Soil carbon stocks in a Sitka spruce chronosequence following afforestation

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Abstract
Increasing concentrations of CO\textsubscript{2} and other greenhouse gases in the atmosphere are leading to concern worldwide due to their contribution to the greenhouse effect. As the body of evidence supporting the need for change from a carbon rich economy/society becomes stronger, international mitigation agreements require high quality and precise information. Following the Kyoto Protocol and EU agreements to reduce carbon production, countries could utilise default values or comparable international data to calculate their carbon budgets. Initially, approximations were successful for generating a guide to a national carbon stock for reporting GHG inventories to the UNFCCC (Tier 1). However, now that the second phase of the Kyoto protocol is running until 2020, greater accuracy is essential and, where possible, nationally specific information is increasingly required (Tier 3, UNFCCC). Forestry and forest soils are seen as a key component in the carbon cycle and depending on their management, can mitigate or contribute to GHG emissions. Litter and soil organic matter (SOM) are two of the major carbon pools required for reporting under LULUCF. In this study, stocks of SOM and litter were recorded along a chronosequence of Sitka spruce (\textit{Picea sitchensis} (Bong.) Carr.) on wet mineral gley soil. Over a 47-year period, the rate of soil carbon sequestration was found to be 1.83 t C ha\textsuperscript{-1} yr\textsuperscript{-1}. Soil microbial biomass was used to estimate highly active SOM. The mineral soils were also fractionated in a density separation procedure to identify light and heavy SOM pools. These estimates can now be used to model carbon budgets of this common soil type currently under forestry in Ireland.

Keywords: Carbon, forest, soil microbial biomass, density fractionation.

Introduction
Globally, the store of carbon (C) in soils is 3.5 times that of the atmosphere and almost five times that of the biosphere (Lal 2008) and, in forests, over two-thirds of the C is contained in soils and associated peat deposits (Dixon et al. 1994). The amount of C stored in the soil is the balance between inputs of organic material from the biota, which depends on the type of vegetation and its productivity at a particular site, and losses, primarily through soil microbial respiration (Zerva et al. 2005). Forests continuously recycle C through photosynthesis, respiration and mineralisation.

However, the net sequestration of C in vegetation, and especially in soil, can range over time periods from years to centuries. Forest soil C stock is controlled by factors such as previous land use (grasslands, cropland etc), tree species, soil cultivation method, soil properties (clay content), stand age, site management,
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topography and climatic zone. Estimates of its size are affected by the methodological approaches used in its estimation (Jandl et al. 2007, Laganière et al. 2010). The residence time of stable fractions of SOC (soil organic carbon) can be >1000 years (von Lutzow et al. 2006) making it a much more stable sink than living plant biomass (Laganière et al. 2010).

Forest soils represent a major C store with stocks exceeding those under most other land-uses, so the stability of this store is of obvious importance to climate change mitigation. Forest management practices that disturb the soil can lead to C loss while climate change can have both positive and negative impacts. Global warming and rising CO2 levels in the atmosphere can enhance forest growth, which in turn could increase soil organic matter through greater litter input. Conversely, increasing soil temperatures are predicted to promote microbial activity and therefore decomposition and loss of soil organic matter (Coûteaux et al. 2001).

Within the United Nations Framework Convention on Climate Change (UNFCCC 1992) each signatory nation must report its C budgets via the greenhouse gas (GHG) inventory. The rules of land-use change and forestry (LULUCF) are particularly relevant to this topic. Initially, Tier 1 approximations (relatively low accuracy) were successful for generating a guide to a country’s C stock for reporting. Now that the second phase of the Kyoto protocol (Kyoto Protocol, 2012) is running until 2020, greater accuracy is required in calculations and, where possible, Tier 3 level information is required. Article 3.3 of the Kyoto Protocol allows changes in C stocks due to afforestation, reforestation and deforestation since 1990 to be used to offset inventory emissions. With the recent increase in afforestation in Ireland, this sector has the potential to offset some of the national GHG emissions.

To understand the dynamics of soil organic matter (SOM), the amounts and the chemical nature of the inputs into the system need to be quantified. The mass, the N and C and lignin content of the litter are measured. These measurements are known to indicate the decomposability of the litter (Berg et al. 1993) and the quality of the SOM produced from this litter (Berg and Ekbohm 1991, Lavelle et al. 1993).

