

Coarse Woody Debris Stimulates Soil Enzymatic Activity and Litter Decomposition in an Old-Growth Temperate Forest of Patagonia, Argentina

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ABSTRACT

In most temperate forest ecosystems, tree mortality over time generates downed logs that accumulate as coarse woody debris (CWD) on the forest floor. These downed logs and trunks have important recognized ecosystem functions including habitat for different organisms and long-term organic C storage. Due to its recalcitrant chemical composition and slow decomposition, CWD can also have direct effects on ecosystem carbon and nutrient turnover. CWD could also cause changes indirectly through the physical and chemical alterations that it generates, although it is not well-understood how important these indirect effects could be for ecosystem processes and soil biogeochemistry. We hypothesized that in an old-growth mature forest, CWD affects carbon and nutrient cycles through its “proximity effects”, meaning that the forest floor near CWD would have altered soil biotic activity due to the environmental and biogeochemical effects of the presence of CWD. We conducted our study in an old-growth southern beech temperate

forest in Patagonia, Argentina, where we estimated and classified the distribution and mass, nutrient pools and decay stage of CWD on the forest floor, and evaluated its impact on litter decomposition, soil mites and soil enzymatic activity of carbon and phosphorus-degrading enzymes. We demonstrate here that CWD in this ecosystem represents an important organic carbon reservoir (85 Mg ha⁻¹) and nitrogen pool (0.42 Mg ha⁻¹), similar in magnitude to other old-growth forests of the Northern Hemisphere. In addition, we found significant proximity effects of CWD, with increased C-degrading soil enzyme activity, decreased mite abundance, and more rapid litter decomposition beneath highly decayed CWD. Considered at the ecosystem scale in this forest, the removal of CWD could cause a decrease of 6% in soil enzyme activity, particularly in the summer dry season, and nearly 15% in annual litter decomposition. We conclude that beyond the established importance of CWD as a long-term carbon reservoir and habitat, CWD contributes functionally to the forest floor by influencing the spatial heterogeneity of microbial activity and carbon and nutrient turnover. These proximity effects demonstrate the importance of maintenance of this ecosystem component and should be taken into consideration for management decisions pertaining to carbon sequestration

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and functional diversity in natural forest ecosystems.

Key words: carbon cycling; β -glucosidase; soil mesofauna; *Nothofagus*; CWD; South America.

INTRODUCTION

Temperate forests are one of the most productive terrestrial ecosystems, and during the course of ecosystem development, overstory species accumulate large amounts of aerial biomass, comprised not only of photosynthetic organs (leaves) but woody structures of large branches and trunks (Chapin and others 2002). Once these trees senesce and die, this woody material can accumulate on the forest floor as coarse woody debris (CWD), where it persists for extensive periods of time on the forest floor (Harmon and others 1986; Spies and others 1988; Woldendorp and Keenan 2005).

Because of its considerable mass and persistence over time, there are various ecological functions that have been identified for CWD. Habitat provision for organisms such as nesting birds, refuge for rodent species from predation, and safe sites for overstory tree regeneration (Harmon and Franklin 1989; Santiago 2000; Jabin and others 2004; Nordin and others 2004; Heinemann and Kitzberger 2006) have all been demonstrated as important roles for CWD in natural temperate forests. In addition, biogeochemical cycles and pools can be affected by the presence of CWD. Due to its slow decomposition and persistence on the forest floor (Beets and others 2008), CWD can represent a substantial reservoir of organic carbon and nutrients in many forest ecosystems (Carmona and others 2002; Currie and Nadelhoffer 2002; Ganjegunte and others 2004), which has been estimated at 142 Pg C globally for mature temperate forests (Schlesinger 1977). In addition, although few studies have incorporated CWD quantitatively in overall C budgets, its exclusion can underestimate the soil C pool by as much as 50% in forests without recent disturbances (Currie and Nadelhoffer 2002).

At the ecosystem scale, variation in CWD is due to forest type, species composition, disturbance regime (natural and anthropogenic), and successional stage. In general, coniferous forests have larger amounts of CWD than deciduous forests, due to better substrate quality and smaller diameter of angiosperm CWD on average (Harmon and others 1986). Models of CWD accumulation over time predict a U-shaped pattern, where early and late successional forests represent peaks of CWD accumulation (Sturtevant and others 1997). However,

by far the largest impact on CWD in forest ecosystems comes from human management, where wood is removed by harvests, either through clear-cutting, burning or salvage logging (Bretz Guby and Dobbertin 1996; Siitonen and others 2000). It is not clear how the removal or alteration of CWD in forest ecosystems could affect biogeochemical cycling in the long-term, particularly due to the fact that very few forests remain that are free from disturbance and human impact.

In addition to direct impacts on aboveground carbon and nitrogen pools, CWD could have indirect impacts on ecosystem processes related to nutrient transformations and energy fluxes in space and time, although the patterns and controls on these processes are much less well understood. As CWD contains substantial amounts of lignin and is generally nutrient poor, carbon turnover, and nutrient mineralization are slow, which could create unique conditions of microsite heterogeneity (Ganjegunte and others 2004; Campbell and Laroque 2007). The long-residence time of highly recalcitrant organic matter could create conditions of divergent carbon qualities at the soil surface when compared to forest floor areas free of CWD. As a result, a chronosequence of CWD decay could alter the input of carbon to the surrounding zone of influence near the downed logs. Similar to the concept of "islands of fertility" in arid ecosystems where soil beneath vegetation patches has higher concentration of organic matter and nutrients, CWD could be viewed as "recalcitrant carbon islands", where large variations in C quality can differentiate microsites in the matrix of the forest floor (Spears and Lajtha 2004). It has been demonstrated that advanced decay stages of CWD can leach significant quantities of organic matter and dissolved organic carbon into the soil (Zalamea and others 2007), that soil chemical characteristics can be altered under its influence, increasing calcium, magnesium, and total N concentrations (Kappes and others 2007), as well as decreasing soil pH (Spears and Lajtha 2004).

