Shaun C. Cunningham, Timothy R. Cavagnaro, Ralph MacNally, Keryn I. Paul, Patrick J. Baker, Jason Beringer, James R. Thomson, and Ross M. Thompson

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Global Change Biology, 2015; 21(4):1552-1566

Reforestation with native mixed-species plantings in a temperate continental climate effectively sequesters and stabilizes carbon within decades

SHAUN C. CUNNINGHAM[1,2*], TIMOTHY R. CAVAGNARO[3], RALPH MAC NALLY[2], KERYN I. PAUL[4], PATRICK J. BAKER[5], JASON BERINGER[6,7], JAMES R. THOMSON[2] and ROSS M. THOMPSON[2]

[1] Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, Vic 3125, Australia
[2] Institute for Applied Ecology, University of Canberra, Bruce, ACT 2617, Australia
[3] School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia
[4] CSIRO Sustainable Agriculture Flagship, GPO Box 1700, Canberra, ACT 2601
[5] Department of Forest and Ecosystem Science, University of Melbourne, Burnley, Vic 3121, Australia
[6] School of Earth and Environment, University of Western Australia, Crawley, WA 6009, Australia
[7] School of Geography and Environmental Science, Monash University, Clayton, Victoria 3800, Australia

* Correspondence: tel. +613 9244 5523, e-mail: shaun.cunningham@deakin.edu.au

Running head: C STOCKS AND POOLS IN MIXED-SPECIES PLANTINGS

Key words: biomass C, carbon sequestration, chronosequence, litter, mixed-species plantings, recalcitrant C, revegetation, soil C

Word count (Introduction-Acknowledgements) = 7454 words

Abstract

Reforestation has large potential for mitigating climate change through carbon sequestration. Native mixed-species plantings have a higher potential to reverse biodiversity loss than do plantations of production species, but there are few data on their capacity to store carbon. A chronosequence (5-45 yr) of 36 native mixed-species plantings, paired with adjacent pastures, was measured to investigate changes to stocks among C pools following reforestation of agricultural land in the medium rainfall zone (400-800 mm yr^{-1}) of temperate Australia. These mixed-species plantings accumulated 3.09 ± 0.85 t C ha^{-1} yr^{-1} in aboveground biomass and 0.18 ± 0.05 t C ha^{-1} yr^{-1} in plant litter, reaching amounts comparable to those measured in remnant woodlands by 20 yr and 36 yr after reforestation, respectively. Soil C was slower to increase, with increases seen only after 45 yr, at which time stocks had not reached the amounts found in remnant woodlands. The amount of trees (tree density and basal area) was positively associated with the accumulation of carbon in aboveground biomass and litter. However, changes to soil C were most strongly related to the productivity of the location (a forest productivity index and soil N content in the adjacent pasture). At 30 yr, native mixed-species plantings had increased the stability of soil C stocks, with higher amounts of recalcitrant C and higher C:N ratios than their adjacent pastures. Reforestation with native mixed-species plantings did not significantly change the availability of macronutrients (N, K, Ca, Mg, P and S) and micronutrients (Fe, B, Mn, Zn and Cu), content of plant toxins (Al, Si), acidity, or salinity (Na, electrical conductivity) in the soil. In this medium rainfall area, native mixed-species plantings provided comparable rates of C sequestration to local production species, with the probable additional benefit of providing better quality habitat for native biota. These results demonstrate that reforestation using native mixed-species plantings is an effective alternative for carbon sequestration to standard monocultures of production species in medium rainfall areas of temperate continental climates, where they can effectively store C, convert C into stable pools and provide greater benefits for biodiversity.

Introduction

The extent of forests around the world has declined by an estimated 16.4 million km^2 (36% of the historical extent) over the last 200 years (Meiyappan & Jain, 2012). This massive forest clearance has resulted in substantial emissions of carbon (C) to the atmosphere, reduced capacity for C storage (Houghton, 2003), and has led to rapid declines in biodiversity (Gaston *et al.*, 2003). Reforestation is the principal means for reversing the loss of native forests and is defined here as replanting trees in areas that were historically forested but that had been cleared for other land uses (IPCC, 2007). Net primary production of forests is estimated globally to be double that of improved pastures and croplands (Fig. 1, Lal, 2004, Pan *et al.*, 2011). Consequently, reforestation could provide an important tool for mitigating climate change in the short-term while fostering a low-carbon economy and improving environmental conditions in the coming decades and centuries (Mackey *et al.*, 2013).

Forests sequester more C than agricultural plants primarily because trees have substantially larger biomass (Fig. 1, Pregitzer & Euskirchen, 2004) and longer life spans (decades to centuries). Productivity of forests and hence C sequestration potential varies widely among climate zones and forest types (1-30 t C ha^{-1} yr^{-1}, Churkina & Running, 1998). Forest productivity increases positively with water availability, temperature (Churkina & Running, 1998) and nutrient availability (e.g. nitrogen (N) mineralization, Schimel *et al.*, 1996). For a given location, monocultures of production trees generally accumulate biomass faster than native tree species due to tree breeding and silviculture (Paquette & Messier, 2010), making monocultures desirable when rapid C sequestration is the goal. Productivity of plantations increases with tree density to a maximum stocking density beyond which overcrowding produces trees with smaller stem and crown diameters, and accelerates tree death from competition (West, 2013).

Similar amounts of carbon are stored in forest soils and forest biomass at the global scale (363 vs 383 Pg C, Pan *et al.*, 2011). However, soil generally provides more stable C storage than plant biomass, which is more susceptible to disturbances, and soil continues to accumulate C after forests mature, unlike plant biomass (Schulze *et al.*, 2000). Estimates of the change in soil C stocks after reforestation based on global data range widely (-10% to +26%), reflecting differences in sequestration among climate regions, soil types, tree species and previous land uses (Guo & Gifford, 2002, Laganière *et al.*, 2010, Paul *et al.*, 2002). Variability in soil C stocks in tree plantings is explained more by climate than former land use or stand age (Marin-Spiotta & Sharma, 2013). Regional meta-analyses show soil C stocks generally show little change until 30 yr after reforestation, particularly on former pastures (Barcena *et al.*, 2014, Hoogmoed *et al.*, 2012) but can increase by 20% within 50 yr (Laganière *et al.*, 2010). Previous land use is an important determinant of the potential for soil C sequestration following reforestation, with increases in stocks in early decades on former cropland (+18% to +26%) but predominantly losses on former pasture (Guo & Gifford, 2002, Laganière *et al.*, 2010). Mature forests have substantially larger soil C stocks than fields (Fig. 1, Lal, 2004) and natural regeneration of abandoned fields can sequester large amounts of soil C after a century (Poulton *et al.*, 2003), suggesting a large sequestration potential for reforestation.

Reforestation may change the molecular form of soil C and, consequently, increase the stability of the existing stock. Trees contain more lignin in their biomass (15%-40%) than do grasses (Novaes *et al.*, 2010), so reforestation of agricultural land will substantially increase woody inputs to the soil. The slow decomposition of woody inputs following reforestation partially explains the initial decrease of soil C on former pastures (Post & Kwon, 2000). Increases in soil C following reforestation can be substantial in the partly decayed material

(Berthrong *et al.*, 2012) whereas increases in the more stable humic material have rarely been shown (Del Galdo *et al.*, 2003). The C:N ratio of soils often increases within decades of reforestation (e.g. Cunningham *et al.*, 2012), which suggests decreased decomposition and increased stability of soil C.