Soil microbial biomass can be a limiting factor in the nutrient cycle, as the vast majority of organic matter (~95% in conifer forest) must pass through microbial tissue in the process of decomposition and mineralisation (Huhta and Koskenniemi 1975, Jenkinson 1977, Petersen and Luxton 1982). High microbial activity indicates balanced nutrient availability and a more readily decomposable litter source (Vance and Chapin 2001). The potential activity of the SOM can be estimated by initially calculating the soil microbial biomass at a site. Therefore, measurement of the C and nutrients contained in the microbial biomass provides a basis for studies of the formation and turnover of soil organic matter, as the microbial biomass is one of the key definable fractions (Ocio and Brookes 1990, Ocio et al. 1991).

SOM is often divided into active, slow and passive pools (Parton et al. 1987) and quantifying the relative proportions of these provides an indication of the lability and stability of the soil C pool. A rough approximation to the distribution of the organic matter into these pools can be achieved through density fractionation (Christensen 1987a,b, 1992). The light fraction contains a considerable part of the
active pool; it contains plant debris, spores, seeds, animal remains and mineral particles adhering to organic fragments. It has high microbial activity and high rates of turnover (Gregorich and Ellert 1993). The rapid mineralisation of the soil's light fraction can be related to the soil microbial biomass; therefore, it is a good estimation of the active fraction of the soil (Janzén et al. 1992). The heavy fraction is considered to be organomineral-complexed SOM which is taken to be comparatively more processed decomposition products with a narrow C:N ratio and a slower turnover rate (Christensen 1992).

Isotopic tracers have been used to show that the light fraction has two or more C pools with differing turnover rates (active and slow). The light fraction is however, sensitive to changes in the active organic matter pool, indicating its substantial presence (Bonde et al. 1992). Thus the light fraction is seen as a transitory SOM pool dominated by decomposing plant and animal residues with a relatively high C:N ratio. It has a rapid turnover containing a large proportion of microbes.

The objectives of this study were: (1) to quantify the change in soil C stock during the life cycle of a Sitka spruce (Picea sitchensis (Bong.) Carr.) forest crop; (2) measure litter quantity and quality; (3) estimate microbial biomass; (4) estimate soil light and heavy fractions; and (5) assess the impacts of afforestation on soil C stocks using the chronosequence method. The results from this study were compared with values from the literature to determine their usefulness in generating predictions in the Irish scenario since unfortunately there are large knowledge gaps in relation to Irish forests.

Materials and Methods
The estimation of the rate of C sequestration in Irish forest soils was carried out using the chronosequence technique (Schlesinger 1990). The C content in the soil under stands of different age was measured and an average soil C increment calculated per year. A chronosequence can be seen as a space-for-time substitute enabling estimation of successional sequestration - that is substituting similar soils/site conditions (space) to obtain a chronosequence of different ages (time) (Allison et al. 2005). In selecting the location of the chronosequence, efforts were made to ensure the sites were as similar as possible, i.e. stands growing under similar environmental and management conditions, with similar soil type, topography, exposure and drainage.

Site description
The main study was located at Dooary Forest, Timahoe, Co. Laois in the Irish midlands, 52° 57’ 00” N, 7° 15’ 00” W. The 30-year mean annual temperature for this area is 9.3°C with a mean rainfall of 850 mm and an elevation of ~260 m. The site was previously unmanaged grassland but was planted largely with single species stands of Sitka spruce at a density of ca. 2,500 stem ha⁻¹.

Within the forested area, four stands were chosen to represent a chronosequence (a reconstructed historical age distribution) of Sitka spruce. The stands were aged 9, 14, 30 and 47 years when sampling began in 2002 (henceforth referred to as D9, D14, D30 and D47, respectively). They were representative of the typical yield class
(18–22 m³ ha⁻¹ yr⁻¹) for Sitka spruce growing on wet mineral soils in Ireland. The D14 stand had a higher than average yield class of 24 m³ ha⁻¹ yr⁻¹ (Tobin and Nieuwenhuis 2007).

An adjacent grassland site was selected to represent a non-forested stand (G0) to assess changes in C sequestration associated with land-use change and the development of the forest. The D9 site had an open canopy at the commencement of the project, with grasses growing in-between and underneath the trees.

The D47 stand was felled in 2002 before any meaningful litterfall collection or microbial biomass measurements were made. A substitute site was then chosen for the litterfall and microbial biomass measurements, which was located in Cullenagh Forest (C45) directly to the north of Dooary. This forest was 45-years-old and also had a yield class of 22 m³ ha⁻¹ yr⁻¹.