In spite of its demonstrated importance in forest ecosystems, it is not well understood how CWD could affect abiotic and biotic characteristics in its sphere of influence. Some authors have suggested that soil contact accelerates CWD decomposition (Naesset 1999; Garrett and others 2010), but the

feedback on soil biotic activity from CWD presence is not clear. In addition, there is evidence that the composition of the soil invertebrate community is altered by the presence of logs (Skubała and Duras 2008; Déchéne and Buddle 2010) and that fallen logs can enhance densities of many macro-arthropods, not only by increasing species diversity but also functional diversity (Jabin and others 2004). CWD may influence invertebrate communities by providing refuge for predatory invertebrates, modifying litter properties or altering the C:N ratio beneath the logs (Evans and others 2003). Although soil mesofaunal groups of oribatids and collembolan play an important role in decomposition and nutrient cycling by fragmenting organic matter and influencing microbial communities, the integrated picture of how these multiple effects translate to variation in ecosystem processes is not clear, particularly as the decomposition process of CWD advances.

The temperate southern beech forests in South America extend from 33° to 56° latitude S; these forests can be dominated by several species of *Nothofagus* spp. (mixed forests) in more favorable zones of the region (Veblen and others 1996). In Argentina, due to their remote location at long distances from urban centers and isolation by the Andes mountain, these forests are largely free of anthropogenic pollution and thus an ideal situation for studies of ecosystem functioning with minimal anthropogenic disturbances (Hedin and others 1995). The evaluation of the importance of CWD as contributions to C and N pools has been evaluated for post-harvest forests in Chile (Carmona and others 2002) and old-growth forests in Valdivia and Tierra del Fuego (Richter and Frangi 1992; Carmona and others 2002; Schlegel and Donoso 2008), but their impact on ecosystem processes has not been studied.

We explored the direct and indirect contribution of CWD to ecosystem functioning in an undisturbed southern beech temperate forest in Patagonia, Argentina. We estimated CWD mass and decay class as well as biological activity with increasing distance from CWD in advanced stages of decay. We hypothesized that CWD in contact with soil for extended periods of time would generate “recalcitrant carbon islands” modulating soil characteristics and soil enzyme activities in its sphere of influence. Specifically, we predicted that overall biotic activity would be stimulated under highly decayed CWD, due to a combination of changes in microenvironmental conditions and labile carbon availability, which would have a significant impact on ecosystem-scale carbon and nitrogen turnover.

METHODS

Study Site

The study was conducted in Lanín National Park in the province of Neuquén, in southern temperate Argentina (40°08'S, 71°30'O). The vegetation is dominated by a mixed deciduous-evergreen beech forest with 3 species of *Nothofagus*: *N. dombeyi* (31.9%), *N. nervosa* (17.3%) and *N. obliqua* (50.8%) (Vivanco and Austin 2008). Stand age is 108 years on average and stand density is 260 stems ha⁻¹ (Vivanco and Austin 2008). The understorey is almost entirely dominated by a single species of bamboo, *Chusquea culeou*, which flowered in a massive event in 2001 in the region (Kitzberger and others 2007; Austin and Marchesini 2012). Mean annual precipitation is 2300 mm according to long-term records from the Estancia Quechuquina, San Martín de los Andes (Vivanco and Austin 2008). Rainfall is highly seasonal, with the peak of precipitation occurring in fall and winter (April–September) and summer drought periods (January–March). Mean annual temperature is 8.8°C. The soils are classified as Thaptic Udovitands, young soils of volcanic origin with high concentrations of organic matter (Broquen and others 2005; Satti and others 2007).

CWD: Distribution and Characteristics

We defined CWD in the study sites as dead tree boles, large branches, and other large wood pieces lying on the ground, with a minimum diameter of at least 7.6 cm. To estimate the volume of CWD, we used a standard line-intersect method where transects were established radially from circular subplots and occurrence and characteristics of CWD were evaluated (Waddell 2002). More specifically, we established five experimental plots throughout the study site (total study site was ~2 ha), with each plot divided in four circular subplots of 36 m in diameter. In each subplot, all CWD was identified along two 18 m radial transects from the center of the subplot (Waddell 2002). We excluded standing dead wood and wood suspended in branches, which was not in contact with soil at the point of intersection with the line transects. Measurements of length, diameter (we further separated into diameter size classes; 7.6–10, 10–20, 20–30, 30–40 and >40 cm), and qualitative characteristics to identify decay stage were completed for each log. Biological degradation on the logs leaves indications that allow for classification in discrete decay stage. CWD was classified into five decay stages according to criteria established by

Carmona and others (2002). In brief, decay stage: (I) logs recently fallen with twigs and leaves, (II) bark present and without leaves, (III) bark often present and wood semi-solid, (IV) bark often absent and wood partly soft, and (V) soft and fragmented wood, and bole shape oval to flat. We determined wood density by taking cylindrical samples of each log and calculating volume and mass for each decay stage, which was later used to estimate biomass of CWD for each decay stage. In addition, we measured total C and N by the dry combustion method with an Elemental Analyzer LECO (Sollins and others 1999).

