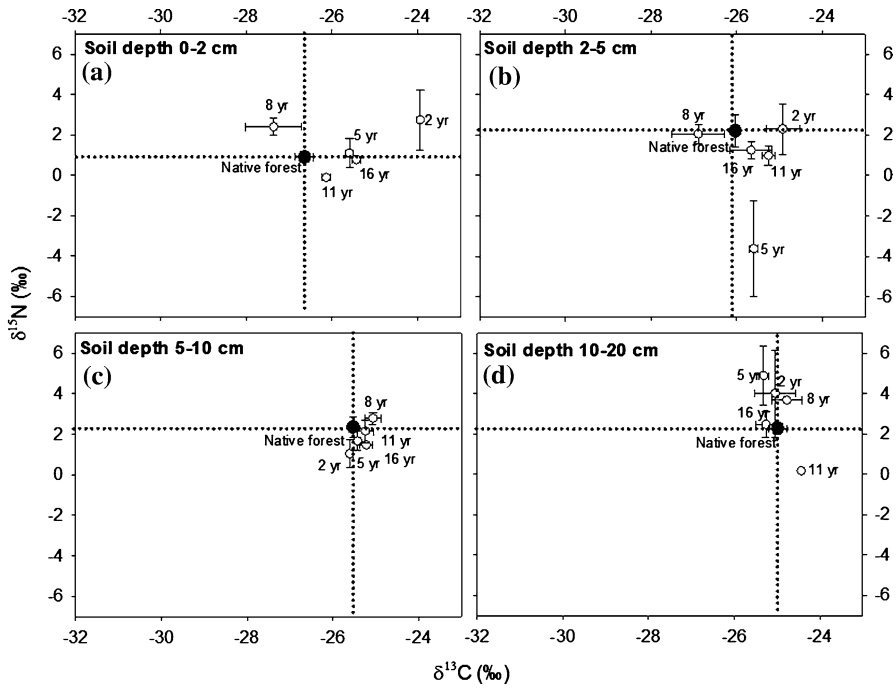

Fig. 6 $\delta^{13}C$ to $\delta^{15}N$ comparison with restoration age at various depth intervals: (**a**) 0–2 cm, (**b**) 2–5 cm, (**c**) 5–10 cm and (**d**) 10–20 cm along a reconstructed jarrah forest chronosequence compared to benchmarked native forest. *Dotted lines* depict the benchmarked native forest levels for $\delta^{13}C$ and $\delta^{15}N$. *Closed symbols*: native forest $\delta^{13}C$; *open symbols*: Restored sites $\delta^{13}C$

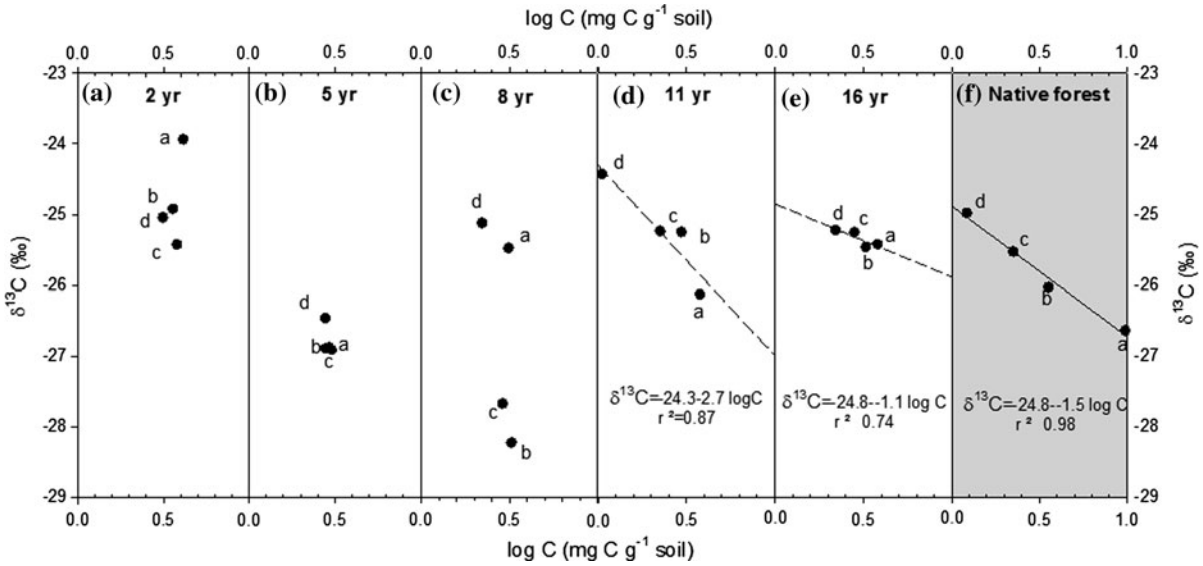

Fig. 7 Linear correlations of the $\delta^{13}C$ and log-transformed C concentrations in the whole soil for increasing age since restoration: (*a*) 2 yrs, (*b*) 5 yrs, (*c*) 8 yrs, (*d*) 11 yrs, and (*e*) 16 yrs compared to benchmarked native forest site (*grey panel*) at the four sampling depths (data labelled as a:0–2 cm, b:2–5 cm, c:5–10 cm, d:10–20 cm). Linear fitted continuous line depicts significant ($P \leq 0.05$) and dotted line depicts a non-significant trend for total soil C with increasing restoration age

1981), but here only a subtle response to depth and age was evident primarily due to lack of any major vegetation shifts between C3 to C4 plants during restoration.

