Litter Removal in a Sclerophyll Forest: Short- and Medium-Term Consequences for Soil Properties

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Centro de Estudios de Zonas Áridas Univ. de Chile Casilla 129, Coquimbo Chile Litter extraction (LE) is a common practice in many forests of the world. This process can cause long-term depletion of C substrates, thereby affecting ecosystem balances. The effects of LE on soil properties such as soil respiration (R_c), soil water content (θ), soil temperature (T), microbial activity, and dissolved organic C (DOC) are not well understood in various forests ecosystems. We investigated the short and medium-term effects of LE on these soil properties in a sclerophyll forest of central Chile. A completely randomized block design was set with three blocks and two treatments, i.e., a control (no LE) and LE totaling six 10- by 10-m plots. The R_s , θ , and T were determined immediately after LE and then at Days 4, 12, 16, and 20. The same properties were determined in the medium term (between Days 448-853). Soil organic C (SOC), basal respiration (C_{min}), microbial biomass C (C_{bio}), and microbial (qCO₂, qMic) and mineralization (qMin) quotients were determined at Day 711 after LE from soil cores obtained at depths intervals of 0 to 3, 3 to 6, and 6 to 9 cm. Soil pore water was extracted from suction lysimeters during the rainfall season of 2011 and analyzed for DOC, specific ultraviolet absorbance, the ratio between the absorbance at 465 and 665 nm in water extracts, and electric conductivity. Litter extraction caused large reductions in R_s in the short term (33%) and smaller reductions in the medium term (21.2%). In addition to the effect of LE, R_s was governed by θ . The SOC, C_{\min} , qCO_2 , qMic, and qMin were unaffected by LE, but DOC significantly decreased with LE by 59.6% (10-cm depth) and 48.8% (30-cm depth). The DOC was comprised of aromatic-rich, low- molecular-weight compounds in both treatments.

Abbreviations: DOC, dissolved organic carbon; EC_p , pore water electrical conductivity; LE, litter extraction; SOC, soil organic carbon; SOM, soil organic matter; SUVA, specific ultraviolet absorbance.

P orest litter is the interface between the aboveground plant biomass and the soil, representing an important C storage compartment (Raich and Nadelhoffer, 1989; Reynolds and Hunter, 2001; Berg and McClaugherty, 2008). Additionally, forest litter creates a protective layer on the topsoil that regulates the soil climate (Sayer, 2006; Berg and McClaugherty, 2008). Anthropogenic disturbances such as forest harvesting or fires alter the forest floor, creating a new soil chemical environment and new microclimatic conditions that finally affect microbial populations and thereby soil processes such as respiration (R_s), mineralization of organic substrates, and synthesis of new organic compounds such as DOC.

The soil microbial biomass (C_{bio}) is an important component of forest ecosystems, regulating plant litter decomposition and consequently acting as both a source and a sink for nutrients (Wardle, 1993; Thirukkumaran and Parkinson, 2000). The microbial processes of mineralization and immobilization, in which

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 $C_{\rm bio}$ is a key factor, affect nutrient availability and consequently forest productivity (Taylor et al., 1999). The soil microbial biomass is also considered a sensitive indicator of organic matter dynamics (Hu et al., 2006). Microbe-related parameters such as the ratio between CO₂–C evolution ($C_{\rm min}$) and $C_{\rm bio}$ (the qCO₂ quotient) or the ratio between $C_{\rm bio}$ and SOC (the qMic quotient) have been widely used as quantitative indicators of microbial efficiency and C dynamics (Putasso et al., 2010). Moreover, these microbial quotients can be indicators of the recalcitrance or availability to soil microorganisms of substrate organic compounds (Moscatelli et al., 2005; Hu et al., 2006).

In the last decades, $R_{\rm s}$ has been considered one of the main fluxes of C in terrestrial ecosystems. Approximately 70% of the CO₂ exchanged between forest ecosystems and the atmosphere comes from the soil (Raich and Schlesinger, 1992; Raich and Potter, 1995; Luo and Zhou, 2006). The release of CO₂ from the soil to the atmosphere depends on both root (autotrophic) and microbial (heterotrophic) respiration. The latter process is mainly associated with plant litter decomposition and the mineralization of soil organic matter (SOM) (Hanson et al., 2000). Environmental factors, such as temperature and water availability, regulate the contribution of autotrophs and heterotrophs to R_s at spatial and temporal scales (Hanson et al., 2000; Raich and Tufekcioglu, 2000). Seasonal variations in R_s are mainly associated with changes in soil temperature and soil water content (Han et al., 2007). Both properties affect soil microbial activity, organic matter decomposition, root growth, and plant productivity (Wiseman and Seiler, 2004; Jabro et al., 2008). In temperate forests, about 50 to 60% of R_s is due to the metabolic activity of roots and associated mycorrhizae. The remaining fraction (40-50%) is generated by the activity of microbial populations that decompose plant debris and SOM (Hanson et al., 2000; Rey et al., 2002; Epron et al., 2004). Thus, the quality and quantity of plant litter and the resulting soil organic matter constitutes an important mechanism controlling $R_{\rm s}$ (Epron et al., 2004; Khomik et al., 2006). Plant litter removal can reduce soil respiration by about 25% (Luo and Zhou, 2006) and up to 33% if litter and more decomposed organic layers such as the Oi and Oa horizons are extracted (Saiz et al., 2007). In quantitative terms, R_s is regulated by microclimatic variables such as air temperature, wind speed, and several soil processes and properties such as the CO₂ production rate, soil-atmosphere CO₂ gradient, pore size distribution (Jabro et al., 2008), soil temperature, soil water content (θ) (Raich and Schlesinger, 1992), N availability (Madritch and Hunter, 2003), cation exchange capacity, soil acidity (Borken et al., 2002), and C_{bio} (Buchmann, 2000).