CWD Microsite Evaluation

Downed logs of decay stage IV and V ($N = 7$ for each decay stage) were selected to evaluate their effect on soil properties. In January and May 2008 (defined as summer and fall seasons), we collected soil samples in a perpendicular transect from the central axis of each log. The point of origin was the center of the log, with two points sampled at 0.5 m intervals to one meter of distance from both sides of the logs. The measurements of the samples at the same distances from both sides of the log were grouped. We defined three positions: below CWD, and 0.5 and 1 m of distance from the log.

A subsample of soil was dried at 105°C for 48 h for determination of gravimetric soil water content for corrections in all the variables. Soil pH was measured in 1:2 aqueous soil extract. Microbial biomass C was measured using the chloroform fumigation-extraction technique (Vance and others 1987). The concentration of C in the unfumigated sample (0.5 M K_2SO_4 extractable C) was used as a proxy of dissolved organic C (Engelking and others 2007). The activity of 1-4- β -glucosidase and acid phosphatase was measured using *p*-nitro-phenyl- β -glucopyranoside and *p*-nitrophenol-phosphate, respectively (Sinsabaugh and others 1999). Phenol oxidase activity was measured using 3,4-dihydroxy-L-phenylalanine (Sinsabaugh and others 1999). This assay was performed with an aliquot of 1 ml for β -glucosidase and acid phosphatase and 2 ml for phenol oxidase, from a soil water suspension (5:100 w/v). The mixture of soil solution and substrate (5 mM) in buffer acetate (50 mM acetate buffer, pH 5) was incubated for 1 h at 38°C. We chose this incubation temperature because some authors suggest high temperatures (37°C) maximize the sensitivity in enzymatic activity (Tabatabai and Dick 2002). The absorbance of *p*-nitrophenol was measured at 410 nm and the oxidized reaction product of phenol oxidase at

460 nm (Spectronic Genesys 2). Enzyme activities are expressed as $\mu\text{mol product g}^{-1} \text{ dry soil h}^{-1}$.

Mesofaunal abundance was measured in CWD of decay stage V ($N = 6$) in the summer season, to coincide with the period of maximum activity (January 2008). After removing the litter layer, we took soil cores with a PVC pipe (6 cm diameter, 5 cm depth) below CWD and 2 m from each stage V CWD (2 cores at each position). Mesofauna were extracted from the samples using Tullgren funnels (Crossley and Blair 1991) during a 96-h period. The extracted invertebrates from two major mesofaunal groups, mites and collembola, were sorted and counted under a stereomicroscope (Nikon SMZ800). Mites were identified to suborder level, and all adult oribatida and collembola were identified to family level (Hopkin 1997; Krantz and Walter 2009) under an optical microscope (Nikon Eclipse E200).

Effects of CWD on Litter Decomposition

We collected recently senesced litter from the dominant evergreen overstory tree, *Nothofagus dombeyi*, for 6 months from early summer to fall using litter nets suspended 2 m from the forest floor between trees in the study site. Litter was sorted by species, and only undamaged intact litter of *N. dombeyi* was separated for use in the litter decomposition experiment. We placed 1 g of litter material in a 2-mm mesh litterbag (10 × 10 cm) and placed duplicate litterbags in January 2007 above, below and beside logs in decay stage IV and V as described in the previous section ($N = 5$ for each decay stage log). We also placed litterbags on the forest floor and buried at 5 cm, both at distances greater than 2 m from CWD ($N = 5$ for each microsite). We collected litterbags annually, in January of 2008, 2009. Once retrieved from the field, litterbags were carefully cleaned, removing any extraneous debris, and after two days of drying in a 65°C oven, were weighed for determination of mass loss. All samples were also corrected for soil contamination by calculating ash free dry mass (AFDM, Stoyan and others 2000).

Data Analysis

Analysis of variance (ANOVA) was used to test for differences in chemical and physical composition of wood in different decay stages, the composition of the soil mesofaunal community and decomposition. We evaluated the effect of the presence of CWD (in decay stage IV and V) on the soil variables with a two-way ANOVA (season and position as factors), and in the case of significant ANOVAs, we conducted post-hoc Tukey tests. Soil organisms

were evaluated with a one-way ANOVA when distinguished by family and a two-way ANOVA for the major decomposer groups, using position and group (Acari and Collembola) as factors. Litter decomposition was analyzed with a one-way ANOVA, using position as a factor. In cases of non-normal distributions, data were log transformed and subjected to parametric tests. We used Statistica 6 software (Stat-soft, Inc., Tulsa, Oklahoma, USA). We evaluated the effect of CWD at the ecosystem scale by comparing the situation with logs (as % cover of CWD $\times$ variable average below CWD) + [(100 – % cover of logs) $\times$ variable average in the matrix) and the situation without logs (as 100% $\times$ variable average in the matrix).

RESULTS

Distribution and Characteristics of CWD

The total CWD in the study site was estimated to be 161 Mg ha⁻¹. CWD was distributed across various decay stages, with stage IV clearly the most abundant (Table 1), representing 55% of total CWD. The average log diameter of CWD for the site was 25 cm (± 15.85), with small (<10 cm) and large (>40 cm) diameter logs very uncommon (data not shown). With decay stage, wood density and chemical characteristics of CWD were variable, but the main differences were observed in the most advanced decay stages. Wood density significantly decreased at decay stage V ($F = 3.61$; $P = 0.012$; Table 1), coupled with increased C ($P < 0.0001$; $F = 53.64$) and N ($P = 0.012$; $F = 4.54$) concentrations in CWD in decay stage V.