Reforestation can substantially change nutrient cycling on agricultural land due to changes in rates of uptake, and quantity and quality of inputs to soils. Losses in soil N are expected after reforestation because concentrations are usually higher on agricultural land than forests (Garten & Ashwood, 2002) due to the addition of fertilizer. Base cations, such as calcium, potassium and magnesium, decrease (> 20%) in the soil after reforestation due to increased translocation to biomass, often resulting in the acidification of the surface soil (-0.3 pH units, Berthrong *et al.*, 2009). Similarly, extended use of saline ground water by trees can be detrimental because it accumulates salt in the biomass and surface soil (Jobbagy & Jackson, 2004).

Reforestation provides many ecological benefits beyond C sequestration, including more habitat and more ecological resources for native species, and improved water quality (Cunningham *et al.*, in review). Monocultures of production species have fast C sequestration rates but typically are colonized by plants and animals that are already abundant in agricultural landscapes (Felton *et al.*, 2010). Planting a range of native tree species within individual plantings and over a region will develop a diversity of forest structures that will increase the heterogeneity of resources and, therefore, the opportunities for a diverse range of native plants and animals to colonize tree plantings (Cunningham *et al.*, in review). Long-term (> 100 yr) plantings are necessary to develop many habitat structures (Vesk *et al.*, 2008). In particular, mixed-species plantings allow for more vertical differentiation of individuals due to differences in architecture and height growth rates, thereby accelerating the development of structural heterogeneity in the developing forest (Oliver and Larson 1996). Reforestation can reduce local runoff (Jackson *et al.*, 2005) but higher tree cover may increase water availability at larger scales (Ellison *et al.*, 2012) and improve water quality (Osborne & Kovacic, 1993).

The C sequestration potential of mixed-species plantings is relatively unknown compared with that of production plantations or native forests. Global meta-analyses of soil C sequestration following reforestation included only six different studies of mixed-species plantings (Silver *et al.*, 2000; Guo & Gifford, 2002; Paul *et al.*, 2002; Laganière *et al.*, 2010). Even meta-analyses deliberately focused on biomass accumulation in mixed-species plantings are dominated by studies of two-species plantings (Forrester *et al.*, 2006, Hulvey *et al.*, 2013, Piotto, 2008). Scenarios based on modelled C sequestration suggest that mixed-species plantings are an economically viable option in lower rainfall regions (< 1000 mm yr^{-1}, Crossman *et al.*, 2011, Polglase *et al.*, 2013). However ,there has been little effort to measure biomass accumulation extensively in mixed-species plantings (Paul *et al.*, 2013, Paul *et al.*, 2014) and even fewer have measured associated changes in soil C (Kasel *et al.*, 2011, Resh *et al.*, 2002).

Here, we present a regional assessment of the potential of reforestation to alter stocks among C pools (biomass, litter and soil) with native mixed-species plantings on agricultural land. We focused on the medium rainfall region (400-800 mm yr^{-1}) of temperate Australia because this land is expected to be a priority for reforestation in the coming decades (Polglase *et al.*, 2013) and comparable climate zones are found in continental areas of Europe, eastern United States, southern Africa and eastern China. A chronosequence (5 to 45 yr) of native mixed-species plantings that was dominated by eucalypts, and included paired measurements of adjacent pastures to account for differences in soil type and land-use history, was measured to determine:

1) Rates and magnitudes of C sequestration in native mixed-species plantings.
2) The site characteristics that are likely to increase C sequestration in native mixed-species plantings.
3) Changes in the stability of soil C stocks and nutrient availability following reforestation with native mixed-species plantings.

Materials and methods

Study area

Tree plantings were on grazing farms in northern Victorian, Australia (36.5 °S 146.0 °E). Prior to European settlement, the region was covered in woodlands (10-30 m tall, 10-30% projective foliage cover, Specht, 1981) dominated by *Eucalyptus* species with grassy understoreys. The region has been cleared extensively since European settlement in the 1840s for dryland agriculture, including cereal crops and pasture for stock. The climate in this region is temperate with seasonal changes in mean monthly maximum temperature (12.6–30.8 °C) and minimum temperature (2.9–16.5 °C), and a winter-dominant mean annual precipitation ranging from 570-715 mm yr^{-1} across the region (1971-2000, BOM, 2014).

Site selection

A total of 39 sites was selected, including 36 tree plantings (1-9 ha) and three remnant woodlands. Tree plantings established along streams were included to investigate potential differences in C sequestration between riparian plantings (*N* = 10 sites) and upland plantings (*N* = 26 sites), given access to additional water in riparian zones.The remnant woodlands were chosen to include species that were common (e.g. *Eucalyptus macrocarpa* (Maiden) Maiden) among the tree plantings of our survey and the region, and as a potential trajectory for plantings at maturity. Like the majority of remnant woodlands in the region, they were likely to have been cleared during the Gold Rush of the 1850s and 1860s, and were logged selectively until reservation in recent decades. The plantings covered the available range of ages (5 to 45 yr in 2010) for native mixed-species plantings on pasture in the region (Table S1). The plantings were established by ripping the soil into furrows, fencing out stock and hand planting tubestock seedlings into the furrows, with no subsequent management. The sites were planted with a mixture of 2–15 regionally endemic trees and shrubs from the genera *Acacia* Mill., *Allocasaurina* L.A.S. Johnson, *Callistemon* R. Br., *Eucalyptus* L'Hér and *Melaleuca* L. The soils at the plantings were predominantly sodosols, except for three of the riparian plantings that were on chromosols, and predominantly had a sandy loam to loamy texture (McKenzie *et al.*, 2000). The dominant eucalypts within the plantings are representative of the historically dominant tree species in the study region, with *Eucalyptus macrocarpa* Hook. in 21 of the upland plantings, its ecological and morphological equivalent *E. albens* Benth. in the 8 upland plantings in the northern part of the study area, and the floodplain species *E. camaldulensis* Dehnh. in the 10 riparian plantings.

Vegetation survey

An inventory of trees and shrubs within the plantings was collected over three months in the austral spring to early summer 2010. Vegetation was surveyed using three randomly placed plots of 900 m^2 at each planting. Plots were predominantly 30 × 30 m but smaller widths (10-15 m) had to be used in narrow plantings. Stem diameter was measured at breast height (1.3 m high) for trees and at the base of shrubs (10 cm high) due to the multi-stemmed form of most shrub species. The species and status (live/dead) of all trees and shrubs was determined. Trees were considered dead if they had no live leaves in their crown (Cunningham *et al.*, 2007). For each planting, total basal area, live basal area, and tree density were calculated from the basal area and status of trees. Aboveground and belowground biomass of each planting were estimated from the basal area measurements using robust species-specific allometrics developed from harvests of native mixed-species plantings in the region (Paul *et al.*, 2014, Paul *et al.*, 2013).

Soil C and plant litter survey

Soil surveys were conducted during the austral winter of 2010. Changes in soil C were estimated at each site of the chronosequence from a pair of plots (400 m^2) within the tree planting and in the adjacent pasture. The adjacent pastures, which continued to be grazed by stock and had fertilizer added, were sampled to determine differences in soil organic C between land uses, and to standardize for potential differences in soil characteristics and disturbance histories among the farms. Measurements in the pasture were used to indicate likely conditions at the reforestation plot if trees had not been planted and not as an estimate of conditions prior to establishment. The pasture plot was located ca 50 m from the planting to limit the influence of the trees, within the same field to minimise differences in previous land-use history and along the same contour to minimize differences in soil type.