Dissolved fractions of soil organic matter play an important role in the movement of C, N, and P from litter to soil (Neff and Asner, 2001; Cleveland et al., 2001), complexation of metal cations in solution (Weng et al., 2002; Antoniadis and Alloway, 2002), increases in the rate of weathering of minerals (Lundström and Ohman, 1990; Neff and Asner, 2001), mineral–organic adsorption reactions (Kothawala et al., 2008), and as a substrate for microbial growth (Smolander and Kitunen, 2002; Neff and Asner, 2001). One of the most important fractions of dissolved organic matter is DOC (Weishaar et al., 2003), which is defined as the organic C that passes through a nonreactive, 0.45-µm filter (Kolka et al., 2008). The primary source of DOC is plant litter because an important fraction of it is highly soluble and able to move into the soil with rainfall. Additional controls on this eluviation-illuviation process are environmental factors such as temperature, soil water content, and the action of microorganisms (Zsolnay, 2003; Cleveland et al., 2001). The concentration and biochemical properties of DOC have been evaluated for land use changes (Kalbitz et al., 1999; Kalbitz, 2001), anthropogenic soil acidification (Zech et al., 1994), and N deposition (Magill and Aber, 2000), as well as under different soil conditions, forest types, and plant tissues (Moore and Dalva, 2001; Glatzel et al., 2003; Ghani et al., 2010). The effect of litter on DOC has also been studied under field conditions where DOC inputs from fresh litter have been evaluated in terms of their interaction with the soil matrix (Fröberg et al., 2007). Controlled laboratory experiments have also contributed to our understanding of the relationship between litter quality and DOC characteristics (Magill and Aber, 2000; Bourbonniere and Creed, 2006). The DOC flux is small compared with other C ecosystem fluxes, but it plays an important role in litter and organic horizon C balances and contributes to in-depth soil processes linked to SOC (Kolka et al., 2008).

Litter extraction for animal bedding was commonly practiced in Europe (Sayer, 2006). In other countries, litter extraction is still a common practice, where plant litter is used as garden mulch (United States) or as a fuel source (China) (Sayer, 2006). The main effects of this practice on soils were summarized in the work of Sayer (2006), who indicated that litter removal causes increased bulk density, enhanced runoff erosion, nutrient depletion, alterations in microbial communities, decreases in R_s , temperature fluctuations, and lower soil water contents during dry periods. In sclerophyll (woody plants with small leathery evergreen leaves) forests of central Chile, litter removal is related to the use of plant litter as a seedbed in gardens. This old and frequent practice has contributed to the degradation of these ecosystems. However, studies regarding the direct effect of litter removal on soil properties do not exist for this type of forest ecosystem. We hypothesized that, in the short and medium terms, plant litter extraction decreases soil respiration and microbial biomass, and changes the quantity and quality of DOC as a consequence of changes in soil water content, temperature, and the depletion of SOC substrates. The aim of this study was to evaluate the short- and medium-term effects of litter extraction (i.e., the first 24 d and after 448 d) on C_{bio} and associated biological indicators, available fractions of N, P, and K, SOC, in situ R_s , soil temperature and θ , as well as the concentration and quality of DOC in a sclerophyll forest of central Chile.

MATERIALS AND METHODS Study Area and Site Description

The study area is located in central Chile $(34^{\circ}7'36'')$ S, 71°11′18″ W, 247 m asl), near the city of Santiago (Fig. 1), and is part of an ongoing research project to investigate the quality and fluxes of SOC as affected by anthropogenic perturbations of sclerophyll vegetation. The climate is Mediterranean, with a mean annual precipitation of 503 mm and maximum and minimum annual air temperatures of 29 and 3°C, respectively (Corporación Nacional Forestal, 2008). The study site is located in a toe slope position (<4% slope) and represents a typical example of the natural vegetation of the region. The site has fewer anthropogenic disturbances than similar ecosystems of the region (i.e., firewood extraction and forest fires occurred around 50 yr ago), with a natural flora comprised of peumo [Cryptocarya alba (Molina) Looser], quillay (Quillaja saponaria Molina), boldo (Peumus boldus Molina), and litre [Lithraea caustica (Molina) Hook. & Arn.]. Annual litter inputs from the canopy to the soil are 314 ± 30 g (dry weight) m⁻² yr⁻¹. Total C and N concentrations (% w/w) in the Oi horizon are 49.86 \pm 1.25 and 0.86 \pm 0.08, respectively. The Oe horizon has C and N contents of 33.56 \pm 5.49 and 1.22 \pm 0.27%, respectively. A summary of the main stand characteristics of the site as well as some chemical characteristics of the litter layers are given in Tables 1 and 2. The soil is classified in the Pachic Humixerepts subgroup and has developed from alluvial granitic deposits, with the particle size distribution dominated by the sand fraction. It has well-developed organic horizons (Oi, Oe, and Oa) on the surface and an A horizon rich in humified SOM.