Effects of CWD on Soil Biotic Activity

Differences in decay stage (IV or V) affected the impact of CWD on soil characteristics, soil biota, and microbial enzymatic activity. Overall, logs in decay stage IV did not generate strong microsite differences. Soil water content, pH, and microbial biomass C were not affected by proximity to CWD

in decay stage IV logs (Figures 1A, C, 2A, C), although dissolved organic C beneath CWD was ~ 1.5 -fold higher than in the other positions. However, soil water content and dissolved organic C in the positions around CWD in decay stage IV were strongly affected by the season, both higher in summer than in fall (Figures 1A, 2A).

In contrast, CWD in decay stage V clearly modified soil properties with significant effects of both position and season (Figures 1B, D, 2B, D). Soil water content beneath CWD was significantly higher than in the rest of the positions in both measured seasons (Figure 1B). Shifts in soil pH were also evident, with more acidic soils under the log than in adjacent positions (Figure 1D). Dissolved organic C beneath CWD in decay stage V was almost threefold higher than in the other positions (Figure 2B), and soil microbial biomass was marginally significantly higher beneath CWD in summer (Figure 2D).

Spatial variation in soil enzyme activity generated by CWD was most notable in decay stage V (Figure 3). C-degrading enzyme activity below and around CWD in decay stage IV was mainly affected by season, with the lowest beta-glucosidase and phenol oxidase activity across all the positions in fall (Figure 3A, B), whereas acid phosphatase activity did not vary across seasons (Figure 3C).

CWD in decay stage V demonstrated a significant effect of position in determining enzyme activity (Figure 3). β -Glucosidase activity demonstrated a significant season–position interaction, with higher activity only in summer below CWD decay stage V (Figure 3D). Phenol oxidase activity also showed a significant season–position interaction, with higher enzyme activity in summer below CWD (Figure 3E). On the contrary, acid phosphatase activity was only affected by position with higher enzyme activity below CWD in both seasons (Figure 3F).

Soil organisms and decomposition were affected by the presence of CWD, particularly for decay stage V. There was a significant reduction in mite and collembola abundance below CWD (Figure 4), and specifically, the abundance of several families

Table 1. Biomass and Characteristics of *Nothofagus* CWD Classified by Decay Stage

Decay stage	CWD (Mg ha ⁻¹)	<i>n</i>	Density (g cm ⁻³)	C (%)	N (%)
I	0.1	13	0.52 (0.004) a	51.8 (0.12) a	0.23 (0.004) a
II	21.5	6	0.46 (0.024) a	50.7 (0.11) a	0.23 (0.016) ab
III	40.7	5	0.50 (0.009) a	52.4 (0.25) a	0.19 (0.008) a
IV	89.2	13	0.44 (0.007) a	50.8 (0.10) a	0.23 (0.012) ab
V	9.4	9	0.38 (0.009) b	59.4 (0.24) b	0.34 (0.028) b

For means of wood density (± 1 SE), the number of replicates are indicated. Means for total C and N (± 1 SE) $n = 3$ with the exception of data of decay stage I ($n = 9$). Lowercase letters indicate significant differences among decay classes for that variable at $P < 0.05$.

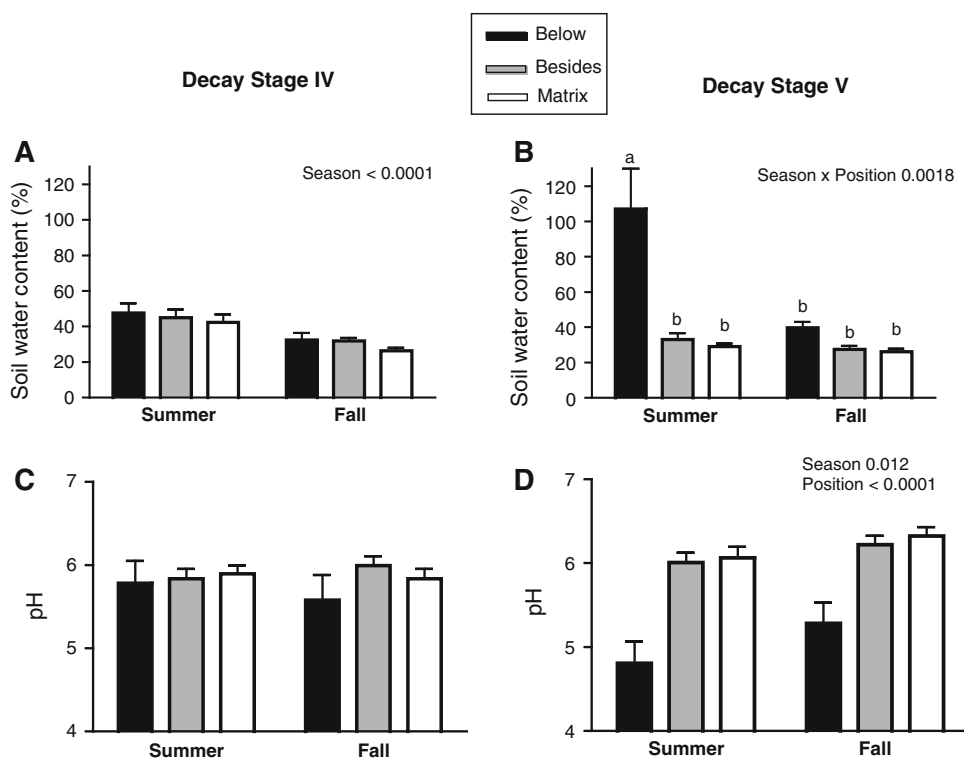


Figure 1. Effect of CWD on soil water content and pH for different positions in relation to CWD in summer and fall. Soil water content for positions around CWD **A** in decay stage IV and **B** in decay stage V. Soil pH for positions around CWD **C** in decay stage IV and **D** in decay stage V. Values are means ($\pm$ SE). Only significant factors for two-way ANOVA of season and position are shown. Different letters indicate significant differences using unequal N HSD.

