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Biological legacies promote succession and soil development on tephra from the Puyehue-Cordon Caulle eruption (2011)

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Abstract Volcanic deposits have frequently been studied from a successional point of view, but the main focus has been on vegetation dynamics, and less frequently on the development of invertebrate and microbial communities and soil properties. Biological legacies, understood as living organisms, seeds, organic debris and biologically derived patterns in soils and understories, are important in succession, and may also influence soil development on young volcanic deposits. The volcanic eruption of the Chilean Puyehue–Cordón Caulle complex (Northern Patagonia) in June 2011 deposited tephra in southern Argentina. Sandy tephra up to 30 cm deep was deposited in the De los Siete Lagos road in Nahuel Huapi and Lanín National Parks, where a road under construction had exposed sub-soil lacking vegetation, while adjacent forest supported a canopy of *Nothofagus dombeyi* with *Chusquea culeou* in the understory. This situation provided a unique opportunity to study soil development and succession on nearby young volcanic deposits with different biological legacies, considering several biological communities. Our hypothesis is that 29 months after the eruption the tephra in the forest would have higher organic C, total N, available P and biological activity than the tephra deposited on the roadside. Plant cover and species richness, invertebrate abundance and richness, as well as substrate respiration, N mineralization and enzymatic activities were highest in the forest. In addition, organic carbon and nutrient incorporation rates in the forest were twice those in the roadside substrate. Nevertheless, two and a half years after the eruption, most variables remained an order of magnitude lower than values expected for temperate forest soils. Surviving canopy and understory play a key role in ecosystem recovery after tephra deposition, providing seeds and organic matter and establishing conditions appropriate for plants, invertebrates, and microorganisms that would in turn accelerate soil development.

Key words: Andean North Patagonia, enzymatic activities, invertebrates, nutrients, soil ecology, vegetation.

INTRODUCTION

Succession is understood in a classical sense as the development of vegetation towards a 'climax' (*sensu* Clements 1916), or in a contemporary framework as the change in species composition (Hobbs *et al.* 2007) and/or three-dimensional structure of a plant community (Pickett & Cadenasso 2005). However, succession may also be defined in a broader sense as the self-organization process by which ecosystems develop structure, functions and diversity from available energy, matter and species (Beyers & Odum 1993).

Volcanic eruptions are a disturbance frequently studied from a successional point of view (Tsuyuzaki 1987; Whittaker *et al.* 1989; del Moral & Lacher 2005). The explosive eruption of Mount St. Helens (USA) in 1980 is an extensively studied case, characterized by the heterogeneity of the disturbance that included a catastrophic lateral blast, a massive debris avalanche, pyroclastic flows, lahars and tephra fallout over ecosystems such as forests, rivers and lakes (Swanson & Major 2005). One of the main conclusions from studying Mount St. Helens, developed in several ecosystems, was the importance of the biological legacy in fostering rapid redevelopment of compositionally and structurally diverse ecosystems (del Moral & Lacher 2005). As defined by Franklin (1990), biological legacies are living organisms, perennating structures, dormant spores and seeds, organic debris, or any biologically derived material in soils and understories. Legacies may also include dead trees and down logs, large soil aggregates and dense mats of fungal hyphae. The extreme case of no biological legacy initiates primary succession, which usually occurs on susbtrates characterized by low fertility, especially of nitrogen (del Moral & Clampitt 1985; Walker & del Moral 2011). Considerable study of primary succession has been performed in young volcanic deposits; however, this has mainly focus on vegetation dynamics (Whittaker *et al.* 1989; del

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Moral *et al.* 2009), and less frequently on the development of other communities, such as invertebrates (Edwards & Schwartz 1981) and microorganisms (Ibekwe *et al.* 2007), and soil properties (Schlesinger *et al.* 1998; Halvorson *et al.* 2005). However, rates of soil formation are expected to be greatest during the early years of soil development and slow gradually thereafter (Bockheim 1980).

Located along southeastern edge of the Pacific Ring of Fire, the Andean Patagonia includes approximately 50 active volcanoes making it an ideal place to study soil development and succession of young volcanic deposits. From west to east, Patagonia includes the Andean cordillera, the lower foothills intersected by glacial lakes and valleys, and the steppe. Patagonia is a temperate to cool-temperate region, where the characteristic of the temperature pattern is the NW-SE distribution of the isotherms, determined mainly by the presence of the Andes. The mean annual temperature ranges from 12°C in the north-east to 3°C towards the south (Paruelo *et al.* 1998). In addition, Patagonia is between the semi-permanent anticyclones of the Pacific and the Atlantic Oceans at approximately 30° and the subpolar low pressure belt at approximately 60°, so that this region is dominated by strong, consistent west winds. Due to the rain shadow effect of the Andes, mean annual precipitation declines from 3000 mm to <500 mm only 80 km to the east in the Patagonian plains (Paruelo *et al.* 1998). A dramatic west-to-east vegetation gradient parallels the precipitation gradient. Intermittent deposition of tephra and humid climate, in conjunction with its influence on vegetation, determine that volcanic soils are formed and maintained only in Andean Patagonia (Broquen *et al.* 2005). These soils, classified as Andisols, develop from the rapid weathering of volcanic glass and show a predominance of non-crystalline clays that give them unique characteristics such as low bulk density and phosphorus availability, and high water retention capacity and organic matter stabilization (Broquen *et al.* 2005).