Samples were collected with a hand auger (diameter 4.2 cm) at random points across the plot. Sampling avoided the rip lines where bulk density was substantially lower because of the soil disturbance. This was considered to be representative sampling because rip lines covered < 10% of the area of a planting. Given that tree roots often concentrate in the looser soil of rip lines (e.g. Falkiner *et al.*, 2006), we may have slightly under-estimated soil C stocks for the tree plantings. At each sampling point, plant litter was collected destructively within a 25 × 25 cm quadrat. Plant litter was defined as any dead biomass that was < 25 mm in diameter and that could be detached with little force by hand from the ground layer. After litter was removed, soils were sampled at the centre of the quadrat from upper (0–5 cm) or lower (5–30 cm) soil layers, with five independent samples collected for each depth. This sampling intensity provides a representative sample of soil C in this region; the probability of estimating within 10% of mean at this level of sampling intensity is ≥ 0.8 (see Cunningham *et al.*, 2012). Additional samples were taken from three of the sampling points for each depth to measure bulk density. These were collected by gently tapping a steel cylinder (96 cm^3) into the soil at the surface for upper soil samples and ca 20 cm depth for lower soil samples. All soil samples were placed into airtight plastic bags, immediately put on ice, and stored at 4 °C upon return to the laboratory.

Chemical analyses for C stocks

Gravimetric moisture was determined after drying ca 20 g subsample of moist soil at 105 ^{o}C for 48 h. The remainder of each sample was air dried, sieved to < 2 mm and roots ≥ 1 mm diameter were removed by manual dry picking for use in subsequent analyses for C and N content. Thoroughly mixed composite samples (20 g composed of ca 4 g from each soil sample) were used to measure pH for each site × land use × depth combination. Soil pH was measured using a conductivity meter (WP-81 meter, TDS, Australia) in a 1:5 soil-water suspension. Soils were acidic (pH = 4.5–6.1) indicating the absence of inorganic C and no need for other pre-processing of samples prior to CHN analysis (Slattery *et al.*, 1999). A 5 g subsample was taken from each sample, plant fragments were removed and the soil was ground to a fine powder. Concentrations of C and N in each subsample were determined from an accurately weighed subsample of 4–5 mg using catalytic combustion and thermal conductivity (vario MICRO cube, Elementar Analysensysteme GmbH, Hanau, Germany), with standards run after 40 samples. Bulk density samples were dried at 105 °C for 48 h and sieved to < 2 mm. Bulk density was calculated by dividing the oven-dried soil mass by the steel cylinder volume. Values of C and N concentration for each soil sample were converted to content (t ha^{-1}) using the mean bulk density from the appropriate site × land use × depth samples and the dimensions of the cores. Plant litter samples were air-dried for two weeks, oven-dried at 60 °C for 48 h and weighed.

Survey of soil C pools and nutrients

From a subset of ten sites (Table S1), more detailed measurements of C pools and nutrients to a depth of 50 cm were taken from different land uses in the austral autumn of 2012. Land-use types were pastures, 10-yr-old, 18-yr-old and 30-yr-old mixed-species plantings, and remnant woodlands, with two sites for each type. Changes in soil variables were estimated at each site from four randomly established plots (400 m^2) within the land-use type and the adjacent pasture. Within each of these sampling plots, five randomly located soil samples were collected from the 0-10 cm soil layer. The five 0-10 cm samples from each plot were bulked in the field and mixed thoroughly to create one soil sample per plot and four replicate samples per site. At the first sampling point in each plot, soil was sampled from the 10-25 cm and 25-50 cm layers. Samples from the three depths were then stored at 4 ^{o}C for transport back to the laboratory.

Chemical analyses for soil C pools and nutrients

Soil samples were sieved to < 2 mm, a subsample was taken to determine moisture content, and the remainder was air dried and used in subsequent chemical analyses, as described above. Concentration of total C and total N was determined from accurately weighed subsamples of 0.4 g using dry combustion (Trumac CNS Analyser, LECO, St Joseph, Michigan, USA) from all three soil layers. Labile C was determined by the amount of C oxidized by permanganate using the procedure of Blair *et al.* (1995). Recalcitrant C was calculated from the difference between total C and labile C.

From the surface (0-10 cm) soil samples, plant-available nutrients were determined colorimetrically using the following standard extractions (Rayment & Lyons, 2011). Available NH_4^+, NO_3^-, Al and S were extracted with 2M KCl, available P was extracted using the method of Colwell (1965), Ca, K and Mg were extracted using the method of Morgan (1941), Cu, Fe, Mn and Zn were extracted using diethylenetriaminepentaacetic acid (Lindsay & Norvell, 1978), B and Si using 0.01 M $CaCl_2$, and Na using 1.0M ammonium acetate. The amount of H was determined by total titratable acid while pH and conductivity were estimated using a conductivity meter (WP-81 meter, TDS, Australia) in a 1:5 soil-water suspension. Concentrations of nutrients were converted to contents (t ha^{-1}) in the surface soil using the bulk density calculated from the mass of < 2 mm soil in a core.

Planting characteristics

Characteristics of the plantings were estimated that were considered potential predictors of C stocks among the sites, which included climate variables, site productivity measures, planting dimensions, arrangement of trees and species mix (Table 1). Monthly climate data were obtained (Queensland Government, 2014) to estimate mean maximum temperature of the growing season (spring to early autumn, *MTGS lifetime*) and *mean rainfall over the lifetime* of the planting as potential indicators of growth rates. *Rainfall of planting year* was calculated from the autumn of the planting year to the following summer because plantings predominantly were established in spring, so this period included an estimate of soil moisture at planting and into the first summer, providing a potential indicator of early recruitment success. A measure of site productivity was provided by the *Forest Productivity Index*, which was predicted by the process model 3-PG based on climate variables, soil variables and leaf area index (Kesteven *et al.*, 2004). *Riparian* was included to test for potential differences in C sequestration between the tree plantings established along streams and the upland plantings. The *area, length and width* of the plantings, *tree density*, width between rows of trees (*row width*), and spacing between trees within a row (*row spacing*) were measured in the field and used as potential constraints on growth rates and, therefore, C sequestration. The proportion of total aboveground biomass made up of dead trees (*% dead biomass*) was calculated as an estimate of stress and mortality within a planting. The proportions of the basal area consisting of trees (*% trees*), of eucalypts (*% eucalypts*) and of acacias (*% acacia*) were used to determine how their dominance affected C sequestration. Similarly, the number of tree and shrub species (*species richness*), *tree species, Eucalyptus species* and *Acacia species* were included to investigate if species choices affected C sequestration. *Basal area* was used as a direct measure of the productivity of a tree planting for models of litter C and soil variables but not aboveground biomass C as this was calculated directly from basal area. Measures of the soil nutrients in the adjacent pasture (*pasture soil C, pasture soil N*) were included in models of soil variables within the tree planting as measures of the potential of a soil to sequester C, using values from the appropriate soil layer.

Statistical analyses

The response variables were C stocks in the aboveground biomass, litter and soil pools, and the availability of soil nutrients after reforestation. Analyses of aboveground biomass and litter C were based on masses from the tree plantings due to the negligible masses (< 0.5 t C ha^{-1}) of these components in the pastures. For the soil variables, the mean difference between forest and pasture at each site was calculated by subtracting the mean value for the adjacent pasture plot(s) from the individual sample values in the land-use change plot. For the pasture-pasture pairs (i.e. no reforestation) in the soil C pools survey, we randomly allocated one site to the pasture category and the other site to the land-use change category. These comparisons between adjacent pasture sites provided an estimate of the inherent spatial variation in soil variables.