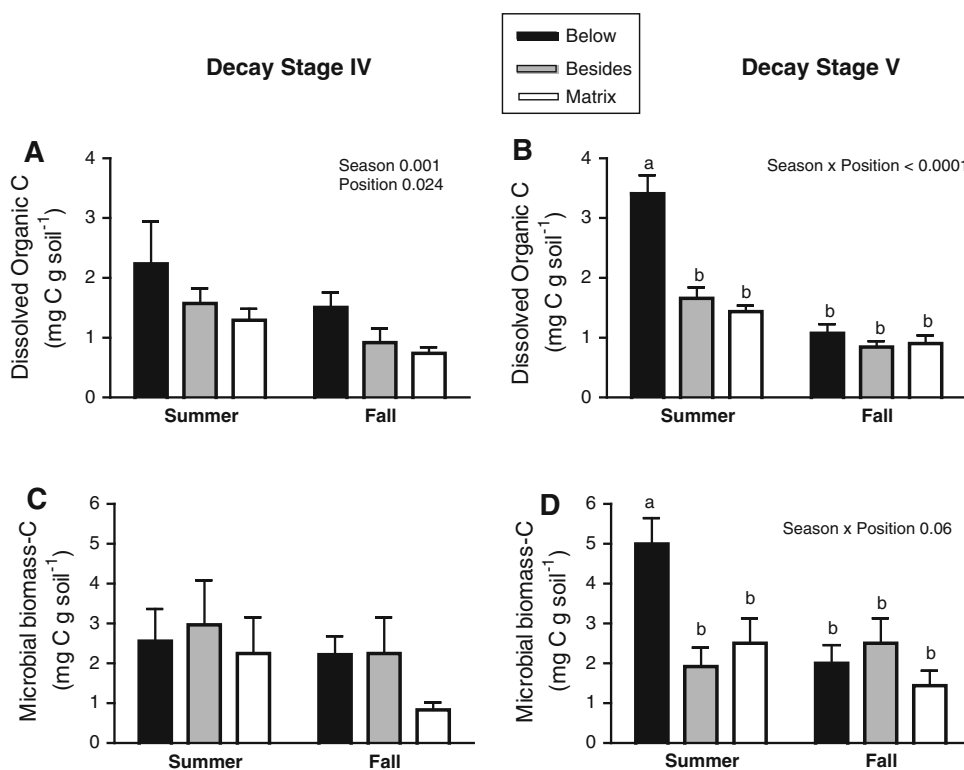


Figure 2. Effect of CWD on soil organic C and microbial biomass-C for different positions in relation to CWD in summer and fall. Soil-dissolved organic C for positions around CWD **A** in decay stage IV and **B** in decay stage V. Soil microbial biomass for positions around CWD **C** in decay stage IV and **D** in decay stage V. Values are means ($\pm$ SE). Post-hoc differences for significant interaction of season and position are shown. Different letters indicate significant differences using unequal N HSD.

of oribatid mites was significantly reduced below CWD (Table 2). In contrast, there was a significant stimulatory effect of proximity to CWD on litter decomposition after 2 years (Figure 5). The most rapid decomposition occurred beneath CWD and

was consistent in both decay stages of IV and V, but only the decomposition under decay stage V logs was significantly faster than the surrounding forest matrix. Surprisingly, decomposition of surface litter did not differ significantly from buried litter, which