Soils are dynamic systems showing a variety of energy and mass fluxes and transformations (Smeck *et al.* 1983). The well-known state factor theory relates soil properties to several factors such as climate, organisms, topography, parent material, and time that control genesis and behaviour of soils and ecosystems (Jenny 1941). Due to the easily weathered nature of volcanic deposits, only a few centuries are needed for the evolution of Andisols (Shoji *et al.* 1993). Therefore, this soil order has usually been the focus of soil genesis studies (Schlesinger *et al.* 1998). The significance of climate and type of tephra in Andisol development has been widely reported. In contrast, the effects of organisms, especially plants and human beings, on the formation and properties of these soils have not been sufficiently emphasized (Shoji *et al.* 1993). We consider that biological legacies through their positive effects on soil organic matter accretion, succession rates, and ecosystem recovery may also be expected to promote soil development in volcanic deposits.

The last important volcanic eruption in Patagonia was that of Puyehue–Cordón Caulle Volcanic Complex (PCCVC) in 2011. The PCCVC is a cluster of Pleistocene to recent eruptive centres located at 40.5°S, 72.2°W that consists of various fissure vents with aligned domes and pyroclastic cones (Lara *et al.* 2004). On 4th June 2011, the PCCVC experienced an explosive rhyolitic eruption, followed by several months of low-intensity ash emissions and effusive activity (Pistolesi *et al.* 2015). Due to the predominance of westerly winds, tephra fallout affected a wide area of Argentina and Chile. On the Argentinean side, the area affected included Nahuel Huapi and Lanín National Parks, where autochthonous forests develop, and a new route was being built at the moment of the eruption. The De los Siete Lagos road, national route 40, was constructed between 2005 and 2015, so during the eruption, there were large areas where vegetation, organic soil and some sub-soil had been removed. So far, studies on the PCCVC eruption have studied the effects of tephra on terrestrial vegetation (Gaitán *et al.* 2011; Ghermandi *et al.* 2015), stream (Lallement *et al.* 2014) and terrestrial fauna (Fernández-Arhex *et al.* 2015).

Along the De los Siete Lagos road, 25–30 cm of sandy tephra was deposited both in sites affected by the road construction and in adjacent areas of protected Andean cold-wet forest (Gaitán *et al.* 2011). In the forest, intact soil and organic layer were buried, but trees and shrubs survived to volcanic eruption (Wilson *et al.* 2013). This situation provided a unique opportunity to study soil development and succession on nearby young volcanic deposits with different levels of biological legacies (i.e. primary and secondary succession) in several biological communities (microorganisms, invertebrates and plants). Our hypothesis is that two and a half years after the eruption, the tephra deposited over the forest would have higher nutrient (N, P) and organic matter content, higher microbiological activity (respiration and mineralization rates, enzymatic activities), and a more abundant and richer invertebrate and plant communities than tephra deposited on roadsides, due to the difference in biological legacy.

METHODS

Study site

The study was conducted in an area affected by the tephra deposition from PCCVC June 2011 eruption in the Nahuel Huapi National Park. The area is a characteristic Andean

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cold-wet forest dominated by *Nothofagus dombeyi* in the canopy and *Chusquea culeou* in the understory (Correa 1984). The annual mean temperature is 8.1°C ranging from a summer mean of 13.5°C to a winter mean of 2.9°C. The mean annual rainfall is 2000 mm, concentrated mainly as rain and snow during the winter. Soils are Andisols according to the USDA Soil Taxonomy (Soil Survey Staff 1999) with high nutrient and organic matter contents; values in nearby forests before eruption were 10.8% of organic C, 0.48% of total N, 7.3 mg kg^{-1} of extractable P, 0.01 dS m^{-1} electrical conductivity and pH of 6.3 (Kowaljow & Mazzarino 2007). Soils have been buried under 25–30 cm of tephra (63–500 μm) with very low contents of N (0.003%), and organic C (0.02%), 3.1 mg kg^{-1} of extractable P, 0.02 dS m^{-1} electrical conductivity and pH of 6.5 (Cremona & Ferrari 2011; Wilson *et al.* 2013).

In December 2012, three 150 m^2 exclosures were established along De los Siete Lagos road, separated by at least 2 km, over the tephra deposited between the road shoulder and the forest. We chose sites undisturbed since the volcanic eruption, where National Parks and National Highway Authority authorized the installation of fencing to demarcate areas safe from subsequent construction works relating to the road. In each exclosure, six 12 m^2 experimental plots were randomly installed to perform different studies. Here, we analyse the tephra from control plots, referred as 'low biological legacy substrate' (LBLS). In December 2012, no vegetation was present on LBLS, scarce herbaceous vegetation was found on the roadside outside experimental exclosures, whereas surviving trees and shrubs were present in the forest. In addition, three areas not affected by road construction, about 100 m from the experimental exclosures, were chosen in natural forest clearings with 50% canopy cover from the adjacent forest to study a 'high biological legacy substrate' (HBLS). All study sites were characterized by a small slope (<15°).