Experimental Setup

Measurements were taken from six 10- by 10-m plots arranged in a complete randomized block design. There were

three blocks representing lower, middle, and upper slopes within the forest stand (Table 1). Blocks were about 10 m apart following a small slope gradient of <4%. Within each block, one plot was a control (C) and one plot had the litter removed (LE). At each plot, six cylinders (polyvinyl chloride [PVC], 10-cm internal diameter, 8-cm length) were randomly installed. The cylinders were inserted manually into the soil to a depth of 6 cm, avoiding major soil and organic layer disturbances. One week after insertion of the collars, soil litter layers (Oi and part of the Oe + Oa horizons) were raked from the LE treatment. For the inner sectional area of the cylinders under the LE treatment, the litter was manually removed. The LE treatment was equivalent to an average extraction (± 1 SD) of 0.92 \pm 0.11 and 1.44 \pm 0.14 kg dry mass m⁻² of the Oi and Oe + Oa horizons, respectively. All cylinders were left undisturbed throughout the experiment.

In Situ Determination of Soil Biological and Physical Properties

Soil respiration (R_s , g CO₂ m⁻² h⁻¹) and volumetric soil water content (θ , m³ m⁻³) in the top 6.5-cm depth and soil temperature (°C) at the soil surface (T_s) and at depths of 6.5 $(T_{6.5})$ and 10 cm (T_{10}) were measured immediately after the extraction of the organic layers (1 Oct. 2009, Day 1) and then at Days 4, 12, 16, and 20 after litter extraction. In the medium term, R_s , θ , T_s , T_{65} , and T_{10} were measured at Days 448 (December 2010) and 552, 608, and 672 (April, June, and August 2011, respectively), and 853 (February 2012) after litter extraction. Soil respiration was measured in each cylinder using a portable, closed chamber (Model SRC-1, PP systems; 10-cm diameter and 15-cm height) connected to an infrared gas analyzer (Model EGM-4, PP Systems; measurement range 0–2000 µmol mol⁻¹). Volumetric soil water content in the top 6.5 cm of soil was determined with a portable capacitance sensor (Sensor Model WET-2, Delta-T Devices; 500-cm³ sample volume) connected to a datalogger (HH2 moisture meter, Delta-T Devices). This sensor also includes a temperature probe, allowing instant soil temperature determinations at the 6.5-cm depth. In addition, the soil permittivity and temperature data obtained by the sensor were used for numerical calculation of the soil pore water conductivity (EC_p, mS m⁻¹; Delta-T Devices, 2005). Soil temperature at a depth of 10 cm was measured using a portable digital thermometer (Checktemp 1, Hanna Instruments). The surface soil temperature was also measured with a portable infrared thermometer (IR Wide Range Non-Contact Thermometer, Extech Instruments), immediately after each measurement of R_s . Soil water content and soil temperature readings were made between 10 and 20 cm



Fig. 1. Location of the study site in central Chile.

Table 1. Dominant tree species, biometric characteristics of the forest stand, and chemical properties of the Oi horizon according to tree species.

Property	Cryptocarya alba	Peumus boldus	Quillaja saponaria	Lithraea caustica	All individuals
Basal area, m ² ha ⁻¹	$6.78 \pm 2.92 \dagger$	5.35 ± 2.22	1.32 ± 1.75	0.84 ± 0.97	14.28 ± 4.56
Diameter at breast height, cm	12 ± 1.45	4.7 ± 0.75	24.97 ± 8.51	8.02 ± 4.29	6.51 ± 1.39
Mean height, m	8.46 ± 5.99	4.95 ± 0.82	7.88 ± 1.7	5.33 ± 1.53	5.06 ± 0.87
Stand density, no. ha ⁻¹	508 ± 357	1908 ± 902	25 ± 27	158 ± 204	2600 ± 978
Leaf area index‡, m ² m ⁻²					1.88 ± 0.19
Ground cover [‡] , m ² m ⁻²					0.54 ± 0.03

 \pm Means of three replicate study plots \pm SD.

‡ Leaf area index and ground cover determined by hemispherical picture analysis.

away from the collars, avoiding the alteration of litter and soil inside them.

Soil Sampling and Laboratory Determination of Soil Chemical and Biological Properties

Soil samples were extracted using a hammer-driven soil core sampler (Soilmoisture Equipment Corp.). Soil cores (5.4-cm diam.) were extracted at a distance of 30 to 40 cm from each PVC cylinder and at depth intervals of 0 to 3, 3 to 6, and 6 to 9 cm. The cores were obtained during Days 4, 12, 24, and 711 (9 Sept. 2011) after litter extraction. Soil samples were stored at 4°C (maximum of 4 d) until laboratory analyses were performed. Soil pH, available N, P, and K, and SOC were determined from these soil cores in both the control and LE treatments. Soil chemical properties were evaluated according to the recommended methods of analysis for Chilean soils (Sadzawka et al., 2004). Briefly, SOC was determined by wet combustion and colorimetric determination of the reduced chromate. Available N (N_{av}) was determined by KCl extraction, steam distillation, and posterior determination of NH3 by titration. Available P (P_{Olsen}) was determined by extraction with 0.5 mol L⁻¹ NaHCO₃ at pH 8.5 and posterior colorimetric determination. Available K (K_{av}) was determined by extraction with 1 mol L⁻¹ NH₄OAc at pH 7.0 and posterior determination by atomic absorption spectrophotometry.