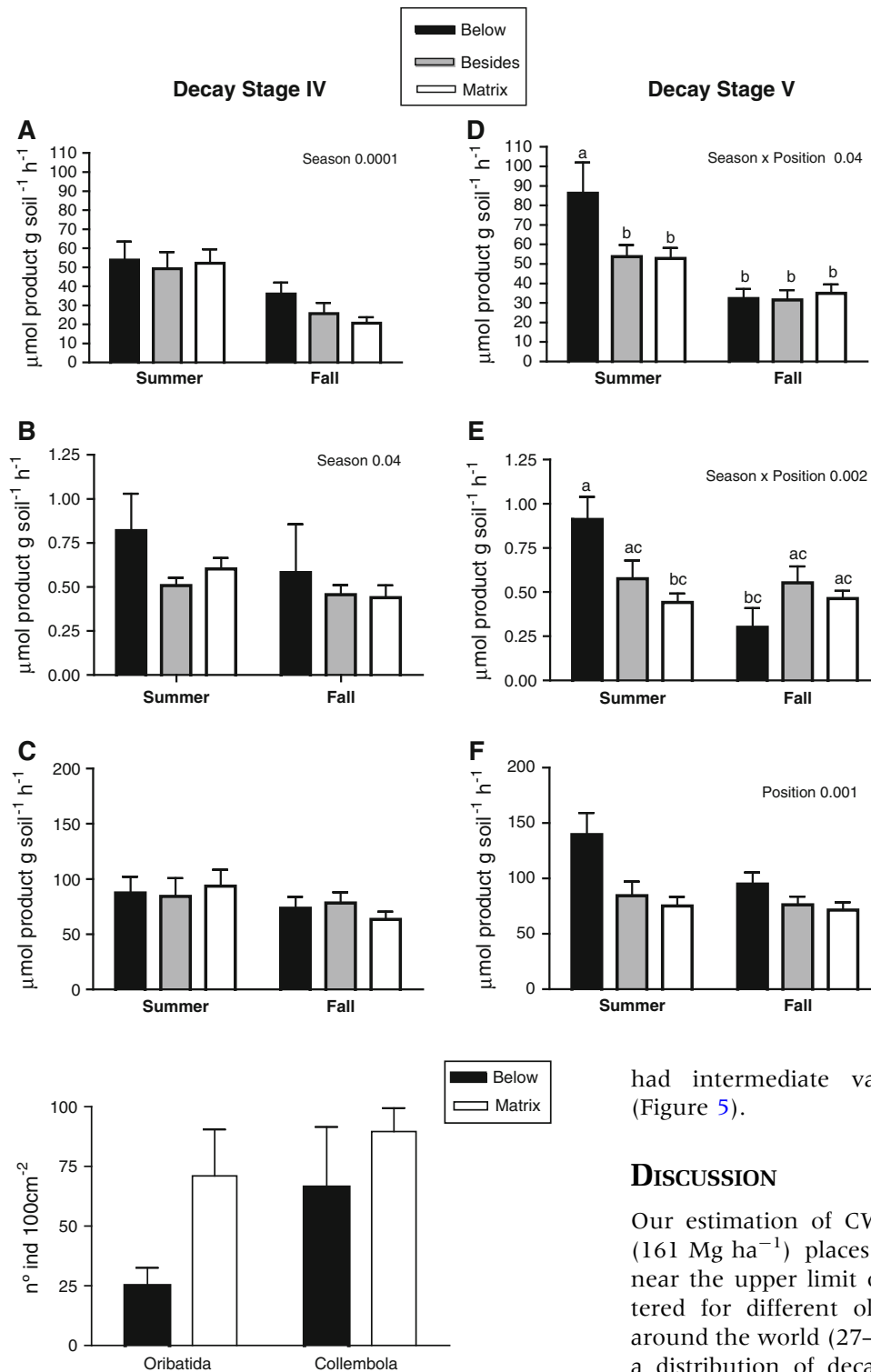


Figure 4. Effects of CWD on soil mesofaunal abundance. Data were analyzed using a two-way ANOVA, using position and decomposer group as factors. Both position ($P < 0.001$) and decomposer group ($P < 0.0001$) were significantly different, with a marked reduction of decomposer organisms under CWD.

Figure 3. Soil enzyme activity for different positions from CWD stage IV and V in summer and fall. β -Glucosidase activity for positions around CWD in decay stage IV (**A**) and in decay stage V (**D**). Phenol oxidase activity for positions around CWD in decay stage IV (**B**) and in decay stage V (**E**). Acid phosphatase activity for positions around CWD in decay stage IV (**C**) and in decay stage V (**F**). Values are means ($\pm$ SE). Post-hoc differences for significant interaction of season and position are shown. Different letters indicate significant differences using unequal N HSD.

had intermediate values across all microsites (Figure 5).

DISCUSSION

Our estimation of CWD in this forest ecosystem (161 Mg ha^{-1}) places this southern beech forest near the upper limit of the range of values registered for different old-growth temperate forests around the world ($27\text{--}179 \text{ Mg ha}^{-1}$, Table 3), with a distribution of decay classes similar to mature temperate forests in the Northern Hemisphere (Harmon and others 1986; McCarthy and others 2001). This study demonstrates that CWD in southern temperate forests clearly represent an important carbon reservoir (85 Mg ha^{-1}), which is similar in magnitude for estimates of organic soil

Table 2. Abundance of Mesofaunal Families (Mites and Collembolas) Below and Away from CWD in Decay Stage V (no. of ind. 100 cm⁻²)

			Below CWD (no. of ind. 100 cm ⁻²)	Soil (no. of ind. 100 cm ⁻²)	<i>P</i> value
Subclass Acari	Order Mesostigmata		29 (±10)	28 (±8)	n.s.
	Suborder Prostigmata		33 (±20)	29 (±7)	n.s.
	Cohort Astigmata		1 (±1)	4 (±2)	n.s.
	Suborder Oribatida	Family Oppiidae	8 (±3)	22 (±6)	0.06
		Family Nodocephidae	1 (±1)	10 (±3)	0.01
		Family Autognetidae	1 (±1)	4 (±2)	0.06
		Immature	7 (±3)	28 (±8)	n.s.
Class		Family Entomobryidae	13 (±6)	6 (±4)	n.s.
Collembola		Family Isotomidae	71 (±9)	47 (±21)	n.s.
		Superfamily Poduroidea	5 (±3)	12 (±7)	n.s.

Means (±SE) are shown.
n.s. indicates no significant difference.

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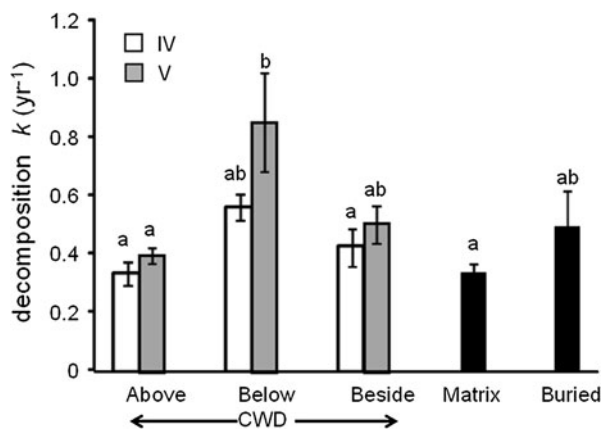