Soil description
The soil is described as being associated with the Raheenduff Series with pockets in the Imperfectly Drained Phase according to Soils of County Laois, from the National Soil Survey of Ireland (Conry 1987). Surveys carried out of the forested area indicate that 90% of the soil is gley with 10% brown earth/podsol mixture. The soil is principally a wet surface-water mineral gley. Mean sand, silt and clay contents of the chronosequence soils were 22%, 36% and 42% respectively; pH was 4.6 and bulk density was 1.018 g cm⁻³ (Saiz et al. 2006, 2007). The C45 site had a sand, silt and clay content of 20, 50 and 30% respectively and a pH of 4.3. It had a bulk density of 1.063 g cm⁻³. This soil was classified as a gleyic brown earth (Saiz et al. 2006). These figures mirror those described in the national soil survey (Conry 1987).

Soil core measurement
Fifteen randomly located soil cores were taken in each stand. The corer was a steel cylinder (5 cm diameter and 30 cm length). The soil was separated into litter, organic and mineral layers visually on site and placed into plastic bags. The soil was refrigerated on the day of collection and processing took place the following day.

Woody debris larger than 1 cm in diameter, stones and living plant and animal material was removed from the soil which was passed through a 5.6 mm mesh. The fresh weight of the soil was then recorded. Two 10 g subsamples were taken from the mineral and organic layers for microbial biomass analysis using the chloroform fumigation technique (Vance et al. 1987). Another 20 g subsample was removed from both layers and was processed for density fractionation. After the subsamples were removed, the remainder was oven dried for 48 hours at 80 ºC, or until weight became constant. The soil was then ground using a pestle and mortar and mixed thoroughly. The moisture content (%) was calculated. A 1 g sample was then taken from each layer and combusted at 540 ºC for two hours.

The percentage loss on ignition was then calculated and a conversion factor of 0.58 (Allen 1989) was used to convert organic matter to C content per gram of soil. Total C content of the soils at each site was estimated for each of the organic and
upper mineral horizons based on loss on ignition, which was calibrated using CHN analysis. Loss on ignition was also calculated for the standing litter layer.

Litter measurements
Litter input was measured each month using litterfall collectors at each site in the chronosequence between 2002 and 2004. In each site, a $30 \times 30$ m plot was selected (away from boundaries to reduce edge effects). Twenty-five litter collectors (25 l plastic buckets of 28 cm diameter) were randomly located within the plot. Each bucket had a 15 cm nail through the centre of its base to secure it to the soil. Four 10 mm holes were drilled in the base of the bucket to allow drainage. Litter was collected once a month for two years from each of the five sites.

The litter was then dried for two days at 60 °C and separated into green needles, dead needles and twig/branch material. For the grassland site, litter estimates were made using the harvest technique of Sims et al. (1978). The same technique was used to estimate the increase in grass litter due to canopy closure in the D9 site. In this instance, a 0.25 m$^2$ quadrant was placed randomly in each plot and all standing vegetation was harvested. This material was dried as above and separated into live and dead material and weighed.

Litter samples were ground for 10 minutes at 900 oscillations per minute using a Retch grinder (Type MM2) until a homogenous powder was formed. The C, N and lignin content of the litter inputs were measured twice at each site of the chronosequence. The C and N concentrations were measured using a CHN analyser. A ground sample was flash combusted at 1,500 °C, followed by gas chromatography in an Exeter Analytical CE440 elemental analyser. All other monthly litter collections were combusted for percentage loss on ignition and total C content was calculated via a conversion constant relative to the previous measurements on the CHN analyser.

The lignin content was estimated following the methods of Allen (1989):

$$\text{Lignin} \% = \left( \frac{\text{corr. Lignin} \times \text{tot.} \times 10^2}{\text{wt. for water extract} \times \text{sample wt.}} \right)$$ (1)

where corr. lignin is corrected lignin. The ash and N-content was calculated for the crude lignin sub-samples. The N value was then multiplied by 6.25 to correct for crude protein. The crude protein and ash was then subtracted from the crude lignin before the lignin % calculation. tot. is the total ether-extracted lignin from the sample.