Microbial biomass C was estimated in soil samples obtained in September 2011 (Day 711 after litter extraction) by the chloroform fumigation method (Horwath and Paul, 1994). A total of 25 g (equivalent dry weight) per core replicate was placed in a mason jar (1000 cm³) with one vial containing 10 mL of 1 mol L⁻¹ NaOH and another vial with 10 mL of deionized water. After placement of the vials, the jars were sealed and stored at 23°C for 10 d. The soil was incubated at 60% of the water retention at 33 kPa, which was determined in soil samples obtained at 0- to 3-, 3-

to 6-, and 6- to 9-cm depth intervals. Samples were slowly saturated from the bottom with degassed tap water and then equilibrated for 96 h at -33 kPa matric potential in a pressure plate extractor (Soil Moisture Equipment Corp.) equipped with a 0.1-MPa ceramic plate. Average soil water retentions at -33 kPa were 0.43 ± 0.03 , 0.25 ± 0.01 , and 0.20 ± 0.01 (kg kg⁻¹ dry soil) for the 0- to 3-, 3- to 6-, and 6- to 9-cm depth intervals, respectively.

Each replicate consisted of a fumigated and an unfumigated subsample. The value of $C_{\rm bio}$ (kg C kg⁻¹soil) was calculated as (Horwath and Paul, 1994)

$$C_{\rm bio} = \frac{C_{\rm f} - 0.22C_{\rm min}}{k_{\rm c}}$$
[1]

where $C_{\rm f}$ (kg CO₂-C kg⁻¹ soil) is the CO₂-C produced from a chloroform-fumigated sample, $C_{\rm min}$ (kg CO₂-C kg⁻¹ soil) is the CO₂-C produced from an unfumigated (control) sample, and $k_{\rm c}$ is the fraction of $C_{\rm bio}$ that is mineralized to CO₂. The value of $k_{\rm c}$ is considered a constant equal to 0.41 (Horwath and Paul, 1994). Following the recommendations of Smith et al. (1995), we used 22% of the measured value of $C_{\rm min}$ for our calculations.

With the data for SOC, C_{\min} , and C_{bio} , the soil ecological indicators microbial metabolic quotient (qCO₂), microbial quotient (qMic), and mineralization quotient (qMin) were calculated as

$$CO_2 = \frac{C_{\min}}{C_{\min}}$$
[2]

qC

Property†	Cryptocarya alba	Peumus boldus	Quillaja saponaria	Lithraea caustica	
Lignin, %	20.64	10.71	19.47	31.60	-
Cellulose, %	19.15	30.40	11.03	18.37	
Hemicellulose, %	10.96	3.67	9.72	10.34	
C, %	52.23	38.83	52.25	54.35	
N, %	0.61	0.63	0.51	0.77	
P, %	0.09	0.07	0.05	0.08	
К, %	0.64	0.46	0.93	0.79	
Ca, %	2.05	2.00	2.65	1.34	
Mg, %	0.20	0.16	0.47	0.19	

Table 2. Chemical properties of different leaf species collected from the Oi horizon.

+ Lignin, cellulose, and hemicellulose determined by the acid-detergent fiber method (Van Soest, 1963); C and N contents determined by dry combustion (Dumas); P determined by dry combustion at 500°C and posterior colorimetry of the H₃PO₄ formed with vanadium molybdate; K, Ca, and Mg determined by dry combustion at 500°C and posterior analysis by atomic absorption spectroscopy.

 $qMic = \frac{C_{bio}}{SOC}$ [3]

$$qMin = \frac{C_{min}}{SOC}$$
 [4]

Values of $C_{\rm bio}$ were expressed in milligrams C per kilogram soil; $C_{\rm min}$ in milligrams $\rm CO_2-C$ per kilogram soil per hour; qCO₂ in grams $\rm CO_2-C$ per kilogram $C_{\rm bio}$ per hour; qMic as a percentage by weight; and qMin in grams $\rm CO_2-C$ per kilogram SOC per hour × 10.

Lysimeter-Extracted Soil-Pore Water Properties

At each plot, two suction lysimeters (Models 1900L06-B02M2 and 1900L12-B02M2, Soilmoisture Equipment Corp.) were randomly installed at depths of 10 and 30 cm in the spring of 2010. Each lysimeter (4.8-cm external diameter) was inserted in a 5-cm-diameter hole that was covered at the bottom with a 0.5-cm-thick layer of glass beads. This layer allowed a good soil–ceramic cup hydraulic contact. Potential preferential flow in the contact zone between the lysimeter tube and the soil was avoided with a polyethylene ring placed at the top of each lysimeter on the soil surface. No water was sampled during the first rainfall season (2010), avoiding the effects of disturbances caused by lysimeter installation.

The soil pore water was extracted during the rainfall season of 2011 (7 July; 4 and 23 August; and 2, 8, and 13 September: Days 643, 671, 684, 690, 700, 706, 711 after litter extraction) using 50-mL soil water samplers (Model 1900K2 extraction kit, Soil Moisture Equipment Corp.). The rainfall amounts and distribution during the experimental setup (2010) and monitoring (2011) were representative of the climate of the area. Water sampling was done around 15 h after each precipitation event. Water samples were immediately stored in amber, 100-mL, polyethylene terephthalate bottles, kept on ice during transport, and refrigerated until laboratory analysis. Each soil water sample was passed through a 0.45-µm glass filter and analyzed for DOC using a total organic C analyzer (Shimadzu TOC-5000A). The reactivity of DOC was evaluated by the specific ultraviolet absorbance (SUVA), which corresponds to the ratio between the absorbance at 254 nm (m^{-1}) and the DOC concentration $(mg C L^{-1})$. This parameter is considered an average absorptivity for all the molecules of the DOC contained in a water sample and has been used as an alternative measurement for DOC aromaticity (Weishaar et al., 2003; Wickland et al., 2007). In addition, the absorbance at 465 and 665 nm was determined to calculate the E_4/E_6 ratio, which has been related to the particle size characteristics of humic substances. This ratio can be an indirect indicator of the molecular weight of organic compounds contained in a water sample (Chen et al., 1977). The absorbance at 254, 465, and 665 nm was determined in each filtered sample using a UV-VIS spectrophotometer (Rayleigh Model UV-1601) and a 1-cm path length quartz cell. Deionized water was used as a blank. The electric conductivity of the filtered water extracts was determined with a conductivity meter (Schott Geräte GmbH, Model CG 853).