Figure 5. Effects of CWD on leaf litter decomposition. Bars indicate mean constants of decomposition after 2 year (k , yr^{-1}) of litter of one of the dominant overstory species, *Nothofagus dombeyi*. Black bars indicate positions on the surface soil and buried at 5 cm depth at a distance >2 m from CWD. Letters indicate significant differences for one-way ANOVA.

carbon (30 cm depth) in this site (120 Mg ha^{-1} , Hess 2013). Moreover, CWD is a substantial long-term N reservoir ($0.42 \text{ Mg N ha}^{-1}$); our estimates exceed those reported by Richter and Frangi (1992) for a *Nothofagus* forest in the extreme southern tip of South America in Tierra del Fuego ($0.152 \text{ Mg N ha}^{-1}$). In contrast with the soil C pool, the N reservoir of CWD is a smaller fraction compared to the storage in the soil N pool (8.5 Mg ha^{-1} , Hess 2013). As has been demonstrated for other old-growth temperate forests, the loss through removal of these reservoirs would substantially affect the carbon and nitrogen balance of the forest.

One of the principal legacies of CWD in forest ecosystems is its persistence in time and its slow decomposition (Harmon and others 1986), which allows for the development of long-term interactions between CWD and soil. The spatial segregation of soil ecosystem processes in this study supports the idea that “recalcitrant carbon islands” of CWD created specific conditions with proximal effects on carbon and nutrient turnover (for example, decomposition rate is almost threefold faster than in the soil matrix). As CWD decay advances, the spatial definition of the generated microsite becomes clearer, as evidenced by the differential effects of decay stage in this study. The time necessary to reach these advanced stages of decay can be close to a century (Mackensen and others 2003; Campbell and Laroque 2007), which may be very important for the detection of long-term interactions among biotic components of this ecosystem (Vivanco and Austin 2008; Austin and Marchesini 2012). Furthermore, it is worth noting that CWD decay stage V is far from the end of the decay process and the long-term impact of CWD may extend well beyond the patterns evaluated in this study. The eventual incorporation of the undecomposed CWD into the soil organic matter pools could generate different legacy effects than those reported here, and would be quite valuable to explore in future studies.

Microbial enzymatic activity and litter decomposition were both enhanced under CWD as compared to the forest soil matrix, particularly in the late stages of CWD decay and during the summer season for potential enzymatic activity. These results suggest that CWD may be important not only for static C and nutrient pools but also

Table 3. CWD in Different Forests Around the World: (A) Data Discriminated by Type of Disturbance: Without Human Disturbance (Old-Growth or Native System) and Plantation or Native Forest with Management Practices; (B) Data Discriminated by Early Successional Stage or Old-Growth Stage

A						
Forest type	Site	Dominant species	CWD definition	Old-growth or natural (Mg ha ⁻¹)	Plantation or managed forest (Mg ha ⁻¹)	Ref.
Temperate forest	Wyoming, USA	<i>Pinus contorta</i>	Diameter > 7.5 cm Downed logs, stumps and snags	29–121	52–123 (logging) 41–284 (fire)	Tinker and Knight (2000)
Temperate forest	Eastern USA	Mixed-oak forest: <i>Quercus velutina</i> <i>Q. rubra</i> <i>Betula lenta</i> <i>Acer rubrum</i> Plantation: <i>Pinus resinosa</i>	Diameter > 10 cm Downed logs, stumps and buried logs	27	40	Currie and Nadelhoffer (2002)
Tropical rainforest	La Selva, Costa Rica	ND	Diameter > 10 cm Downed logs	46	ND	Clark and others (2002)
Temperate forest	Kočevski Rog, Slovenia	<i>Abies alba</i> <i>Fagus sylvatica</i>	Diameter > 5 cm Downed logs, snags and stumps	40–179	49–57	Debeljak (2006)
Temperate forest	Australia	Natural forest Plantations	Diameter > 10 cm Downed logs	19–134	66.5 and 10.3	Woldendorp and Keenan (2005)
Temperate forest	Argentina	<i>Nothofagus nervosa</i> <i>N. obliqua</i> and <i>N. dombeyi</i>	Diameter > 7.6 cm Downed logs	161	ND	This study

Table 3. continued

Forest type	Site	Dominant species	CWD definition	Early-successional stage (Mg ha ⁻¹)	Old-growth stage (Mg ha ⁻¹)	Ref.
Temperate rainforest	Southern, Chile	<i>N. nitida</i> <i>Drimys winteri</i> <i>Podocarpus nubigena</i> <i>Laureliopsis philippiana</i>	Diameter > 10–5 cm Downed logs	9–42	65–31	Carmona and others (2002)
Temperate rainforest	Valdivian forest, Chile	<i>N. dombevi</i> <i>Laureliopsis philippiana</i> <i>Saxegothaea conspicua</i> <i>Dasyphyllum diacanthoides</i>	Diameter > 20 cm and length > 1 m Downed logs and snags	89 (<i>Nothofagus</i> -dominated stand)	59 (mixed-species without <i>Nothofagus</i>)	Schlegel and Donoso (2008)
Temperate forest	Kočevski Rog, Slovenia	<i>Abies alba</i> <i>Fagus sylvatica</i>	Diameter > 5 cm Downed logs, snags and stumps	40 (juvenile)	96 (optimal)	Debeljak (2006)
Boreal forest	Canada	<i>Picea mariana</i> <i>Populus tremuloides</i> <i>Pinus banksiana</i>	Diameter > 5 cm Downed logs and snags	80 (18 years)	10 (77 years)	Bond-Lamberty and Gower (2008)

affecting dynamic processes of turnover, including litter decomposition of overstory trees.