The soil $\delta^{13}C$ values of surface soil (0–2 cm) will be most strongly influenced by increasing plant litter inputs (Krull et al. 2005). The ensuing heterotrophic bacteria and fungi mediated microbial break down of

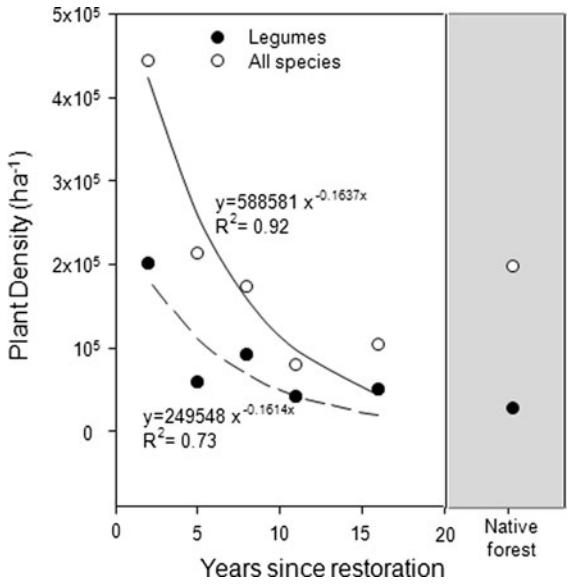

Fig. 8 Legume and plant density changes with restoration age compared to benchmarked native forest site (*grey panel*)

organic particulates preferentially enhances the light ^{12}C atoms over ^{13}C resulting in a more depleted $\delta^{13}C$ in the surface soils (Nadelhoffer and Fry 1988). This preferential isotopic fractionation is more evident for the recently restored sites than older sites in the surface soils. Change in litter quality in older restored sites (Fig. 1) may be responsible for corresponding changes in soil isotopic C signature. Contrary to this, disruption in litter supply following the disturbance event, has resulted in the native forest residual C for the 2-year-old restored site to become gradually enriched in $\delta^{13}C$ by preferential decomposition of depleted labile fraction over enriched recalcitrant fraction (Grandya and Neff 2008). The lower 5–10 cm and 10–20 cm depths were only marginally influenced by the developing vegetation based on the generally stable $\delta^{13}C$ values across the restored sites. In contrast, most of the 10–20 cm restored sites showed enriched $\delta^{15}N$ isotopic values possibly from leaching of the nitrate anion originating from the large proportion of N-fixing species dominated litter (Vervaet et al. 2002).

Interestingly, correlating soil $\delta^{13}C$ with total C concentration revealed the re-establishment of the isotopically depleted labile to enriched refractory C continuum with soil depth for the older restored sites (11 and 16 yrs). This signified that from a pedogenic point of view, the restored soils are developing towards the original native soil C profile. While for the younger restored sites this continuum was not established due to an obvious lack of any relationship between soil isotopic shift $\delta^{13}C$ regressed against the log-transformed soil C with soil depth. The regression slope, or 'isotopic discrimination factor' (Nadelhoffer and Fry 1994; O'Leary 1981), can be used as a proxy measure of soil carbon turnover rates (Garten 2006; Garten and Hanson 2006).

Conclusions

The restored forest chronosequence revealed several important insights into how soil C is developing with age in the top 0–20 cm. Litter accumulation increased rapidly with stand development and outpaced the native forest litter accumulation in 12 years after restoration. The surface soils, in general, showed a corresponding increase in total C in response to the litter input. This increasing trend is not clearly observed at the lower soil depths (5–10 and 10–20 cm). Furthermore, accumulation of C was observed with increasing restoration age in the two SOM size-fractions (>200 and 200–53 μm) and surprisingly for humified <53 μm size-fraction in the surface 0–2 cm depth. Soil C:N showed a decreasing trend with restoration age for all depths except in surface 0–2 cm; this was attributed to the huge pulse of N-fixing pioneer species dominating the initial years of the restored forest. Interestingly, a reverse trend was observed for the 200–53 μm SOM fraction with increasing restoration age, probably attributed to C mineralisation on release of this intra-aggregate particulate organic matter from the physical protection of macroaggregates, following soil disturbance. Correlating the soil $\delta^{13}C$ with total C concentration revealed the re-establishment of the isotopically depleted labile to enriched refractory C continuum with soil depth for the older restored sites. This implied that from a pedogenic point of view, the restored soils are developing towards the original native soil carbon profile.

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