Data Analysis

All the analyses were undertaken using R (Version 2.15, R Development Core Team, 2010; www.r-project.org/). All nonstatistical independent replicates (pseudo-replicate measurements) were averaged to yield one value per plot at each sampling date. Variables were tested for normality and homogeneity of variance, and transformations were made as necessary to meet the underlying statistical assumptions of the models used. Repeated measures analysis of variance was performed to test the main and interactive effects of litter extraction and sampling date (or soil depth) on soil chemical and biological properties. When significant differences were found, we used adjusted *P* values to control the familywise error rate using Bonferroni's test. Analysis of covariance was used to test whether slopes and intercepts of the linear relationships between R_s and θ were significantly different between the control and the LE treatment.

RESULTS AND DISCUSSION Soil Respiration, Water Content, and Temperature

Soil respiration was significantly affected by the main effect of litter extraction (P < 0.001), time (P < 0.001), and the litter extraction \times time interaction (P < 0.001). This shows that R_s was greater in the control than the LE treatment and that such effect was attenuated from the short to the medium term. From Days 4 to 20, soil LE reduced R_s from an average (±1 SE, n =15) 0.63 \pm 0.02 in the control to 0.42 \pm 0.04 g CO₂ m² h⁻¹ in the LE treatment (Fig. 2). From Days 448 to 853, LE reduced $R_{\rm s}$ from (±1 SE, n = 15) 0.31 ± 0.05 in the control to 0.24 ± 0.03 g CO $_2$ m 2 h $^{-1}$ in the LE treatment. These 33.3% (short-term) and 21.2% (medium-term) average R_{c} losses are in the range (20-40% decrease) also reported for other forest ecosystems (Buchmann, 2000; Vasconcelos et al., 2004; Saiz et al., 2007; Sayer et al., 2007) and can be attributed to the depletion of readily decomposable C substrates of the litter layers (Sayer et al., 2007; Wenjie et al., 2008).

In addition to the effect of substrate availability on soil respiration, values of R_s with time (considering the short- and medium-term data) were analyzed in terms of their correlation with θ and $T_{6.5}$. Variations in R_s were mainly explained by θ (r = 0.75, P < 0.001) followed by $T_{6.5}$ (r = -0.30, P < 0.012). A high correlation between R_s and θ has been previously reported for semiarid and Mediterranean ecosystems in which θ was considered the critical environmental determinant (Conant et al., 2004; Rey et al., 2011). Analysis of covariance showed that slopes (P < 0.001) and intercepts (P < 0.001) of the linear relationship between R_s and θ were significantly different between the LE and control treatments. Thus, we fitted individual models for the LE and control treatments. When R_s was correlated to θ and T_{65} , the latter became nonsignificant (P = 0.10), and therefore we present the simpler version (Fig. 3). In Mediterranean ecosystems, the correlation between R_s and the combination of the effects of soil temperature and θ can be closely associated; however, some of the studies in these ecosystems have also indicated that the best predictions of R_s are obtained with θ alone (Reichstein et al., 2002), while other studies have found the best correlations with soil temperature (Pavelka et al., 2007) or have indicated that temperature itself is not a good predictor of R_{s} (Davidson et al., 2000; Rey et al., 2002).

Soil Chemical and Biological Properties Available Nitrogen, Phosphorus, and Potassium and Pore Water Conductivity

After 4 d of treatment application, LE did not cause a significant variation in available N (P = 0.235). The same trend was



Fig. 2. Rainfall, soil respiration (R_s), volumetric water content (θ) in the top 6.5 cm of soil, and soil temperatures at the surface (T_s) and 6.5- ($T_{6.5}$) and 10-cm (T_{10}) depths under the control and litter extraction (LE) treatments. Values are presented as means (\pm 1 SE) for each sampling date and treatment. Significant differences between the litter extraction vs. the control treatment are shown as *P* range: ns, nonsignificant; *significant at *P* < 0.05; **significant at *P* < 0.001.

observed after 12, 24, and 711 d of LE (14 and 27 Oct. 2009 and 9 Sept. 2011), which indicates that the remaining topsoil organic matter under the LE treatment was able to counteract the effect of litter depletion, maintaining mineralization rates found under the control treatment (Table 3). Variations in nutrient levels between treatments can be indirectly detected by measuring the soil pore-water electric conductivity (EC_p). In our study, we determined EC_p at the 6.5-cm depth in the short term (based on the numerical approximation used by the WET-2 sensor). The results obtained for EC_p were in agreement with the available N, P, and K results. Values of EC_p were only affected by time (*P* < 0.01) but not by LE (*P* = 0.20) or the time × LE interaction (*P* = 0.46). Values of EC_p were (±1 SE, *n* = 15) 5.6

 \pm 0.1 mS m⁻¹ for the LE treatment and 6.1 \pm 0.2 mS m⁻¹ for the control treatment. Values of EC_p increased in the short term in both the control and LE treatments, which can be related to the slight decrease in θ , which causes a natural increase in the concentration of solutes. In addition, the biological transformation of SOM causes the release of H⁺ ions, which can compete with the nonacid ions for the colloidal exchange sites, favoring their release to the soil solution and thereby increasing EC_p (García-Gil, 2001).