Linear regression was used to determine the significance and rate of change in C stocks (aboveground biomass C, litter C and soil C in the various layers) with planting age. Boosted regression trees were used to explore if planting characteristics (Table 1) accounted for sources of residual variation not explained by planting age. Boosted regression trees are a type of machine learning that overcomes the inherent inaccuracies in seeking a single parsimonious model by constructing an ensemble of regression trees, which relate values of a response (leaves) to its predictors through a series of binary decisions or branches (Friedman, 1991). They are known to select relevant environmental variables and predictions are generally superior to general linear models (Elith *et al.*, 2006). Boosted regression trees were built using the ‘gbm’ package (Ridgeway, 2013) in R (R Development Core Team, 2010), with 10,000 trees built, a learning rate of 0.001, 50% of the data set selected randomly to build each tree, 75% of the selected data used for training and no interactions incorporated among variables. Results of boosted regression trees are presented by the relative influence of each variable on the prediction of the response variable, which is calculated from the number of times it is used in the ensemble of trees and the improvement in model fit. For those predictor variables with a high relative influence (>> 10%), partial dependence plots were built, which showed the effect of a variable on the response after accounting for the mean effect of all other variables in the model. Changes in soil C pools and plant available nutrients following land-use change were explored with one-way ANOVA. The categorical variable was land-use type: pasture, 10-yr-old, 18-yr-old and 30-yr-old mixed-species plantings and remnant woodland.

Results

Changes in carbon stocks following reforestation

There was a substantial amount of C sequestered in the aboveground biomass of the tree plantings, with 140.9 ± 17.4 t C ha^{-1} after 45 yr ($\bar{x}$ ± SD, Fig. 2a). The mean rate of C sequestration in the aboveground biomass was estimated to be 3.09 ± 0.85 t C ha^{-1} yr^{-1}. Within 20 yr, the aboveground biomass C of the tree plantings was similar to that of the remnant woodlands (53.3 ± 4.9 t C ha^{-1}). There was a similar linear increase in litter C with planting age (Fig. 2b), with an annual mean rate of 0.18 ± 0.05 t C ha^{-1} yr^{-1}. The tree plantings reached a comparable litter mass to the remnants (6.9 ± 1.0 t C ha^{-1}) within 36 yr and contained 9.3 ± 3.0 t C ha^{-1} at 45 yr.

Soil C did not show consistent changes over the 45 yr of the chronosequence, with no significant increase ($P < 0.05$) with planting age in either of the soil layers (0-5 cm and 5-30 cm). The general response of soil C across the soil profile (0-30 cm) after reforestation was substantial variation (increases and decreases relative to the adjacent pasture) for the first 20 yr followed by substantial increases at 45 yr (+13.7 ± 5.9 t C ha^{-1}, Fig. 2c). Although there were only two 45-yr-old plantings, this increase with age is supported by the substantially higher soil C in the remnant woodlands compared with their adjacent pastures (+36.6 ± 20.1 t C ha^{-1}). There was a significant increase in the C:N ratio of the surface (0-5 cm) soil with planting age, which was consistent with the higher C:N ratio of the remnant woodlands compared with their adjacent pastures (Fig. 2d).

Site characteristics associated with increased C sequestration

Other characteristics of the tree plantings explained some of the variation in measures of C sequestration (Table 1). Total biomass C was positively associated with the density of trees (Fig. 3b), with a predicted mean increase of 20 t C ha^{-1} from 600 to 1000 trees ha^{-1}. Litter C was influenced positively by the basal area of a planting (Fig. 3d), with a predicted increase of 1 t C ha^{-1} with an increase in basal area from 10 to 40 m^2 ha^{-1}. The increase in soil C (0-30 cm) following reforestation was most strongly influenced by the total N content of the adjacent pasture soil and to a lesser extent by the Forest Productivity Index (Fig. 3e, f). A soil N content of ca 4 t ha^{-1} was predicted to be a threshold for C sequestration in soil following reforestation, with increases more likely below this value and decreases above (Fig. 3e). Sites with higher values of the Forest Productivity Index were predicted to be more likely to have increases in soil C (Fig. 3f). The C:N ratio of soil was more likely to increase in tree plantings with a higher basal area. There was little association between measures of C sequestration and climate variables, planting dimensions, arrangement of trees, species mix or being located in a riparian zone (Table 1).

Changes in soil C pools and nutrient availability following reforestation

Changes in soil C following reforestation were found to be significantly different ($P < 0.05$) among the pastures, tree plantings and remnant woodlands in the surface soil (0-10 cm, Fig. 4), but not in the deeper 10-25 cm and 25-50 cm soil layers (Tables 2, S2). The remnant woodlands had higher total C and C:N ratio in the surface soil relative to the adjacent pastures. The 30-yr-old plantings showed similar increases, but there was little evidence of changes in soil C in the 10 and 18-yr-old plantings and pastures (Fig. 4a, d). The same patterns were found for labile and recalcitrant C, with remnant woodlands having significantly higher contents than their adjacent pastures and 30-yr-old plantings having similar increases (Fig. 4b, c). All land uses (tree planting, remnant woodland and pasture) had similar differences to their adjacent pasture in the availability of macronutrients (N, K, Ca, Mg, P and S) and micronutrients (Fe, B, Mn, Zn and Cu), content of plant toxins (Al, Si), acidity (pH, H) and salinity (conductivity) to that of the paired pastures (Tables 2, S2).

Discussion

Reforestation of agricultural pastures with native mixed-species plantings led to substantial increases in the amount of C stored in the plant biomass, litter and soil in as little as three decades. Carbon stored in the biomass and litter of the tree plantings reached amounts equivalent to remnant woodlands within the region after 20 yr and 36 yr, respectively (Fig. 2). Soil C was slower to accumulate with increases relative to adjacent pastures observed only after 45 yr (Fig. 2). At 30 yr, the mixed-species plantings had increased the stability of the soil C store, with higher amounts of recalcitrant C and higher C:N ratios than in the adjacent pasture (Fig. 4). There was little evidence for associated changes in the availability of other soil nutrients after reforestation with native mixed-species plantings (Table 2). Our survey suggested that rates of C sequestration in biomass and the litter layer following reforestation may be increased by planting trees at relatively high densities (up to 1,000 trees ha^{-1}), while the rate of soil C sequestration may be increased by planting on more productive locations or on pastures with lower levels of N (Fig. 3).

Rates and magnitudes of C sequestration in mixed-species plantings

The aboveground biomass accumulation of 140.9 ± 17.4 t C ha^{-1} after 45 yr of reforestation (Fig. 2a) approaches that of temperate forests > 200 yr old (Pregitzer & Euskirchen, 2004). Temperate continental native forests and plantations in Asia are estimated to store an average of 120 and 200 t C ha^{-1}, respectively (IPCC, 2006), although substantially larger amounts have been estimated for forests of southeastern Australia (Keith *et al.*, 2010, Kilinc *et al.*, 2013). Carbon sequestration in the native mixed-species plantings was comparable to the regionally important plantation species *Eucalyptus cladocalyx* F. Muell., which can accumulate 117-129 t C ha^{-1} after 32-45 yr (Paul *et al.*, 2008). The annual sequestration rate calculated from our chronosequence (3.1 ± 0.9 t C ha^{-1} yr^{-1}, Fig. 2a) was comparable to that of young (< 10 yr) plantations of the widespread production species *Eucalyptus globulus* Labill. when grown in areas with a similar rainfall (3.8 t C ha^{-1} yr^{-1}, Miehle *et al.*, 2009). These biomass accumulation rates found in short-rotation plantations of production species are likely to decline if plantations are allowed to mature (ca 80 yr, Ryan *et al.*, 1997). Native mixed-species plantings appear to be comparable to monoculture plantations for sequestering carbon in medium rainfall zones such as ours (400-800 mm yr^{-1}).