Vegetation and leaf litter

The plant cover and richness were estimated every 2 months from September 2013 to September 2014 with six randomly located quadrants (50 × 50 cm^2) per plot. Plant cover per square metre (m^{-2}) for each species was calculated. Local dichotomous keys were used to identify species of plants (Correa 1984). In March 2014, a soil corer (6 cm diameter, 10 cm depth) was used to obtain three litter samples in each forest site. Litter depth was assessed with a metric tape, and the material was dried at 60°C to estimate litter density as gram per cm^3.

Invertebrates

In November 2013 (spring) and March 2014 (summer), small shovels were used to collect leaf litter before sampling the tephra (no leaf litter was present in LBLS). Soil corers (6 cm diameter, 20 cm depth) were used to extract 5-cm deep tephra samples. Each sample was a composite of six subsamples of tephra (140 cm^3 tephra per subsample) or leaf litter (150 cm^3 litter per subsample). All samples were randomly taken. In the laboratory, leaf litter and 300 cm^3 of tephra were examined to separate big invertebrates (>2000 μm). In order to extract smaller invertebrates, leaf litter and tephra were mounted in separate Berlese funnels (15 cm diameter, 20 cm depth, 2 mm mesh screens, without heating elements) over plastic containers with 70% alcohol, which were kept for 15 days at 25°C to prevent violent sample dehydration of invertebrates. The density of invertebrates was estimated per square metre (N m^{-2}), and local dichotomous keys were used to identify species (Krantz & Walter 2009), under stereoscopic microscope and microscope.

Tephra substrate

A portion of the 5-cm deep tephra sampled as mentioned in the previous section was sieved through 2-mm mesh for chemical and biological analysis. A part (300 cm^3) was preserved at 4°C to estimate gravimetric humidity, ammonia, nitrate and enzymatic activities and to initiate potential N mineralization and respiration assays within 48 h from sampling. The other part (250 cm^3) was air dried at 25°C for 5 days for electrical conductivity, pH, organic C, extractable P and total N analyses.

Bulk density (g cm^{-3}) was estimated in three samples taken in May 2014 from experimental plot and forest sites, employing a metal soil corer (6 cm diameter, 5 cm depth); samples were dried at 105°C and weighed. In November 2014, total tephra depth was measured in small pits at seven randomly selected points nearby each roadside exclosure and forest experimental sites.

Chemical and biological analyses

Aqueous extracts were used to estimate pH (1:2.5, substrate:water ratio) and electrical conductivity (1:5, substrate:water ratio). Phosphorous extracted in 0.5 M $NaHCO_3$ (1:20, substrate:solution ratio) was determined by the molybdate ascorbic acid method (Olsen & Sommers 1982), total N by semi-micro Kjeldahl (Page *et al.* 1982), and organic C by the Walkley-Black wet digestion method (Nelson & Sommers 1996). Subsamples were analysed for moisture content (dried to 105°C for 72 h).

The activity of β-glucosidase, acid phosphomonoesterase, leucine-aminopeptidase, and phenol oxidase was assessed in suspensions of tephra and water (1:10). Aliquots of sample suspension (1 ml) were incubated at 20°C with 1 ml of each enzyme substrate at specific conditions; a sample control (sample suspension + buffer) and a substrate control (substrate + buffer) were also incubated (Sinsabaugh *et al.* 1999). Enzyme assays began within 48 h of sample collection; however, leaf litter enzyme assays were performed with air-dried material stored for a year. Soil enzyme activity was expressed on both tephra mass and organic matter. Organic matter per gram of tephra was estimated from organic C multiplied by a conversion factor of 1.72 (Nelson & Sommers 1996). In addition, enzymatic activities per gram of tephra and litter were multiplied per density and layer depth to estimate enzymatic activities per cm^2.

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Potential microbial respiration and N mineralization were assessed in field moist substrate, rewetted to field capacity (15% by weight) when necessary. Potential microbial respiration was estimated from the emission of CO_2 by incubating 100 g samples in 1.5 L tightly sealed glass jars at 25°C in the dark for 16 weeks. The CO_2 was trapped in glass vials with 20 mL of 0.2 M NaOH placed in each jar. Vials were replaced and titrated with HCl 0.1 N at 4, 10 and 16 weeks (Kowaljow & Mazzarino 2007). At each sampling date, the jars were left open for approximately 1 h, and soil moisture was adjusted to field capacity. Potential microbial respiration was estimated as the cumulative CO_2 during the incubation period. Potential soil N mineralization was estimated by incubating 100 g moist samples aerobically at 25°C and dark in 0.25 L plastic jars for 16 weeks (Satti *et al.* 2007). Soil moisture was gravimetrically adjusted every week. At several sampling dates (0, 4, 10, 16 weeks), samples were extracted with 2 M KCl (1:5, tephra:solution ratio). Then, NO_3^--N and NH_4^+-N were determined in the extracts by copperized Cd reduction and the Berthelot reaction, respectively. Potential N mineralization was estimated as the inorganic N (NO_3^--N + NH_4^+-N) at each sampling date (t_x) minus the initial concentration at t_0.

Statistical analyses

The effect of biological legacy on soil variables, enzymatic activities, vegetation and invertebrate abundance and richness was analysed per sampling season. The effect of biological legacy on plant cover and richness was analysed per sampling time (September and November 2013, January, March, May, July and September 2014). The effect of biological legacy on potential N mineralization and respiration rates was analysed for each time (2, 4, 6, 12, 16 weeks). Mann–Whitney *U* test was used in all cases, and analyses were performed with SPSS 14.0 for Windows.