At the end of the experiment, available P and K were influenced only by the main effect of soil depth but not by LE or their interaction. Because P and K are less mobile than N, the effects were not as marked as for N, although available P and K tended to decrease in the medium term.

Soil Microbial Biomass and Related Ecological Indicators

In the medium term, $C_{\rm bio}$ significantly decreased with soil depth (P < 0.004) but not with LE (P = 0.339) (Table 3), while their interaction was not significant (P = 0.55). The C_{bio} value was significantly greater ($n = 6, \pm 1$ SE) in the top 3-cm depth (1796 \pm 532 mg C kg⁻¹ soil) compared with 3 to 6 cm (804 \pm 169 mg C kg^{-1} soil) and 6 to 9 cm (519 \pm 109 mg C kg^{-1} soil) depth intervals. This is consistent with the normal trend of reduced substrate availability with soil depth found under forest ecosystems. Despite the fact that studies about the effects of litter extraction on biological properties are still scarce (Mariani et al., 2006), several studies have indicated that microbial activity, particularly near the soil surface, can decrease as a result of SOM depletion (Castillo and Joergensen, 2001; Tan et al., 2005) or extreme changes of microclimatic conditions and soil physical properties (Mariani et al., 2006). Wang et al. (2010) found that litter removal affected C_{bio} , but the effect was controlled by plant community composition and

community productivity. Other studies have found no significant effect of litter removal on $C_{\rm bio}$ (Li et al., 2004; Mariani et al., 2006). In our case, the depletion of organic substrates caused by LE did not affect the microbial biomass. It seems, therefore, that the topsoil resiliency of this forest ecosystem allowed maintenance of the microbial population.

Soil organic C, basal respiration (C_{\min}) , the metabolic quotient qCO₂ (the ratio between C_{bio} and C_{\min}), the microbial quotient qMic (the ratio between C_{bio} and SOC), and the mineralization quotient qMin (the ratio between C_{\min} and SOC) were unaffected by LE (Table 3). This indicates that the removal of important amounts of soil organic matter under this type of forest ecosystem did not significantly change the inherent



Fig. 3. Relationship between soil respiration (R_s) and volumetric water content (θ) at the 6.5-cm depth in the control and litter extraction (LE) treatments.

characteristics of the remaining SOM as well as the activity of the microbial population. Other studies have found significant variations in some of these properties as a result of changes in the quality of the SOM. For instance, Vance and Chapin (2001) found lower qMic values under taiga forest floors with increased C/N ratios (a well-known indicator of SOM recalcitrance). Liao and Boutton (2008), studying the response of the soil microbial biomass to woody plant invasion of a grassland savanna in Texas, also found a decrease in qMic as a result of increased resistance to decomposition of organic matter inputs from wooded areas compared with grassland areas.

Soil-Pore Water Properties

In the medium term, the electric conductivity of the lysimeter extracts was not significantly affected by litter extraction (P = 0.39 and 0.76 for the 10- and 30-cm depths, respectively). Overall mean values were 12.7 ± 0.7 and 11.1 ± 0.9 mS m⁻¹ for the control and LE treatments, respectively. Significant variations in EC were found only with time (P < 0.01) for lysimeter extracts at the 30-cm depth, which is attributable to the different solute composition of the soil-pore water as a result of differences in the amount, rate, and time between each precipitation event. Low soil water flows associated with small precipitation events allowed the soil water to interact with the matrix and evolve in terms of its ionic composition (Worrall et al., 2002). The nonsignificant effect of time on the lysimeter water extracts from the top 10-cm depth can be explained by the high capacity of SOM to control adsorption– desorption reactions between the solid and liquid phases.

Lysimeter water extracts were slightly acidic and not significantly affected by LE (P = 0.11 and 0.84 for the 10- and 30-cm depths, respectively). The potential release of H⁺ ions was apparently counterbalanced by the high buffer capacity that the remaining SOM gave to the soil. In the top 10-cm depth, pH values were 6.61 ± 0.09 and 6.71 ± 0.1 for the control and LE treatments, respectively. At the 30-cm depth, pH values were in the same range, with values of 6.70 ± 0.10 and 6.60 ± 0.08 for the control and LE treatments, respectively. Variations in pH were found with time (P = 0.019 and 0.011 for the 10- and 30-cm depths, respectively).

Dissolved organic C was significantly reduced in both depths (P = 0.061 and 0.03 for the 10- and 30-cm depths, respectively) independent of rain events (P = 0.26) (Fig. 4). In the

Table 3. Soil biological and chemical properties in the control and litter extraction (LE) treatments at three soil depths.