Within 20 yr, the mixed-species plantings reached similar amounts of aboveground biomass C to the remnant woodlands (53.3 ± 4.9 t C ha^{-1}). Past selective logging of these woodlands has kept them developmentally restricted, with their current structure dominated by small trees (median dbh = 15 ± 2 cm) with few large trees (maximum dbh = 63 ± 18 cm, 3 large trees (dbh > 50 cm) ha^{-1}) whereas remnant trees of up to 2 m diameter occur in fields and roadsides (unpublished observations). In contrast, the 45-year-old plantings were dominated by smaller trees (median dbh = 14 ± 6 cm) but had substantially more large trees (44 ± 10 large trees (dbh > 50 cm) ha^{-1}). Therefore, remnant woodlands of the region have the capacity to accumulate substantially more biomass C (at least 120 t C ha^{-1}) as they are released from this form of unsustainable harvesting.

Substantial amounts of C were stored in the litter layer beneath the mixed-species plantings, with 9.3 ± 3.0 t C ha^{-1} after 45 yr (Fig. 2). This mass was larger than that measured in the remnant woodlands (2.9 ± 0.9 t C ha^{-1}) but again this reflects the degraded state of the latter. A foothill forest dominated by the same eucalypt species and in the same region in which our study was conducted contained 46% more litter mass (13.6 ± 1.5 t C ha^{-1}, Adams & Attiwill, 1986). These observations suggest that the litter layer of both the 45-yr-old plantings and remnant woodlands have the potential to accumulate more litter in the coming decades before reaching equilibrium. In contrast, regional plantations of *E. cladocalyx* only attained litter masses of 3.7 and 4.5 t C ha^{-1} at stands ages of 48 and 75 yr, respectively (Paul *et al.*, 2008).

In comparison to biomass and litter, which are more susceptible to disturbance (e.g. fire), soil generally provides a more stable C store and continues to accumulate C after forests reach maturity (Schulze *et al.*, 2000). We found that reforestation with native mixed-species plantings on former pastures produced highly variable changes in soil C in the first three decades (Fig. 2c). Early increases in soil C may have been masked by differences in soil C content before planting and subsequent changes to contents in the adjacent pasture after planting. Ideally but rarely done, changes in soil C should be assessed by repeated surveys of the same sites before and for a long time after reforestation (Poulton *et al.*, 2003). Although only two mixed-species plantings > 33 yr were available for measurement, a consistent increase of 13.7 ± 4.9 t C ha^{-1} occurred at 45 yr, which was supported by the higher soil C content of the remnant woodlands to their adjacent pastures (Fig. 2c). Furthermore, the remnants are likely to be < 160 yr due to past widespread clearance and were selectively logged until recently, so they may have a potential to accumulate more soil C (Schulze *et al.*, 2000). Production plantations in the region are harvested predominantly before 45 yr, with no significant increase in soil C content after reforestation with *E. globulus* after 10 yr (Mendham *et al.*, 2003) or *E. cladocalyx* after 26 yr (Harper *et al.*, 2012). Harvesting of plantations leads to removal of biomass and losses of soil C, with losses from soil unlikely to recover during short rotations (Nave *et al.*, 2010) and, therefore, leading to substantially less C sequestration than more permanent plantings.

Meta-analyses have reported results that are consistent with our survey. A global meta-analysis of reforestation showed that soil C concentration increased only after 30 yr (Paul *et al.*, 2002). A review of reforestation, including predominantly data for *Eucalyptus* species in Australia, found little evidence for an increase in soil C concentration within 30 yr (Hoogmoed *et al.*, 2012). A meta-analysis dominated by studies of young plantings (< 30 yr) of conifers in New Zealand found a 10% decrease in soil C content after reforestation of pasture (Guo & Gifford, 2002). No significant increase in soil C content after reforestation of pasture was recorded in a broader analysis of tree species and stand ages (Laganière *et al.*, 2010). The largest proportional increases in soil C after reforestation of pasture generally are found in lower rainfall areas (Berthrong *et al.*, 2012, Guo & Gifford, 2002). Our results and the above findings suggest that increases in soil C following reforestation of pastures are more likely after 30 yr in medium rainfall zones (400-800 mm yr^{-1}).

Stability of soil C and nutrient availability following reforestation

Reforestation with native mixed-species planting increased the stability of soil C stocks after 30 yr, with higher amounts of recalcitrant C and higher C:N ratios than the adjacent pasture soil (Fig. 3). There was increased labile and recalcitrant C after 30 yr compared with the adjacent pasture in our chronosequence, a result corroborated by the significantly higher stocks in the remnant woodlands compared to their adjacent pastures (Fig. 4). Increases in soil C following reforestation can be substantial in the 'light' fraction of partly decayed material (Berthrong *et al.*, 2012), whereas increases in the more recalcitrant 'heavy' fraction of humic material are uncommon. Increased amounts of recalcitrant soil C have only been shown in a 20-yr-old deciduous *Quercus-Tilia* planting (Del Galdo *et al.*, 2003) but not in younger deciduous *Poplar* (De Gryze *et al.*, 2004) or evergreen *Eucalyptus-Acacia* plantings (Kasel *et al.*, 2011). Taken with our survey, these limited data suggest that recalcitrant C takes at least two decades to increase following reforestation. The C:N ratio of soils often increases after reforestation at a similar rate to recalcitrant C (e.g. Cunningham *et al.*, 2012, De Gryze *et al.*, 2004), which suggests reduced decomposition and increased stability of soil C. The substantially higher amounts of recalcitrant C and higher C:N ratios in forests than on agricultural lands (De Gryze *et al.*, 2004, Murty *et al.*, 2002) support the future potential of these developing plantings to increase and stabilize the soil C stock. Physical protection of soil C may be as important as its molecular form (Dungait *et al.*, 2012) and reforestation can improve aggregate stability within 20 yr (Saha *et al.*, 2007).

There were no significant changes in nutrient availability following the early development (10-30 yr) of tree plantings on former pastures (Table 2). Decreases in soil N are expected due to uptake by trees, shrubs and soil microbes (Berthrong *et al.*, 2009) and the cessation of agricultural inputs. Pastures of the region generally were unimproved, as were our surveyed farms, with no fertilizer additions or N-fixing legumes and consequently had relatively low soil N (3.3 ± 1.9 t ha^{-1}), making large decreases following reforestation less likely. Reforestation can result in substantial translocation of base cations (Ca, K and Mg) from the soil to biomass and salt (Na) from ground water to surface soils, resulting in soil acidification and salinization, respectively (Berthrong *et al.*, 2009). No such effects were found after 30 yr of reforestation with native mixed-species plantings (Table 2). Our farms were deliberately chosen to not have shallow, saline water tables. A lack of soil acidification in our survey was consistent with significant acidification following reforestation with *Eucalyptus* only occurring in higher rainfall areas (1100-4600 mm yr^{-1}, Berthrong *et al.*, 2009) and there being little difference in acidity between pastures and forests of the region (Prosser *et al.*, 1993).