RESULTS

Plant community

The plant cover was low both in LBLS as in HBLS (<20%), but there were significant differences between sites (Fig. 1). In LBLS, cover was very low (<1%) and included only the vascular plant *Rumex acetosella* (Polygonaceae). Throughout the survey period, HBLS mean plant cover was between 12% and 20% and richness between 4 and 8 species. This vegetation was dominated by vascular plants; however, mosses and liverworts also grew over the HBLS tephra. Native *Nothofagus dombeyi* and *Berberis darwinii* (Berberidaceae) seedlings were dominant, while *Chusquea culeou* and *Maytenus chubutensis* (Celastraceae) were always present but in low cover. Finally, four exotic and scarce plant species were found in HBLS (Table 1).

Invertebrate community

A total of 83 invertebrates were sampled. No invertebrates were found in either LBLS or HBLS in spring 2013. During the summer 2014, the LBLS invertebrate density remained undetectable; however, the HBLS density increased above zero. At both sampling periods, there were invertebrates in the leaf litter from HBLS (Table 2). These invertebrates belonged to 18 taxa, mainly Opiidae (Acari Oribatida; 19% of total individuals sampled in the two sampling occasions), Isotomidae (Collembola Entomobryomorpha; 10%) and Isotogastruridae (Collembola Poduromorpha; 10%). Aranae was also a well-represented group (10%).

Physicochemical properties

In November 2014, the tephra depth was 11 ± 2 cm (mean ± standard deviation) in LBLS and 13 ± 3 cm in HBLS. The bulk density for the first 5 cm of tephra was 0.70 ± 0.03 and 0.67 ± 0.09 g cm^{-3} in LBLS and HBLS, respectively. Besides, HBLS was covered by a 2 ± 1 cm layer of litter which density was 0.1 ± 0.7 g cm^{-3}.

The HBLS presented the highest moisture and lowest temperature in spring and summer. No significant differences in electrical conductivity and pH were observed between LBLS and HBLS in any season (Table 3), but on both sampling dates, HBLS presented the highest values of organic C, total N and extractable P (Table 4).

Biological properties

The potential respiration was higher in HBLS than in LBLS, while N mineralization was higher in HBLS only in summer, which was the sampling date with the highest potential microbial respiration and net N mineralization (Fig. 2, Table 4).

All enzymatic activities were higher in HBLS than in LBLS, either expressed per gram of tephra or gram of organic matter (Fig. 3a,b). In addition, high enzymatic activities per square centimetre were found on the forest litter that presented higher leucine-aminopeptidase activity than HBLS. There were no differences in β-glucosidase and acid phosphomonoesterase activities between litter and HBLS. Finally, phenol oxidase activity was similar to that in LBLS (Fig. 3c). Some seasonal effect was detected on acid phosphomonoesterase and leucine-aminopeptidase activities referred to organic matter, which increased in summer in HBLS, while phenol oxidase activity per gram of organic matter decreased.

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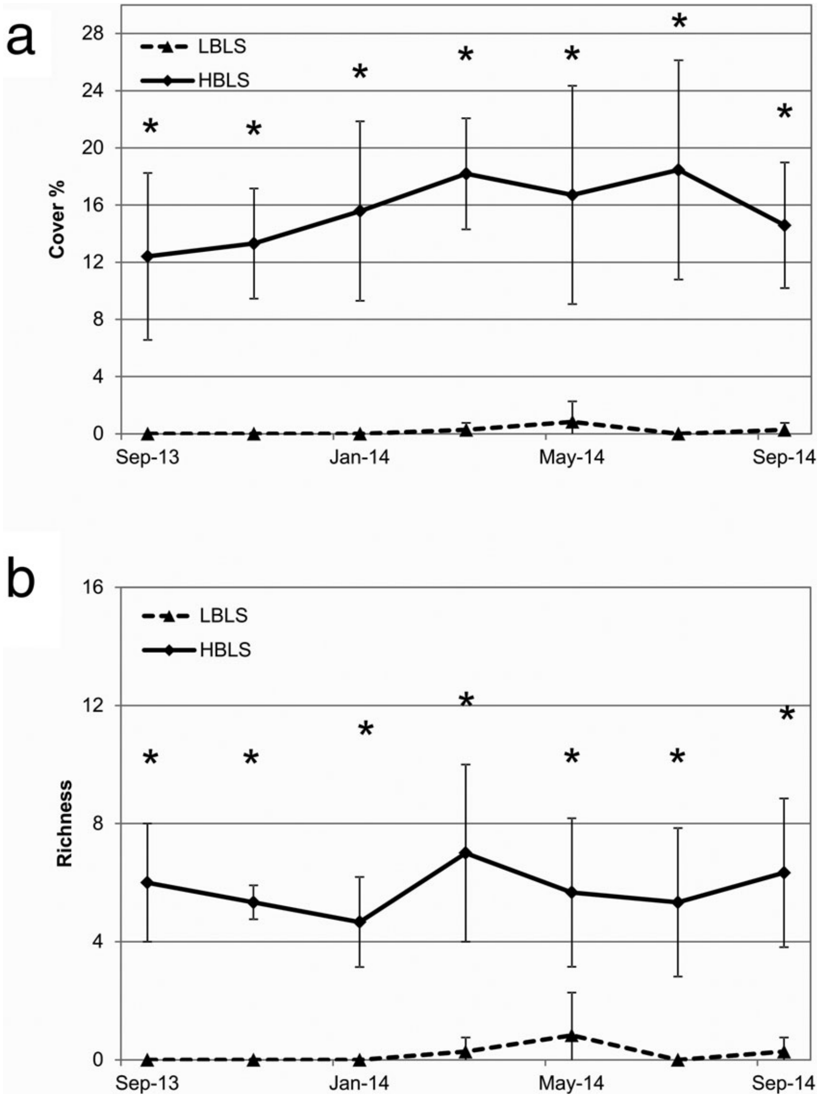

Fig. 1. Mean plant (a) cover and (b) richness along studied period, September 2013–2014. *Significant differences ($P < 0.05$).