	0–3 cm		3–6 cm		6–9 cm		ANOVA statistics [‡]		
Son propertyr	Control	LE	Control	LE	Control	LE	L	S	L×S
$C_{\rm bio'}$ mg C kg ⁻¹ soil	1991 ± 422§	1602 ± 142	896 ± 87	714 ± 89	572 ± 33	467 ± 78	ns	A-B-B¶**	ns
SOC, % (w/w)	14.3 ± 4.1	13.0 ± 1.9	5.4 ± 0.8	5.0 ± 0.5	3.3 ± 0.1	3.0 ± 0.3	ns	A-B-B***	ns
C _{min′} mg CO ₂ −C kg ^{−1} soil h ^{−1}	1.53 ± 0.49	1.32 ± 0.1	0.66 ± 0.09	0.54 ± 0.04	0.39 ± 0.03	0.34 ± 0.03	ns	A-B-B***	ns
qCO ₂ , g CO ₂ –C kg ⁻¹ C_{bio} h ⁻¹	0.74 ± 0.08	0.84 ± 0.06	0.72 ± 0.05	0.798 ± 0.11	0.70 ± 0.07	0.90 ± 0.26	ns	ns	ns
qMic, %	1.49 ± 0.13	1.28 ± 0.13	1.68 ± 0.10	1.44 ± 0.14	1.72 ± 0.12	1.52 ± 0.11	ns	A-AB-B*	ns
qMin, g CO ₂ –C kg ⁻¹ SOC h ⁻¹ ×10	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.00	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.03	ns	ns	ns
$N_{av'} \text{ mg kg}^{-1}$ (4 d after extraction)	7.4 ± 1.4	13.5 ± 4.2	4.5 ± 1.3	5.7 ± 0.1	3.0 ± 1.0	3.0 ± 0.6	ns	A-B-B*	ns
$N_{av'}$ mg kg ⁻¹ (12 d after extraction)	7.5 ± 2.1	8.5 ± 1.8	3.7 ± 0.6	6.7 ± 1.4	3.1 ± 0.1	4.6 ± 0.8	ns	A-AB-B*	ns
$N_{av'}$ (mg kg ⁻¹ (24 d after extraction)	10.3 ± 1.5	11.7 ± 2.2	6.6 ± 0.5	7.1 ± 1.2	4.8 ± 0.5	7.0 ± 1.3	ns	A-B-B*	ns
$N_{av'}$ mg kg ⁻¹ (711 d after extraction)	3.6 ± 1.3	0.4 ± 0.1	2.6 ± 1.5	1.8 ± 0.6	7.0 ± 2.6	1.9 ± 1.3	ns	ns	ns
$P_{Olsen'}$ mg kg ⁻¹ (711 d after extraction)	7.3 ± 1.8	6.4 ± 1.4	3.9 ± 1.4	3.1 ± 1.2	2.8 ± 1.4	1.8 ± 1.4	ns	A-B-B***	ns
K_{av} mg kg ⁻¹ (711 d after extraction)	135.3 ± 5.9	123.1 ± 3.1	82.8 ± 2.2	72.8 ± 6.0	60.1 ± 2.3	55.7 ± 3.0	ns	A-B-C***	ns

* Significant difference between LE and control at *P* < 0.05; ns, not significant.

** Significant difference between LE and control at P < 0.01.

*** Significant difference between LE and control at P < 0.001.

+ $C_{\text{bio'}}$ microbial biomass C; SOC, soil organic C; $C_{\text{min'}}$ basal respiration; qCO₂, microbial metabolic quotient; qMic, microbial quotient; qMin, mineralization quotient; N_{av} available N; P_{Olsen}, available P; K_{av} available K.

* Separation of means was determined by a Bonferroni test when applicable over the main effects of soil depth. L, main effect of litter removal; S, main effect of soil depth; L'S, interactive effect of L and S.

§ Means \pm SE.

¶ Different uppercase letters indicate significant differences at P < 0.05: (0–3 cm)-(3–6 cm)-(6–9 cm).



Fig. 4. Variation of dissolved organic C (DOC), specific ultraviolet absorbance (SUVA), and the E_4/E_6 ratio of soil-pore water samples extracted during the 2011 rainfall season under the control and litter extraction (LE) treatments. Error bars indicate ± 1 standard error of the mean.

top 10-cm depth, DOC values were 63.71 ± 8.54 and 25.74 ± 1.56 mg L⁻¹ for the control and LE treatments, respectively. At the 30-cm depth, DOC was in a similar concentration, with values of 50.94 ± 4.0 and 26.07 ± 2.04 mg L⁻¹ for the control and LE treatments, respectively. This 59.6% (10-cm depth) and 48.8% (30-cm depth) decrease can be explained by the high potentially soluble fraction of the Oi and Oa horizons that makes litter a main source of DOC (Magill and Aber, 2000; Moore and Dalva, 2001) that moves down the soil profile with precipitation. Other studies have found decreased DOC after peat bog disturbances (Glatzel et al., 2003) or after forest harvesting (Kalbitz et al., 2000), in which the main factor was also the depletion of easily decomposable organic matter.

Table 4. Main soil physical characteristics of the study site.

Depth	Dull danster	Particle size distribution+					
interval	bulk density	Sand	Silt	Clay			
cm	g cm ⁻³						
0-28	$1.05\pm0.17\ddagger$	62.4 ± 7.6	26.4 ± 4.4	11.2 ± 3.8			
28-43	1.23 ± 0.09	58.8 ± 2.1	25.5 ± 2.0	15.7 ± 1.3			
+ Particle size distribution determined by the hydrometer method.							
± Mean + 9	SD						

Microbial communities can play an important role in DOC balances (Neff and Asner, 2001), either as consumers or as sources of DOC (Kalbitz et al., 2000). In our case, microbial communities were not significantly reduced; therefore, the consumption of organic substrates and further production of DOC was not apparently affected by microorganisms and their activity.

The nonsignificant differences in DOC between the two measured depths imply that an important fraction of the DOC produced in the soil surface can be translocated to the 30-cm depth. It seems that chemical controls of DOC retention, such as adsorption and precipitation reactions, are exceeded by physical controls such as the water infiltration flux. Other studies have shown that DOC concentrations decrease with depth. In these cases, the clay content and mineralogy have played a key role (Neff and Asner, 2001). In our study, clay content, particularly in the top 30 cm of the soil, was low (Table 4), allowing the translocation of DOC by means of convective transport through macro- and mesopores (Kalbitz et al., 2000).