Establishment choices to increase C sequestration

Growth and hence C sequestration are influenced by choices made during the establishment of tree plantings. First, where trees are planted (climate and nutrients) will determine the rate of C sequestration. On a global scale, productivity of forests tends to increase with water availability, temperature (Churkina & Running, 1998) and nutrient availability (e.g. N mineralization, Schimel *et al.*, 1996), with higher C sequestration in warmer, wetter and more nutrient-rich environments (Laganière *et al.*, 2010, Paul *et al.*, 2002). We found no association between biomass accumulation and climate variables (Table 1), which was not unexpected given the small differences in climate among farms (annual maximum temperature = 20.2-22.3 °C, annual precipitation = 570-715 mm yr^{-1}). Soil C sequestration was negatively associated with soil N in the adjacent pastures (Fig. 2e), suggesting that reforestation of more productive pasture leads to a loss in soil C in the first 30 yr following reforestation. More productive pastures are likely to have high initial soil C, potentially increasing the losses in soil C observed in early decades after reforestation (Paul *et al.*, 2002). The capacity of the land to grow trees, estimated by the Forest Productivity Index (Kesteven *et al.*, 2004), was related positively to increases in soil C following reforestation (Fig. 2f), supporting the silvicultural approach of planting trees on productive land to maximize biomass accumulation (West, 2013). Habitat features associated with large trees often are rare in agricultural regions, so reforestation on the most productive sites may accelerate development. Planting in the riparian zone, where water availability is higher, had negligible influence on C sequestration in native mixed-species plantings (Table 1). Although riparian plantings increase C stocks (Smith *et al.*, 2012), our survey suggested that the rates of sequestration were no faster than upland plantings.

The choice of species in tree plantings is important in C sequestration. Monocultures of production trees generally accumulate biomass faster than native tree species due to tree breeding and silviculture (Paquette & Messier, 2010). Some sets of tree species will accumulate similar amounts or more biomass when planted as a mixture than when planted in monocultures (Hulvey *et al.*, 2013). In general, the largest increases in soil C are found after reforestation with broadleaf species (27%), with intermediate responses under eucalypts (12%) and little change under conifers (2%, Laganière *et al.*, 2010). Plantings that include nitrogen-fixing tree species can have higher productivity (Forrester *et al.*, 2006) and higher retention of original soil C stocks (Resh *et al.*, 2002). For example, higher soil C content occurs when the N-fixing tree *Acacia mearnsii* De Wild. is included in plantations of *E. globulus* (Forrester *et al.*, 2013) and in native mixed-species plantings (Kasel *et al.*, 2011). However, the use of N-fixing trees does not necessarily ensure increased soil C sequestration because the effect depends on species and location (Hoogmoed *et al.*, 2014).

The lack of a species effect on C sequestration in our chronosequence (i.e. richness or abundance of taxa) suggests an inherent capacity of these medium rainfall (400-800 mm yr^{-1}) locations to sequester C. This is consistent with the theory that the optimal leaf area or basal area is determined by the water availability of an area (Hatton *et al.*, 1997). Consequently, although production species may accumulate aboveground biomass faster, with an associated higher water use, than native mixed-species planting, C sequestration in the long-term will be similar, with water stress moderating the productivity of plantation species. Furthermore, native mixed-species plantings are likely to be more self-sustaining in the coming decades and centuries than production species because of the long-term adaptation of the component species to the regional climate and disturbances (e.g. fire, drought, herbivory). Some *Acacia* species in the plantings (e.g. *Acacia dealbata*) recruited prolifically from root suckers during a recent extended drought (1997-2010). Developing (< 20 yr) native mixed-species plantings in a nearby region showed rapid recovery of structure and cover, with no loss of species, within 5 yr of fire (Pickup *et al.*, 2013).

Planting configuration is an important determinant of the resultant forest structure and C accumulation. It is well-established that increasing tree density leads to higher biomass production but overcrowding produces trees with smaller stem and crown diameters, and accelerates tree death due to competition (West, 2013). We found increases in biomass C with increases of density up to 1,000 tree ha^{-1} (Fig. 2b), which is the density that increases growth rates and survival of trees during drought periods in nearby regions (Horner *et al.*, 2009). A density of 1,000 tree ha^{-1} may be desirable for C sequestration and other benefits such as reduced soil erosion and improved water quality but high densities can substantially reduce runoff and provide poorer habitat for native plants and animals (Cunningham *et al.*, in review). Controlling for planting age, a higher basal area increased litter C and the C:N ratio of the soil (Table 1), which is consistent with litterfall, litter inputs to soil and uptake of N increasing with biomass (Lonsdale, 1988).

Conclusions

Policy responses to global changes in climate increasingly favour the planting of trees to sequester and store atmospheric carbon. There is the potential for considerable biodiversity benefits from reforestation when native mixed-species plantings are used. However, there has been relatively little study of the capacity of these kinds of plantings to sequester carbon, and limited understanding of the time scales and environmental variables that may influence rates of sequestration. Our survey of an extended chronosequence of reforested pastures (5-45 yr) suggests that reforestation with native mixed-species plantings is an effective alternative for sequestering carbon to plantations of production species in medium rainfall (400-800 mm yr^{-1}) regions. Carbon sequestration after 45 yr (biomass = + 141 t C ha^{-1}, litter = + 9 t C ha^{-1}, soil = + 14 t C ha^{-1}) was comparable to the production species planted in the region. These mixed-species plantings showed signs of increased stability of soil C stocks within 30 yr and no negative effects on nutrient availability, acidity or salinity in the soil. Carbon sequestration in future plantings of native mixed-species is likely to be increased by planting at moderate densities (1,000 tree ha^{-1}), resulting in higher basal areas. The largest gains in soil C after reforestation with native mixed-species plantings are likely to be on more degraded pastures in more productive regions (based on climate and soil type). In medium rainfall regions of temperate continental climates, native mixed-species plantings present an effective alternative for carbon sequestration to monocultures of production species due to their additional biodiversity benefits, including developing a variety of habitat structures that promote colonization by native plants and animals, to the expected changes to C and water cycling when trees are planted.

Acknowledgements

This research was funded by the Australian Research Council Linkage Program (LP0990038), Victorian Department of Sustainability and Environment Victoria, Goulburn-Broken Catchment Management Authority (CMA), Victorian EPA, Kilter Pty. Ltd and the North Central CMA. Jason Beringer, Ross Thompson, Tim Cavagnaro and Patrick Baker were supported in part by ARC Future Fellowships (FT110100602, FT110100957, FT120100463 and FT120100715, respectively). Ralph Mac Nally was supported in part by an ARC DORA Fellowship (DP120100797). We thank the landholders for providing access to their farms. Big thanks to Scott Mc Donald, Kristen Metzeling and Sarah Fitzpatrick for their assistance and good cheer in the field and laboratory.

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Table 1 Relative influence of planting variables in boosted regression trees for total biomass C, litter C, and the change (Δ, planting – pasture) in soil C and C:N. Variables with substantial influence (» 10%) are indicated in bold. Nutrient in the adjacent pasture were only included in trees for soil C and C:N. Plantings variables that were known to have direct relationships with the response variable were excluded (e.g. basal area was used to calculate biomass C).