DISCUSSION

Two and a half years after the eruption, vegetation, invertebrate, and microbial communities were more developed in HBLS, where organic C and nutrient content were twice the value of original tephra and LBLS. The forest biological legacy demonstrably promoted organic matter accumulation and tephra weathering, leading to a facilitation of the establishment of plants, invertebrates and microorganisms that would in turn accelerate the development of this substrate into an Andisol.

Three years after the eruption, plant cover remained nearly absent in LBLS and was about 20% in HBLS. The poor growth of plants on tephra has been previously reported and attributed both to physical structure as to N limitation (del Moral & Magnusson 2014). According to our estimates of nutrient content, N would be a limiting factor for De los Siete Lagos road revegetation.

Ground cover in the HBLS was low and dominated by *Nothofagus dombeyi* seedlings. Vogel (1996) reported a massive establishment of seedlings in 25 cm deep tephra deposits in forests dominated by *Nothofagus* after the eruption of Hudson volcano. This positive effect on seedlings is explained by the recruitment strategy of *Nothofagus* set off by a high light availability after a disturbance (Veblen *et al.* 1979). The sparse vegetation cover in the LBLS primary succession, represented only by a few individuals of the invasive ruderal *Rumex acetosella*, may be attributed to poor substrate conditions (nutrient content, temperature, humidity) and the isolation from seed and propagule sources (del Moral *et al.* 2009).

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Table 1. Mean plant cover per species and biological legacy along studied period, September 2013–2014

					LBLS						
		Origin	Habit	Life history	September 2013	November 2013	January 2014	March 2014	May 2014	July 2014	September 2014
Acaena ovalifolia Ruiz & Pav.	Rosaceae	N	H	P							
Adenocaulon chilense Less.	Asteraceae	N	H	A							
Aristotelia chilensis (Molina) Stuntz	Elaeocarpaceae	N	Sh	P							
Berberis darwinii Hook.	Berberidaceae	N	Sh	P							
Blechnum penna-marina (Poir.) Kuhn	Blechnaceae	N	H	P							
Buddleja globosa Hope	Scrophulariaceae	N	Sh	P							
Chusquea culeou E. Desv.	Poacea	N	H	P							
Cerastium arvense L.	Caryophyllaceae	N	H	P							
Cirsium vulgare (Savi) Ten.	Asteraceae	E	H	A							
Lomatia hirsuta (Lam.) Diels (Ruiz & Pav.) R. T. Penn.	Proteaceae	N	T	P							
Maytenus chubutensis (Speg.) Lourteig, O'Donell & Sleumer	Celastraceae	N	T	P							
Nothofagus dombeyi (Mirb.) Oerst.	Nothofagaceae	N	T	P							
Osmorhiza chilensis Hook. & Arn.	Apiaceae	N	H	P							
Prunella vulgaris L.	Lamiaceae	E	H	P							
Ribes magellanicum Poir.	Grossulariaceae	N	Sh	P							
Rosa rubiginosa L.	Rosaceae	E	Sh	P							
Rumex acetosella L.	Polygonaceae	E	H	P				+	1		+
Bryophyta: Bartramia ithyphylla (moss) + *Leptoscyphus* sp. (liverwort)		N	–	–							

Among species growing over tephra, it is important to highlight exotics such us *R. acetosella*, *Cirsium vulgare* and *Rosa rubiginosa*. Exotic species cover was always lower than 2% but considering that total cover was never higher than 20%, exotic species represented an important part of the plant community. In our study site, tephra deposition eliminated all herbaceous vegetation. Hence, shrub exotics (e.g. *R. rubiginosa*) may be survivors or have germinated from seeds, but herbaceous exotics (e.g. *C. vulgare*, *R. acetosella*) have all derived from imported seeds, as the tephra layer is too thick to allow germination of seeds from the old soil. All these exotic species were present in the National Park before the volcanic eruption (Correa 1984). However, they may take advantage of this disturbance to expand its distribution along the road or in forest gaps. After 3 years, none of them dominated the forest plant community, but that *R. rubiginosa* is an invasive species in Patagonia, and *R. acetosella* was the only species colonizing LBLS makes necessary to keep annually monitoring these species cover on disturbed sites.