There are several potential mechanisms that could explain the enhancement of microbial enzymatic activity and decomposition due to CWD proximity effects. One is the buffering of environmental variation, evidenced by significantly higher soil moisture and continued high enzyme activity during the summer drought period (Figures 1, 2). Second is the increased DOC found in soils beneath CWD, which has been observed in other studies with woody debris addition (Lajtha and others 2005), and could have directly stimulated microbial activity due to increased labile carbon availability. Species identity and decay class appear to be important in determining the quality of SOM fractions found in soil beneath CWD (Zalamea and others 2007), and more detailed studies are needed to identify the relative importance of species identity on DOC leachates, and the effect of species identity was not directly evaluated in this study.

Finally, the presence of CWD could alter the composition and efficiency of the decomposer organisms. These effects could be due to direct effects of increased labile carbon with stimulation of microbial biomass as mentioned previously, indirect effects due to the presence of recalcitrant carbon compounds stimulating enzyme production (Allison and Martiny 2008), changes in microbial community composition due to the variability in substrate availability (Busse 1994), or alterations of the trophic interactions among organisms. Additions of woody debris to the forest floor increased soil fungal/bacterial ratio as well as increased respiration and soil microbial biomass in temperate conifer forests (Brant and others 2006), which is similar to patterns of increased activity observed in this study. Furthermore, the patterns of enzyme activity suggest that soil contact with CWD may select for microbial communities with higher capacities to metabolize complex compounds. Direct evidence for this increased capacity is the significant increase in phenol oxidase activity beneath CWD, a carbon-degrading enzyme that targets recalcitrant organic matter. Moreover, the ratio of β -glucosidase:phosphatase enzyme activity (carbon acquiring vs. phosphorus acquiring activity) did not change with distance from CWD in spite of significant changes in activity (Figure 3, ratios not shown). These results suggest that resources invested by the microbial community for P uptake are a constant proportion of the investment in obtaining C (Sinsabaugh and others 2008).

Oribatid mite and collembolan soil communities were also affected by the presence of CWD, although

the overall impact of CWD was to reduce mesofaunal abundance. The most represented families of oribatids were less abundant below CWD, which has been demonstrated in several studies (Seastedt and others 1989; Siira-Pietikäinen and others 2008), but contrasts with other studies that demonstrated no effect of CWD on mite abundance (Evans and others 2003; Déchéne and Buddle 2010) or increased abundance in the litter layer beneath CWD (Jabin and others 2004). Collembola and mites may serve as prey for species typically associated with CWD refuges, such as spiders and pseudoscorpions (Jabin and others 2004; Castro and Wise 2010), which could explain the reduced abundance of these soil fauna beneath CWD. Nevertheless, these observational differences in mite abundance may reflect a complexity of trophic interactions that extend beyond the direct effects of the presence of CWD (Castro and Wise 2010). For example, reduced predation of microbial communities by oribatid mites and collembola could contribute to increased microbial biomass and activity, suggesting that CWD may be affecting multiple biotic interactions simultaneously (Castro and Wise 2010). Although it is not entirely clear what is causing the changes in the soil decomposer community, viewed from the perspective of the effects of soil fauna on litter decomposition, which was significantly stimulated beneath CWD, these results are consistent with studies that have indicated an inverse relationship of mite abundance and rates of litter decomposition (Neher and others 2012).

Taken together, CWD altered a number of microenvironmental characteristics with consequences for carbon turnover and soil biotic activity. To evaluate the impact at the ecosystem scale, one way to consider the importance of CWD for ecosystem functioning is to quantitatively evaluate the impact of CWD removal in this ecosystem. In the study area, the total cover of CWD in decay stages IV and V is nearly 9%. Extrapolating the changes in enzymatic activity, for example, would mean that without CWD, potential β -glucosidase and phenol oxidase activity would be reduced by 3 and 6%, respectively, at the ecosystem scale, most notably due to changes in the summer season. The impact on litter decomposition would be even greater; the absence of CWD could decrease litter decomposition by 13% overall in comparison with a homogeneous forest floor. In a single year, these differences may not be detectable considering the large size of the organic matter pools in this ecosystem, but in the medium to long-term, these effects could substantially alter nutrient availability for the vegetation, and the quality and quantity of soil organic

carbon and nutrient pools. The proximity effects on ecosystem processes appear to be an unappreciated consequence of the presence of CWD in these old-growth ecosystems, but could clearly have relatively large impacts on biogeochemical cycles in the long-term.

CONCLUSIONS

CWD is an important component of many forest ecosystems and its value as specialized habitat and carbon reservoirs has been documented (Harmon and others 1986; Spies and others 1988; Currie and Nadelhoffer 2002). In this study, we demonstrate additional, previously unidentified roles of CWD-affecting soil organisms and ecosystem processes in its zone of influence. We conclude that CWD represents a substantial C and N pool in this forest ecosystem, as has been observed in other temperate forest ecosystems, and its presence in the ecosystem has significant effects not only on biogeochemical reservoirs, but on dynamic microbial and biotic processes affecting carbon and nutrient turnover. Moreover, the effect of CWD depends on the decay class, demonstrating that the spatial variability resulting from the presence of CWD is a long-term interaction that creates and maintains spatial variability in ecosystem processes. The removal of CWD due to harvest could have substantial impacts on C and N reservoirs as well as C turnover and microbial activity in this ecosystem. This study demonstrates that the presence and persistence of CWD in forested ecosystems have an ecological importance that should be taken into consideration for management decisions pertaining to carbon sequestration and functional diversity in natural forest ecosystems.

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