	Relative influence in tree			
Variable	biomass C	Litter C	Δ soil C (0-30 cm)	Δ C:N (0-5 cm)
Planting age (yr)	**47.56**	**38.52**	6.68	**18.28**
Mean temp. growing season over	7.09	2.05	1.80	4.34
Mean rainfall over lifetime (mm yr^{-1})	5.93	7.95	2.72	5.37
Rainfall planting year (mm)	2.08	1.04	2.11	6.14
Forest Productivity Index	4.81	1.86	**13.30**	0.85
Potential available water top 1 m	0.95	1.95	0.74	4.87
Riparian	0.09	0.00	0.34	0.05
Pasture soil N, 0-30 cm (t ha^{-1})			**24.79**	
Pasture soil C, 0-30 cm (t ha^{-1})			8.54	
Pasture soil N, 0-5 cm (t ha^{-1})				2.63
Pasture soil C, 0-5 cm (t ha^{-1})				1.84
Litter mass (t ha^{-1})			1.35	4.59
Planting area (ha)	0.75	2.00	1.49	1.57
Planting length (m)	4.25	0.38	3.88	1.08
Planting width (m)	3.14	1.77	3.36	1.17
Basal area (m^2 ha^{-1})		**19.02**	0.90	**20.27**
Density (trees ha^{-1})	**9.60**	1.89	1.44	1.12
Row width (m)	1.62	4.80	3.14	5.83
Row spacing (m)	1.01	1.61	7.17	0.53
% dead biomass	2.00	5.16	3.26	2.81
% trees	2.72	1.91	2.08	0.14
% *Eucalyptus*	2.80	1.08	1.10	5.19
% *Acacia*	1.50	0.00	2.13	0.53
Species richness	0.65	0.86	0.40	1.89
No. of tree species	0.71	4.48	0.95	2.92
No. of *Eucalyptus* species	0.41	1.67	1.62	5.79
No. of *Acacia* species	0.33	0.00	4.72	0.20

Table 2 Results of one-way ANOVAs comparing changes (land-use – adjacent pasture) in soil variables among different land uses (pasture, 10-yr-old plantings, 18-yr-old plantings, 30-yr-old plantings and remnant woodlands. $N = 2$ sites per land use and d.f. = 4. Significant differences among land uses are indicated in bold.

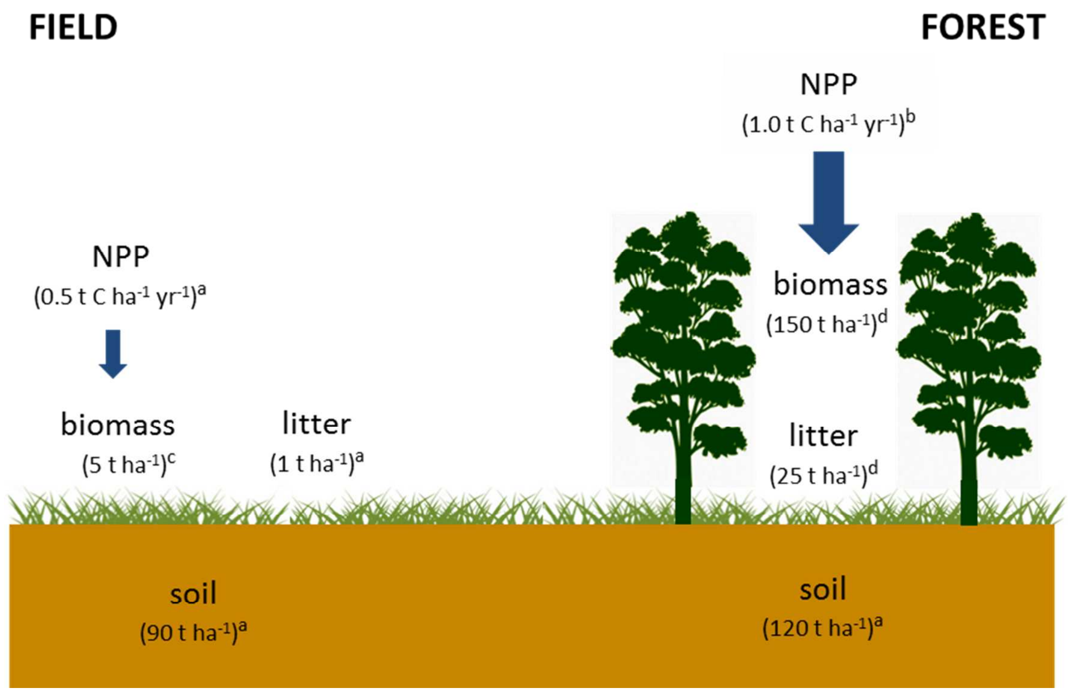
Variable	*F*	*P*
Surface soil (0-10 cm)		
Total C	8.49	**0.02**
Labile C	7.65	**0.02**
Recalcitrant C	7.99	**0.02**
Total N	3.91	0.08
C:N	54.98	< **0.01**
NH_4^+	0.29	0.88
NO_3^-	0.20	0.93
K	1.13	0.44
Ca	0.52	0.73
Mg	0.62	0.67
P	0.57	0.70
S	2.65	0.16
Fe	2.76	0.15
B	1.38	0.36
Mn	0.88	0.54
Zn	0.90	0.53
Cu	0.82	0.56
Na	1.09	0.45
Si	3.63	0.09
H	0.53	0.72
Al	0.74	0.60
Conductivity	1.34	0.37
pH	0.14	0.96
Total soil profile (0-50 cm)		
Total C	1.81	0.26
Labile C	1.11	0.44
Recalcitrant C	1.87	0.25
Total N	0.34	0.85
C:N	1.76	0.27

Fig. 1 Schematic of carbon stocks in fields and mature forests illustrating the potential for carbon sequestration after reforestation of agricultural land. NPP = net primary production. Estimates for stocks and NPP are given in brackets and were calculated from the following sources: [a] Lal, 2004; [b] Pan *et al.,* 2011; [c] Bondeau *et al.* 2007; [d] Pregitzer and Euskirchen, 2004.

Fig. 2 Relationship between planting age and a) aboveground (AG) biomass C, b) litter C, c) change (Δ, land-use – adjacent pasture) in soil C content and d) change in C:N ratio across the chronosequence (N = 36 plantings). Values for the three remnant woodlands (REM, °) are included for comparison. Solid lines are linear regressions with the 95% confidence intervals indicated by dashed lines. For soil variables, the horizontal dashed line indicates the boundary between decreases and increases relative to the adjacent pasture.

Fig. 3 Planting variables with a high relative influence (>> 10%) on the boosted regression tree for biomass C (a, c), litter C (b, d), change (Δ, planting – pasture) in soil C (0-30 cm, e,f) and change in C:N (0-5 cm, g,h). N = 36 plantings.

Fig. 4 Changes (Δ, land-use – adjacent pasture) in characteristics of the surface soil (0-10 cm) including a) total C, b) labile C, c) recalcitrant C and d) C:N ratio. Land uses include pastures (PAS), 10-year-old plantings (P10), 18-year-old plantings (P18), 30-year-old plantings (P30) and remnant woodlands (REM). Values are means of 2 sites with standard errors, and results of one-way ANOVAs are provided. The dashed line indicates the boundary between decreases and increases relative to the adjacent pasture.