Visible and ultraviolet (UV) spectroscopy can give some insights regarding the structure of DOC. In case of UV light transmission, aromatic molecules have the greatest absorptivities, with wavelengths λ between 200 and 380 nm, while other structures are translucent (Weishaar et al., 2003). This characteristic of the selected UV spectra allows the characterization of DOC in terms of its aromaticity (Weishaar et al., 2003). Specific UV absorbances (the ratio between the absorbance at 254 nm and the DOC concentration in water extracts) were high in both sites, indicating an elevated aromatic content (Fig. 4). This is consistent with other pore water studies in forest soils (Wickland et al.,

with other pole water studies in folest solis (witch and et al., 2007). At both the 10- and 30-cm depths, SUVA values were not significantly different between the control and LE treatments (Fig. 4). Because the more labile SOM fraction (Oi horizon) was depleted in the LE treatment, we expected that the DOC produced from the remaining SOM fraction would exhibit a smaller fraction of aromatic compounds. However, the elimination of the litter layer decreased DOC but did not provoke a significant change in its aromaticity. The SUVA values were affected by rain events but only at the 10-cm depth (P = 0.025 and 0.70 for the 10- and 30-cm depths, respectively). It seems that the quality of DOC in terms of the presence of more or fewer aromatic compounds can be affected by time, particularly near the soil surface. Studies of the effects of soil water content, soil temperature, and amount and intensity of precipitation, as well as the role of microorganisms on DOC composition will help to elucidate this question.

In the visible spectra, the fractionation of DOC by E_4/E_6 ratios (the ratio between the absorbance at 465 and 665 nm in water extracts) did not reveal any major difference between the control and LE treatments at either depth (P = 0.22 and 0.46 for the 10- and 30-cm depths, respectively). Significant variations in this ratio were not found with time (P = 0.18 and 0.45 for the 10- and 30-cm depths, respectively). Overall mean values in

the control treatment were 16.17 \pm 5.05 and 21.43 \pm 4.65 for the 10- and 30-cm depths, respectively. In the LE treatment, this ratio was 19.49 \pm 4.9 (10-cm depth) and 16.81 \pm 4.48 (30-cm depth). It seems that the E_4/E_6 ratio is not as sensitive as SUVA values to changes in the quality of DOC. These values are in the range found in other forest ecosystems such as beech (Fagus sylvatica L.), oak (Quercus robur L.), grand fir [Abies grandis (Douglas ex D. Don) Lindl.], and Norway spruce [Picea abies (L.) H. Karst.] forest sites (Strobel et al., 2001). The E_4/E_6 ratio is inversely related to the molecular weight of the humic fraction (Collier, 1987) and therefore might indicate the dominance of either fulvic or humic acids (Chen et al., 1977; Swift, 1996). In our case, the E_4/E_6 ratios were >5 (Fig. 4), which indicates the predominance of low-molecular-mass humic substances (i.e., fulvic acids) (Swift, 1996). The fulvic acid predominance is in agreement with the high SUVA values found because fulvic acids are rich in aromatic compounds (Schnitzer, 1986). Moreover, the pH values of water extracts were slightly >6.5, a value considered the upper limit for humic acid solubility.

SUMMARY AND CONCLUSIONS

Wildland areas of central Chile, where sclerophyll forests are a main component, are highly affected by anthropogenic disturbances. The effects of such disturbances are not well understood, thereby increasing the need for a better comprehension of the dynamics of chemical, physical, and biological properties under increasing human pressure and climate change. Soil litter extraction decreased soil respiration in the short (Days 0-24 after extraction) and the medium term, which was attributed to the elimination of readily decomposable C substrates that constitute the O horizons. Volumetric water content was the second factor affecting soil respiration in both treatments. Soil organic C, basal respiration, microbial biomass, and their related metabolic quotients (qCO₂, qM_{ic}, and qM_{in}) were not affected by litter extraction. It seems that the removal of important amounts of SOM did not affect the inherent characteristics of the remaining SOM or the amount and activity of the microbial population.

Dissolved organic C significantly decreased as a result of LE. In both treatments, DOC was comprised of aromatic-rich, low-molecular-weight compounds (i.e., fulvic acids) that apparently did not react with the soil matrix (i.e., adsorption–desorption reactions) but can be of particular importance in controlling other soil processes such as acidity, cation exchange, and translocation of metals (Qualls et al., 2003). The potential effects of the decrease in DOC in these as well as in other soil processes should be taken into consideration in future studies.

Specific UV absorbances as well as the E_4/E_6 ratios were not affected by LE. This demonstrates that LE caused a change in the amount but not in the quality of the DOC. Differences in SUVA values with time near the soil surface (i.e., the 10-cm depth) suggest a potential effect of other time-related properties, such as variations with time in the soil water content and temperature, the amount and intensity of precipitation, or changes in soil biological activity with time. New studies are therefore needed in this direction as well.

Despite the apparent high resiliency of the soil system to LE, these results should be considered with caution. Our results represent the effect of only one litter removal, at a site that had not been subject to disturbance for 50 yr, and thus represents the most conservative scenario. It is known that sclerophyll forests of central Chile can be affected by LE up to seven times in a century. A frequent alteration of the topsoil will impact humification processes and therefore the development of biologically resistant organic matter characterized by turnover times of 25 yr (Parton et al., 1987) or the generation of chemically recalcitrant SOM, also known as passive SOM, characterized by turnover times >250 yr. The elimination of these C pools and its effect on the microbial population and soil C fluxes are still unknown for this type of forest ecosystem.

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