Microbial biomass C
The chloroform fumigation-extraction method (Vance et al. 1987) was used to measure the microbial biomass C in the soil. This method determines the microbial C by estimating the difference between fumigated and non-fumigated soil samples. Four sub-samples of soil were obtained from each soil unit, two from the mineral horizon and two from the organic horizon (approximately 10 g dry weight equivalent each). Biomass C was calculated using the following equation:

$$\text{Biomass C} = \left( \mu \text{g C g}^{-1} \text{ soil (fumigated)} - \mu \text{g C g}^{-1} \text{ soil (non-fumigated)} \right) / 0.45$$ (2)
The proportion of microbial C evolved as \( \text{CO}_2 = 0.45 \) for 10-day incubations at 25 °C (Jenkinson and Ladd 1981)

Density fractionation

Density fractionation separates macro organic matter from the mineral components of the soil (Six et al. 2001). This was done using water or heavy liquids, such as in this case sodium polytungstate (SPT) that are of greater density than the organic matter. The floating particles are considered as the light fraction (Christensen 1992). The light and heavy C fractions were separated following the methods of Compton and Boone (2002) which uses centrifugation and a liquid with a density of 1.6 g cm\(^{-3}\). The resulting heavy and light materials were dried, weighed and ground. The C and N content of each fraction was measured using a CHN analyser. The total stock was estimated by multiplying the C and N concentrations by the weight of the original samples (which were area-based).

Results

Soil C stocks

The stocks of C in the soil varied considerably between the closed canopy sites in the chronosequence, but generally increased with time and were always greater than 100 t C ha\(^{-1}\) under a closed canopy (Figure 1). Generally the increase in soil C stocks over time varied between 0.2 to 1.8 t C ha\(^{-1}\) yr\(^{-1}\). The lower C stock and greatest stock changes occurred in the D30 stand (Figure 1), which had a sloping topography, was more exposed and had lower soil moisture content. In contrast, the higher accumulation of soil C in D9, D14 and D47 stands may have been associated with the flatter topography and higher soil moisture contents.

![Bar chart showing soil carbon stock (t C ha\(^{-1}\)](chart.png)

**Figure 1:** Total soil C (t C ha\(^{-1}\), ± S.E.) in the combined mineral and organic layers at each site in the chronosequence.
The standing litter in each site increased with age. There was an accumulation on the forest floor especially with the onset of canopy closure where the rate of decomposition was less than that of litterfall. In the youngest site D9, there was still living under-storey vegetation and an open canopy, which partially explains the lack of accumulation at this site. The oldest site (D47) had greatest variation due in part to standing water and patches of bare soil where no litter had accumulated (Figure 2).

Density fractionation
The heavy fraction of the soil increased over the chronosequence, 76 t C ha\(^{-1}\) in the G0 site to 153 t C ha\(^{-1}\) in the D47 site, whereas the light fraction fluctuated between 18 and 36 t C ha\(^{-1}\) (Figure 3). The heavy fraction at the D47 site was found to be a third or more greater than at the other sites, ~150 t C ha\(^{-1}\) versus ~100 t C ha\(^{-1}\). The light fraction was very similar in the two youngest sites G0 and D9 (20 and 16 t C ha\(^{-1}\)), increased at the D14 site (32 t C ha\(^{-1}\)) and then stabilised at the two older sites (23 and 29 t C ha\(^{-1}\)).

Litter inputs
Litter input was measured at the four forested sites in the chronosequence. Monthly litter C inputs tended to be greatest from March to May associated with resumption of metabolism leading up to the time of bud burst (Figure 4). The input was greatest in 2002/2003 in the 14-year-old site as canopy closure had resulted in all the needles of the lower branches dying and no thinning event had occurred. In 2003/2004 all sites had increased litterfall rates, particularly the closed canopy sites (D14, D30 and C45). C45 was felled in September 2004 and no further litterfall values were obtained.
The largest annual input (4.04 t C ha\(^{-1}\) yr\(^{-1}\)) was recorded in the 14-year-old site (Figure 5). The closed canopy sites had more than double the cumulative litter of the open canopy site (D9) and grassland site (G0). This reflected the fact that almost all needles attached to branches were still alive at D9, with little or no senescence on lower branches. This resulted in a litter stock similar to G0 as the understory grasses were contributing the greatest proportion of the standing litter stock of D9. The

**Figure 3:** Separated light (blue) and heavy (maroon) soil fractions (t C ha\(^{-1}\), ± S.E.) at each of the sites in the chronosequence at Dooary.

**Figure 4:** Monthly litterfall at each of the four forested sites in Dooary (t C ha\(^{-1}\) month\(^{-1}\)).
annual litter input values for D9 (Figure 5) did not include the dead grass C input. This represented an additional C input of ca. 3 t C ha⁻¹ yr⁻¹, based on a decrease in measured grassland biomass of 1.5 t C ha⁻¹ yr⁻¹ and a shoot to root ratio of 0.5.