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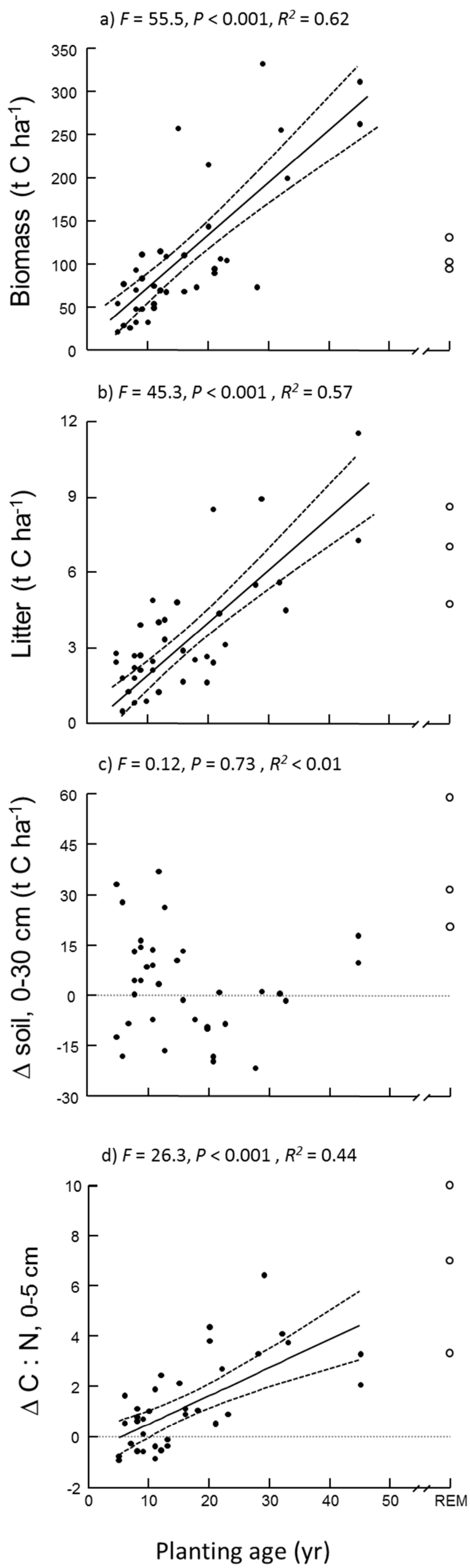

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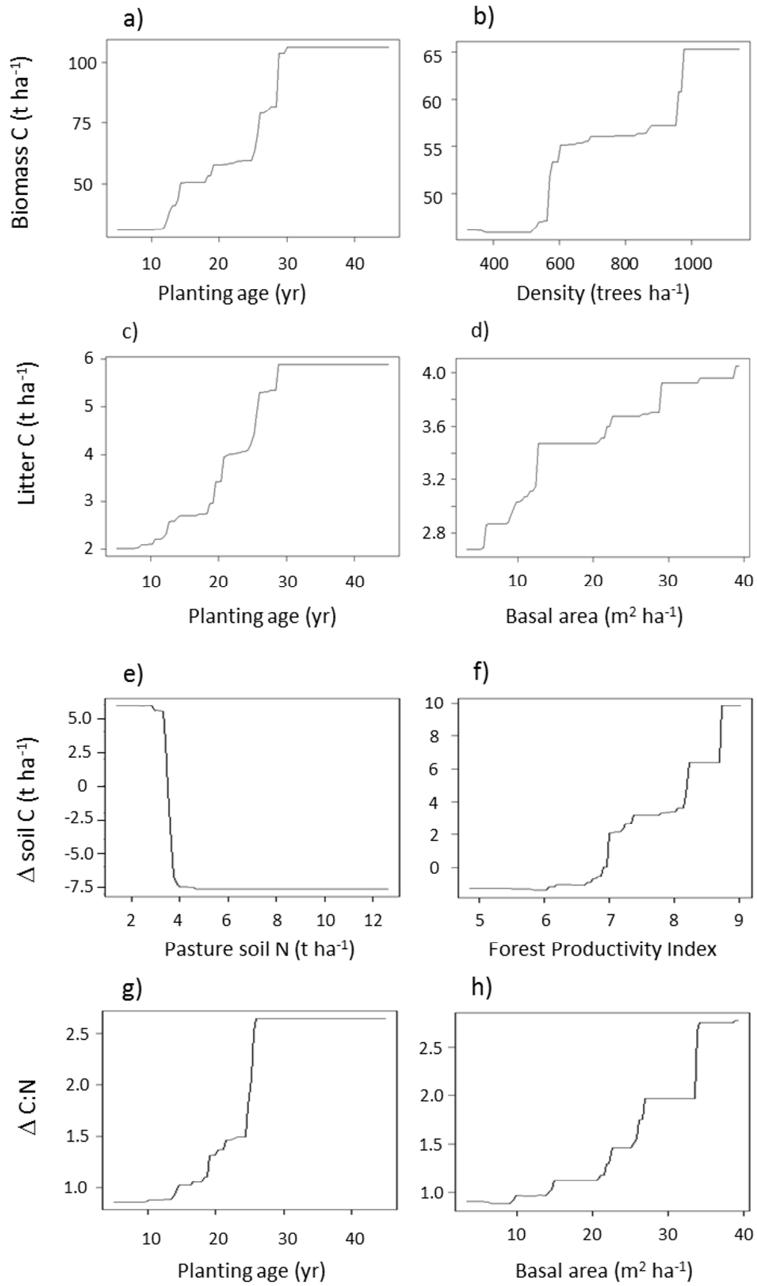
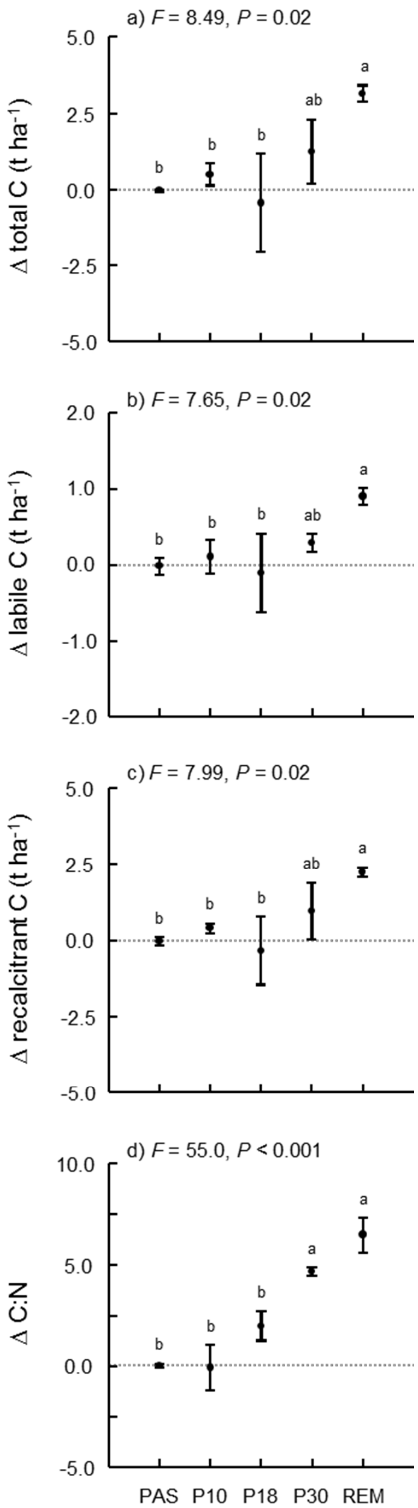


Fig. 3 Planting variables with a high relative influence (>> 10%) on the boosted regression trees for biomass C (a, b), litter C (c, d), change (Δ, planting – pasture) in soil C (0-30 cm, e, f) and change in C:N (0-5 cm, g, h). N = 36 plantings. These are partial dependence plots, which show the effect of a planting variable on a C pool after accounting for the mean effect of all other variables in the ensemble tree.



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