No invertebrates were found in LBLS and very low abundances were detected in HBLS. This may be expected because tephra is known to lead to increased morbidity and mortality of arthropods (Fernández-Arhex *et al.* 2015). Some major causes proposed for this effect are desiccation induced by cuticular abrasion and excess salivation, respiration stress by impairment of spiracular function, and gut activity disruption by the accumulation of tephra boli (Edwards & Schwartz 1981). As for invertebrates in litter over HBLS, densities were low compared with those reported for forest floors (Reynolds *et al.* 2003). This may also be explained by the negative effect of tephra on arthropods as small size tephra is transported by air and water, and may be found over litter. An additional explanation is that buried soil invertebrates are not able to penetrate the 15–30 cm tephra layer, and therefore, all sampled invertebrates are from a recently developed community, initiated by colonizers transported by air (Hodkinson *et al.* 2002) and organisms that survived in organic debris refuges from nearby trees and shrubs.

The mean tephra depth declined by almost half after 2 years. A notable characteristic of PCCVC eruption was the extensive redistribution of tephra after 6 months. This process occurred especially in the steppe and was attributed to the action of strong winds in conjunction with a low plant cover, small tephra size and scarce precipitation (Gaitán *et al.* 2011). In Patagonia forests, a much lower effect of strong winds on tephra transport may be expected, due to the protective effect of trees, higher tephra size and humidity. However, some transport by air and water may also have occurred. In addition, we cannot exclude that compaction and infiltration by tephra into the buried soil may have contributed to reduce the tephra layer from 30 to 15 cm after a couple of years. Tephra pH and conductivity in LBLS and HBLS were similar to those in the original material and local forest volcanic soils (Satti *et al.* 2007; Cremona & Ferrari 2011). In addition, the HBLS showed the highest water, organic C, total N and available P contents. The forest effect on substrate moisture may be attributed to shading by surviving trees. Differences in organic C and nutrient contents may be explained by canopy leaching and by decomposition of the litter that currently covers the tephra. In any case, HBLS organic C and N were an order

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Table 1. Continued

	HBLS						
	September 2013	November 2013	January 2014	March 2014	May 2014	July 2014	September 2014
Acaena ovalifolia Ruiz & Pav.				1	1		
Adenocaulon chilense Less.	+						
Aristotelia chilensis (Molina) Stuntz			1	1	+	+	
Berberis darwinii Hook.	2	2	4	2	4	4	2
Blechnum penna-marina (Poir.) Kuhn			1	+	+	1	+
Buddleja globosa Hope							+
Chusquea culeou E. Desv.	1	+	1	+	1	1	1
Cerastium arvense L.				+	+	+	1
Cirsium vulgare (Savi) Ten.	+	1	+	+	+	+	
Lomatia hirsuta (Lam.) Diels (Ruiz & Pav.) R. T. Penn.	+	+			+	+	+
Maytenus chubutensis (Speg.) Lourteig, O'Donell & Sleumer	1	1	+	1	1	+	1
Nothofagus dombeyi (Mirb.) Oerst.	6	7	8	9	5	7	7
Osmorhiza chilensis Hook. & Arn.	+						+
Prunella vulgaris L.				+			
Ribes magellanicum Poir.							1
Rosa rubiginosa L.		+	+	+			
Rumex acetosella L.	+			+			+
Bryophyta: Bartramia ithyphylla (moss) + *Leptoscyphus* sp. (liverwort)	3	2	+	1	3	4	2

+, cover <0.5%; N, native; E, exotic; C, cosmopolitan; H, herb; Sh, shrub; T, tree; P, perennial; A, annual.

Table 2. Mean density (N m^{-2}) and number of taxa of invertebrates in volcanic ash and leaf litter, from low and high legacy sampling sites

	Density (N m^{-2})				Number of taxa (N)			
	Volcanic ash		Leaf litter		Volcanic ash		Leaf litter	
	Spring 2013	Summer 2014	Spring 2013	Summer 2014	Spring 2013	Summer 2014	Spring 2013	Summer 2014
LBLS	n/d	n/d	–	–	n/d	n/d	–	–
HBLS	n/d	67 (47)	262a (45)	716a (125)	n/d	2 (1)	3b (2)	8a (2)

n/d, non-detectable. Values are means (SD). Different letters indicate significant differences between seasons ($P < 0.05$).

of magnitude below values reported for local forest volcanic soils. Available P values were similar to those reported for the original tephra deposited in 2011 and for local Andisols, which are characterized by low P availability depending on development and amorphous allophane content (Satti *et al.* 2007).

As for biological soil properties, potential N mineralization rates estimated by aerobic incubations in controlled humidity and temperature conditions, and respiration rates are indicators of both organic substrate quality and microbial activity (Vitousek & Matson 1985). Both were expected to increase in the forest as microorganisms and organic substrates are considered biological legacies. The fact that N mineralization was higher in HBLS in summer but not in spring, may be attributed to lower microbial activity during low temperature months, as could be expected from the lower potential respiration and enzymatic activities found in spring.