The C, N and lignin contents of the litter inputs were estimated on three occasions over the 2-year period at all sites (Table 1). The four forested sites did not vary significantly for the litter quality variables. The grassland had slightly lower concentrations of C and N, 43.14% and 1.02% versus 47.12–47.94% and 1.06–1.37%. The grassland litter (G0) was more decomposed due to its better quality, with a narrower CN ratio and low lignin content which was ~25%, as distinct from >40% in the spruce needles.

**Microbial biomass C**

The greatest concentration of microbial biomass in the chronosequence was in the organic horizon in the grassland site, G0, 4,991 μg C g⁻¹ dry soil which is over three times the next highest value of 1,427 μg C g⁻¹ dry soil in the organic horizon of D30

<table>
<thead>
<tr>
<th>Age</th>
<th>C</th>
<th>N</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>G0</td>
<td>43.14</td>
<td>1.02</td>
<td>24.91</td>
</tr>
<tr>
<td>D10</td>
<td>47.12</td>
<td>1.14</td>
<td>41.79</td>
</tr>
<tr>
<td>D15</td>
<td>47.58</td>
<td>1.37</td>
<td>41.69</td>
</tr>
<tr>
<td>D30</td>
<td>47.94</td>
<td>1.10</td>
<td>40.10</td>
</tr>
<tr>
<td>C45</td>
<td>47.62</td>
<td>1.06</td>
<td>41.41</td>
</tr>
</tbody>
</table>

Table 1: Quality of litter collected from the 5 chronosequence sites, % C, N and lignin in dry mass.
Figure 6: Microbial biomass ($\mu g \text{ C } g^{-1} \text{ dry soil}$, ± S.E.) in organic (lilac) and mineral (maroon) soil horizons for the five sites in the Dooary chronosequence.

(Figure 6). The organic horizon values for D9, D14 and C45 were quite similar to each other and were approximately half the concentration at D30. The greatest concentration of microbial biomass in the mineral horizon was again at the G0 site (2,127 $\mu g \text{ C } g^{-1} \text{ dry soil}$). This was much smaller than that contained in the G0 organic horizon. The same occurred at the D9, D14 and D30 sites, where organic horizon microbial biomass concentrations were approximately twice those in mineral horizons. The C45 site was different in that the concentration in the organic horizon was 705, compared to 244 $\mu g \text{ C } g^{-1} \text{ dry soil}$ in the mineral horizon.

The amounts of microbial biomass present in the soil profile were calculated by combining the values above with the known weights of the soil. Across the chronosequence, the total stock of microbial biomass in both horizons was markedly different, with that in the mineral horizon being much larger, except at the D9 site where the amounts were similar (Figure 7 A). The large amount of microbial biomass in the G0 site is further emphasised when the site totals are compared across the five sites (Figure 7 B). The G0 site contains 362.8 g C m$^{-2}$, which is three times the amount in the D30 site (128.9 g C m$^{-2}$) and in the D9 site (121.4 g C m$^{-2}$).

Discussion
The total soil C stocks increased from 97 t C ha$^{-1}$ in the grassland site to 183 t C ha$^{-1}$ in the 47-year-old stand of Sitka spruce (Figure 1) which indicates that Sitka spruce plantations on this soil type have a potential to sequester approximately 80 t C ha$^{-1}$ over the lifetime of a crop. This represents a sequestration rate of 1.83 t C ha$^{-1}$ yr$^{-1}$, which is high in comparison to some other studies; e.g. 0.34 t C ha$^{-1}$ yr$^{-1}$ on acidic soil after 57 years under Norway spruce in Italy (Thuille and Schulze 2006) and 0.36 t C ha$^{-1}$ yr$^{-1}$ on a pseudogley soil under Norway spruce in Demark (Vesterdal et al. 2002). The higher accumulation at Dooary may be expected given the relationship between factors such as rate of productivity and decomposition.
across Europe (Berg et al. 1993, 1999, Bottner et al. 2000, Coûteaux et al. 2001). In higher latitudes trees may not produce as much biomass and consequently litter, even when the decomposition rate is restricted by the lower temperatures compared with low latitude sites.

The mean C stock in global temperate biomes is approximately 100 t C ha⁻¹ (Lal 2005), but the mean appears to be somewhat higher in forests with mean stocks of 130 t C ha⁻¹ reported for global mixed cool temperate forest soils using 56 samples (Adams 2004) and a median C pool in European Norway spruce forests estimated as 140 t ha⁻¹ (de Vries et al. 2003). In general, the values reported in the present work are within the ranges of values found in the literature for older sites such as the 47-year-old stand (D47). The stocks for the first 20 years in the sites assessed were very variable (Figure 1). This may have been due to site preparation associated with afforestation which disturbed the soil C content by exposing lower soil layers to mixing with upper soil layers. However, stock quantities are within the ranges reported for other forests. In NE England 140 t C ha⁻¹ were reported in a 40-year-old stand of Sitka spruce and 250 t C ha⁻¹ in a 30-year-old reforested stand (Zerva et al. 2005). A Norway spruce plantation on glacial clay soils had 209 t C ha⁻¹ in sites aged 55–60 years (Berg et al. 2001) and a greater stock was found in a mixed broadleaf stand in cool temperate Japan with a mean of 318.3 t C ha⁻¹ in the mineral soil (Jia and Akiyama 2005).

However, in terms of C sequestration it would be desirable that a large proportion of the soil C would be held in the stable fractions of the SOM. This is represented in this study by the heavy fraction. The percentage of C held in the heavy fraction (84%) at the D47 site is similar to, but at the lower end, of the values reported in the literature. For example, 80.9% of the C was found to be in the heavy fraction in a study of 46 to 72-year-old forests on loam-dominated soils at seven sites in Washington and Oregon (natural range of Sitka spruce) (Swanson et al. 2002). However, Gregorich et al. (1994) found the heavy fraction to represent up to 99.1% of the soil C in clay loam soils in forests dominated by Acer spp. in Ontario, Canada. But the stock (140.64 t C ha⁻¹ in the heavy fraction) was comparable to that found at the D47 site where the heavy fraction was 153.457 t C ha⁻¹ (Figure 3).

Similarly, for a tallgrass prairie in Kansas and an abandoned pasture in Costa
Rica much greater proportions of the soil C were in the heavy fraction, 99.53 and 99.75% (Strickland and Sollins 1987) in comparison with the grassland site in this study where on 73% was contained in the heavy fraction. However, in this instance the G0 soil had a much higher stock of stable C (76.18 t C ha⁻¹) (Figure 3).

Thus the C dynamics in the soils of this study appear to be different than in other circumstances. The size of the soil organic matter (SOM) pool represents a balance between the organic matter input as litter and the rate of decomposition. Litterfall rates, together with the rates of decomposition will determine the size of the C litter pools and the amount of litterfall is positively correlated with NPP (Vilà et al. 2004), while the rate of decomposition is determined largely by climate, the chemical quality of the litter input and the decomposer organisms present in the system.

The amounts of litterfall at the sites are similar to those reported in the literature, both for monthly litterfall and peak patterns between months and generally between species. The annual litterfall was closely related to changes in leaf area index and net primary production (NPP) over the chronosequence (Tobin et al. 2007). The total inputs of 4.43, 2.02 and 1.93 t C ha⁻¹ yr⁻¹ in 34-, 39- and 47-year-old stands respectively reported in Carey and Farrell (1978) are also similar to the amounts measured in the D30 and C45 sites, which were 3.7 and 2.8 t C ha⁻¹ yr⁻¹, respectively (Figure 5). Sitka spruce stands aged 25–30 across Scotland and Northern England had a lower litterfall of 1.6 t C ha⁻¹ yr⁻¹ (assuming C was 50%).

An afforested site in Thuringia, Germany of Norway spruce had a similar yearly litter input for 30- and 57-year-old stands, 2.5 and 3.2 t C ha⁻¹ yr⁻¹, respectively (Thuille and Schulze 2006). Sitka spruce on 30 to 34-year-old stands with similar precipitation and temperature in Denmark had an average input of 1.76 t C ha⁻¹ yr⁻¹ for five years 1989–1994 (Pedersen and Bille-Hansen 1999). However, this was on very sandy soil (89.2% sand).

In addition, the amount of standing litter at the mature site D47 (Figure 2) appeared to be very similar to values reported for other forests. Smith and Heath (2002) reported a mean value for forest floor litter of 33 t C ha⁻¹ with a minimum of 4.6 and a maximum of 68.1 t C ha⁻¹ for a mature spruce, fir (Abies spp.), hemlock (Tsuga spp.) forest type in north-eastern United States. This mirrors the findings of D47 with a mean of 21 (and a minimum of 15 and maximum of 39) t C ha⁻¹. The forest floor C stocks of a boreal pine forest chronosequence in western Canada were 16 t C ha⁻¹ for a 30-year-old stand and 14 t C ha⁻¹ for a 35-year-old stand, which are very similar to D30 (Figure 2) and ranged from 12 to 22 t C ha⁻¹ for a 50-year-old stand, comparable again to D47 (Figure 2) (Nalder and Wein 2005).