Enzymatic activities in tephra were an order of magnitude lower than those generally found in forest soils (Sinsabaugh *et al.* 2008), however, the HBLS enzymatic activities were greater than those found in LBLS. Forest litter enzymatic activities were similar to those reported for other forest floors (Berenstecher *et al.* 2016). Enzymatic activity on an organic matter basis reflects a microbial community property, since it expresses the nutritional status of the organic matter from the perspective of the microbial community. The fact that enzymatic activities referred to organic matter were significantly lower in the forest floor than in the tephra suggests that litter is richer in recalcitrant organic matter. In addition, expressing activity on a soil mass basis allows calculating the rate of consumption of organic substrates on an area basis. This ecosystem-level measure may be estimated multiplying the activity per soil mass by the bulk density and soil horizon depth. When tephra and forest litter density were used to estimate enzymatic activities per square centimetre, results suggested that litter would be providing an important amount of enzymatic activity and products to the

Table 3. Physicochemical characteristics of volcanic ash in studied sites and sampling times

	Temperature (°C)		Moisture (%)		EC (dS m^{-1})		pH	
Treatments	Spring 2013	Summer 2014	Spring 2013	Summer 2014	Spring 2013	Summer 2014	Spring 2013	Summer 2014
LBLS	13.5 Ab	16.7 Aa	21 Ba	7 Ab	0.014 Ba	0.009 Ab	6.5 Aa	5.9 Ab
	(0.8)	(0.9)	(1)	(2)	(0.002)	(0.001)	(0.1)	(0.2)
HBLS	9.5 Bb	14.3 Aa	35 Aa	21 Ab	0.018 Aa	0.014 Ab	6.4 Aa	5.6 Bb
	(0.8)	(0.7)	(5)	(7)	(0.001)	(0.002)	(0.1)	(0.1)

Values are means (SD). Different capital letters indicate significant differences between biological legacy levels, while different small letters indicate significant differences between seasons ($P < 0.05$).

Table 4. Volcanic ash carbon, nitrogen, and phosphorus in studied sites and sampling times

	Organic C (g kg^{-1})		Total N (g kg^{-1})		Extractable P (mg kg^{-1})		Inorganic N (mg kg^{-1})		Pot. N mineralization (mg kg^{-1})	
	Spring 2013	Summer 2014	Spring 2013	Summer 2014	Spring 2013	Summer 2014	Spring 2013	Summer 2014	Spring 2013	Summer 2014
LBLS	0.4 Ba	0.6 Ba	0.01 Ba	0.01 Ba	1.9 Ba	1.9 Ba	0.3 Ab	1.1 Aa	1.1 Aa	2 Ba
	(0.1)	(0.4)	(0.01)	(0.01)	(0.4)	(0.4)	(0.2)	(0.3)	(0.8)	(2)
HBLS	1.3 Aa	1.9 Aa	0.05 Aa	0.07 Aa	4.4 Aa	5 Aa	1.3 Aa	1.1 Aa	4.5 Ab	12 Aa
	(0.3)	(0.9)	(0.02)	(0.03)	(0.3)	(1)	(0.9)	(0.5)	(3.6)	(3)

Values are means (SD). Different capital letters indicate significant differences between biological legacy levels, while different small letters indicate significant differences between seasons ($P < 0.05$).

substrate below. The highest enzymatic activity in HBLS may be prompted directly by higher organic substrate availability as well as indirectly by higher microorganism abundances in the forest. In any case, our results indicate that fungi and bacteria decomposing the litter in De los Siete Lagos road forest would be providing an important amount of nutrients and organic C to the tephra below, thus promoting soil formation in HBLS.

Biological legacies have two components: (i) living organisms that survive in refuges and act as colonizers, and (ii) dead individuals and organic matter that provide complexity, food, and nutrients (Franklin 1990). Tsuyuzaki (1987) recognizes four mechanisms promoting the succession in the volcano Usu: (i) vegetative recovery of buried plants, (ii) seed immigration, (iii) introduction of plants, and (iv) exposure of the buried seeds. Buried plants and seeds are a special case of biological legacy as they are survivors that do not survive in refuges from tephra deposition, but buried under the tephra layer. Then, they are part of legacies promoting succession but whether they contribute as living organisms acting as colonizers, or as dead material providing nutrients to plants with roots in the old soil depends on the stochastic event of exposure. Soil invertebrates and microorganisms buried by tephra would be in a similar situation. In De los Siete Lagos road, forest trees and shrubs survived the volcanic eruption so that they should be considered biological legacy themselves. However, trees would have also provided refuges for the survival of invertebrates and microorganisms living on the mantle of dead organic material associated with mats of epiphytes ('canopy soil'), found on the branches of trees in temperate rainforests, that may act as colonizers of the new forest floor (Lowman & Wittman 1996; Dangerfield *et al.* 2017). In addition, buried soil litter may be part of the dead biological legacy as it would release nutrients that return to the surface through primary production and decomposition over the tephra layer. Finally, we have observed removal of tephra along drainages by surface water runoff, especially in roadsides where substrate is prone to erosion. This process may be locally important and determine whether buried herbaceous vegetation, soil seeds, invertebrates and microorganisms are able of acting as colonizers. This would depend on the probability of being exposed before dead. Nevertheless, as they are soil organisms, it may be expected that survive under tephra for a long time.