In three 47 to 51-year-old stands of Norway spruce in Denmark, forest floor values were 13.14, 17.01 and 47.87 t C ha⁻¹ on very sandy soils, indicating great within-site variability (Vesterdal et al. 1995). In another study three 33- to 34-year-old sites stands of Sitka spruce in Denmark had 7.21, 20.69 and 16.56 t C ha⁻¹ in the standing litter on the forest floor (Vesterdal et al. 1998). This was comparable to the D30 site. In general, reports from Sitka spruce stands were similar to the results shown in Figure 2, but trends within particular sites varied slightly. It is therefore most likely that the decomposition process is the key factor leading to the differences in the proportions of stable SOM.
The mean C and N concentrations in the Dooary forest litter (Table 1) were 47.5% C and 1.20% N, which are comparable with the 48.4% C and 1.27% N reported by Carey and Farrell (1978). Pedersen and Bille-Hansen (1999) reported comparable mean N% in litterfall of 1.34% in Sitka spruce stands over a 5-year period (35-years-old). Miller et al. (1996) reported much lower N levels (0.69–1.07%) for Sitka spruce litter from stands situated across Scotland and Northern England in an age group of 25–30 years. There the percentages were weighted means to correct for the seasonality in N%, where levels decrease in the autumn and winter months, which may be a reason for the large discrepancy. The N% in needle litter was also reported to be 1.34% for green needle litter in Berg et al. (1993). Sitka spruce stands in Danish 33 to 34-year-old stands on sandy soils had 40.6, 41.9 and 46.2% C in the litter and 1.48, 1.65 and 2% N (Vesterdal 1998).

Mean lignin content was 41.2% for the forested sites of the chronosequence (Table 1). This is at the medium range of the spectrum of values reported in the literature. The lignin content of spruce litter in England was 48.5% (Rowland and Roberts 1994), which is one of the higher values. Rutigliano et al. (1996) reported lignin values of 40.47% lignin in fir in Italy. However, Norway spruce litter in southern Sweden ranged from 35%–36% (Lundmark-Thelin and Johansson, 1997, Berg 2000). *Pinus sylvestris* needles from SW England contained lignin values of between 20 and 32% (Sanger et al. 1996). Again Berg et al. (1993) reported lower values between 23.1–28.8% for Scots pine in East central Sweden.

This higher lignin content indicates greater C sequestration potential for this litter due to the inherent recalcitrance of lignin. This may also be a reason for the higher N% levels as the N is lignin associated and not as readily available to decomposers. Therefore, the SOM content at these sites is greater than might be expected. Further studies of C sequestration potential at other Irish forest stands of different species would require specific litter input data.

Soil microbial biomass affects the rate of decomposition. In the organic horizon of the grassland (G0), the soil microbial biomass was 4,991 µg C g⁻¹ dry soil (Figure 6) which is higher than many of the values reported in literature. Four pastures in New Zealand had values which ranged from 1,125–1,724 µg C g⁻¹ dry soil in the 0–10 cm horizon but was 479–575 µg C g⁻¹ dry soil at a depth of 10–20 cm (Sparling 1992). The higher values were associated with the clay-dominated soils as opposed to the silt/loam soils. The soils in this study had a high clay content and this could explain the high microbial biomass content.

However, other studies have reported higher values on upland soils. Williams et al. (2000) reported 2,900 µg C g⁻¹ dry soil for unimproved upland grassland and 1,600 µg C g⁻¹ dry soil for an improved upland grassland in Scotland. These values are closer to those reported in this study where the chronosequence mean elevation was ca. 260 m. However, the values reported in Figure 6 are higher than the 847 µg C g⁻¹ dry soil for a grazed site and 1,035 µg C g⁻¹ dry soil for a managed fertilised grassland from Wales (Bardgett and Leemans 1995). The microbial biomass reported at Rothamsted Highfield Permanent Grassland Experiment was 948 µg C g⁻¹ dry soil (Wu et al. 1995). That site also received an annual application of N
fertilizer, however the sites in the current study had had little intensive management and it is highly unlikely that any fertilizer was added.

Bardgett and Shine (1999) found that increased diversity in plant litter increased the microbial biomass content in temperate grasslands. In a microcosm experiment the microbial biomass was 743 µg C g⁻¹ dry soil for two species litter and 1,628 µg C g⁻¹ dry soil for six species litter, indicating a possibility of greater species diversity in grasslands being indicative of lower management intensity and lack of fertilisation (Rodwell 1992, Smith, 1994). The G0 site in this study was on marginal land with minimal management and contained a variety of grass and rush species. Therefore, plant diversity could also have contributed to the large microbial biomass.

The soil microbial biomass of mature spruce forest (acidic type) in Taiwan ranged from 308 µg C g⁻¹ in the subsoil (21–40cm) to 870 µg C g⁻¹ in the topsoil (0–20cm), the organic layer contained 216–653 µg C g⁻¹ dry soil (Yang et al., 2003). The 870 µg C g⁻¹ for a dry soil is comparable to the organic horizon of the C45 site (705 µg C g⁻¹ dry soil). The subsoil value of 308 µg C g⁻¹ dry soil reflects the mineral horizon value in the site C45 (244 µg C g⁻¹ dry soil), also indicating microbial populations decrease with increasing soil depth.

This reduction through the profile was also reported by Sparling et al. (1992) where the 0–10 cm depth had almost double the microbial biomass of the 10–20 cm depth sampled. The seasonal variation in microbial biomass could also be a factor; all forest samples were taken in the winter/spring time. Yang et al. (2003) found differences of up to a third in microbial biomass content when sampling at different times of the year. Bardgett et al. (1997) found grassland microbial biomass levels increasing by up to a half in the summer in comparison to the other three seasons. The grassland cores in this study were taken during the summer.

Mature Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) forest on volcanic soils in Washington had microbial biomass of 1,700 µg C g⁻¹ dry soil, double the amount found at C45 (organic horizon) in Figure 2.5. Mature (90 and 115-year-old) Norway spruce in Lower Saxony, Germany had considerably higher values than reported here in the organic horizon: 2,651 and 4,374 µg C g⁻¹ dry soil versus 705 µg C g⁻¹ dry soil at C45 (Figure 2.5). The mineral horizon values are more comparable, 224 µg C g⁻¹ dry soil in site C45 (Figure 5) compared to 209 and 119 µg C g⁻¹ dry soil in the study in Germany (Borken et al., 2002). These differing microbial biomass values in this study compared to other studies appears to be the key as to why there are generally higher SOM values in the forest soil in Ireland.

The microbial biomass total of 362.8 g C m⁻² for the G0 site was also on the higher end of the scale for unimproved grassland sites (Figure 7 B). Soil microbial biomass increases as soil fertility decreases moving from improved to unimproved grasslands (Grayston et al. 2001). Ten unimproved grassland sites in Scotland had a microbial biomass mean of 89.4 g C m⁻² (Grayston et al. 2004). However, it should be noted that these cores were taken to a depth of only 5 cm. Bardgett et al. (1997) showed the microbial biomass was positively correlated with SOM content across a range of upland grassland types, and with other studies of a wide range of managed and natural ecosystems (Wardle 1992, Zak et al. 1994).
One possible explanation for the higher grassland values of C content could have been that soil was sampled to a lower depth in this study. The addition of more soil lower in the profile would lead to increasing the microbial biomass content on an area basis. Bardgett et al. (1997) reported reductions in microbial biomass down through the soil profile in grasslands. There the cores were taken to 15 cm depth, whereas the soil core used in this study was 30 cm in length.

In summary, the grassland values are on the higher end of the scale of microbial biomass C and this may be due to a combination of higher elevation, lack of management, increased diversity, increased clay content in the soil and the sampling date being in the summer season. The forest values were difficult to compare to other studies due to a paucity of studies in Sitka spruce forests in Ireland. In some cases the values were similar in regenerating sites and samples taken in the winter. In other comparisons the forest sites were different to other sites and species but never by a large magnitude. In all cases the soil microbial C decreased down through the soil profile. In this instance the soil microbial C appears crucial in explaining the large pool of SOM accumulated over the chronosequence. With the lower soil microbial biomass in the forests it could be hypothesised that the recalcitrant parts of the litter with higher lignin content is unavailable to these communities. The result was that the soil C increased over the timeframe of the chronosequence.

Conclusions
The results of this study supports the view that land-use change from marginal grassland to Sitka spruce forest can lead to significant C sequestration over the lifetime of the forest. This C increase in national forest stocks offers huge potential for the mitigation of GHGs, especially as much of the afforestation in Ireland will be classified as Kyoto forest. These data should allow more accurate modelling of the most common Irish forest ecosystem and, coupled with the microbial biomass data and soil fractionation data reported here, allow reporting to the UNFCCC at Tier 3 level.

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