Sandy tephra is vesicular and unconsolidated, which contributes to high plant available water holding capacity and high specific surface area which in turn leads to rapid release of nutrients by weathering (Shoji *et al.* 1993). Even P in recently deposited tephra is considerably soluble in dilute acid solutions

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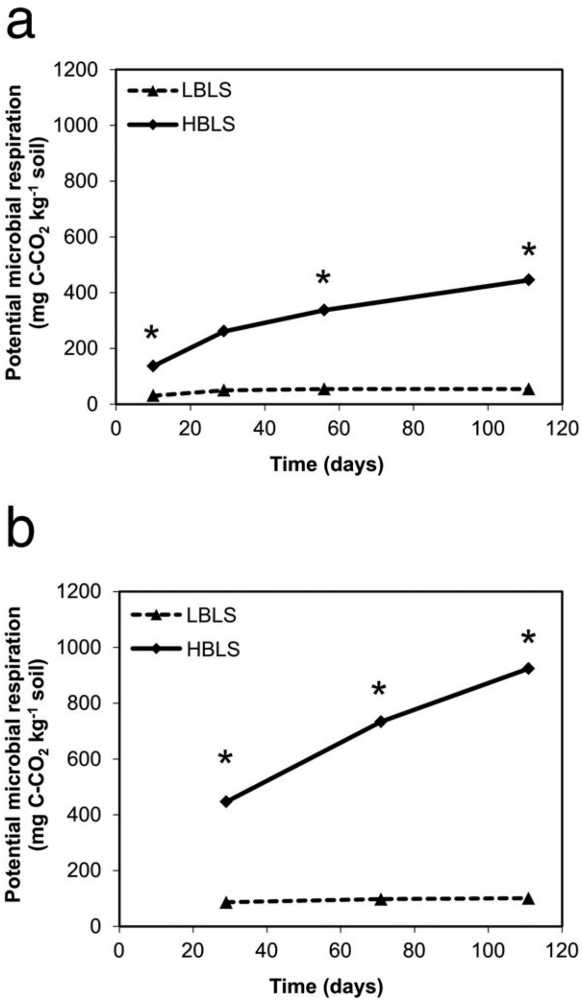

Fig. 2. Mean potential microbial respiration (as cumulative C mineralization during 16-week incubation) in (a) Spring 2013 and (b) Summer 2014. *Significant differences ($P < 0.05$).

due to the release by weathering and the lack of active Al and Fe components, which render P unavailable by sorption. Then, the important limiting nutrient for revegetation is N (Shoji *et al.* 1993). This was confirmed by the N content of the PCCVC tephra. However, LBLS revegetation may also be limited by the low water content, especially in summer, high irradiance, lack of seed rain, and absence of refuge for seeds and seedlings on roadsides.

According to Shoji *et al.* (1993), Andisol age required for the accumulation of 6% organic C is 360 years (10°C-normalized age), that is, 0.017% per 10°C-year. Similar estimates were made at Mount St. Helens, 14°C mean annual temperature, where Halvorson *et al.* (2005) reported an increase of 0.69% in 13 years, that is, 0.05% per year. Considering that mean annual temperature in our system is 8°C, our estimations in spring 2013 of 0.04% and 0.13% organic C (i.e. 0.016% and 0.053% per year) in LBLS and HBLS, respectively, agree with the general trend.

Vitousek *et al.* (1983) studied several soil chronosequences from montane rainforest soils on Hawai'i, including sites derived from sandy tephra. As for the mineral part of tephra derived soils, a mean of 382 and 22 kg ha^{-1} year^{-1} of C and N, respectively, accumulated during the first 191 years of soil development. Schlesinger *et al.* (1998) studied Krakatoa sandy tephra deposits, estimating a minimum input of 450–1270 and 30–85 kg ha^{-1} year^{-1} of C and N, respectively, during 110 years of soil development. In the road De los Siete Lagos, considering an bulk density of 0.7 ± 0.1 g cm^{-3} and a tephra depth of 11 cm, we may estimate that after 29 months of the tephra deposition 128 and 3 kg ha^{-1} year^{-1} of C and N, respectively, accumulated in LBLS; and 493 kg ha^{-1} year^{-1}, and 19 kg ha^{-1} year^{-1} inputs of C and N, respectively, in LBLS. Vitousek *et al.* (1983) and Schlesinger *et al.* (1998) studied closed canopy sites, so lower LBLS accumulation rates in De los Siete Lagos road may be expected. The highest values of C accumulation were observed in HBLS despite higher mean annual temperature in Hawai'i and Krakatoa, but this may be expected because rates of soil formation are often greatest during the early years of soil development and slow gradually thereafter (Bockheim 1980). In addition, Haruki and Tsuyuzaki (2001) studied the tephra deposited in 1977–1978 by the volcano Usu, reporting that in 1986 substrate presented a mean accumulation of 0.187 g kg^{-1} of N (i.e. 0.020 g kg^{-1} year^{-1}) that generally agrees with our estimation of 0.004 and 0.021 g kg^{-1} year^{-1} of N for LBLS and HBLS, respectively.

Finally, Schlesinger *et al.* (1998) studied several P fractions in Krakatoa tephra, including HCO_3-extractable (plant available-P) which was found to be 36–79 mg kg^{-1}, that is 0.33–0.72 mg kg^{-1} year^{-1}. After 29 months, we estimated 1.9 and 4.4 mg kg^{-1} of P available to plants (i.e. 0.79 and 1.8 mg kg^{-1} year^{-1}) in spring 2013 LBLS and HBLS, respectively. Then, HCO_3-extractable P input in De los Siete Lagos was twice the lowest estimation for Krakatoa island. This higher availability of P in our study may be explained by the absence of allophanes that retain phosphorus in Krakatoa (Schlesinger *et al.* 1998) and developed volcanic soils (Satti *et al.* 2007).

This study shows that the biological legacy controlled both ecological succession and soil parameters in tephra from the PCCVC. Two and a half years after the eruption, vegetation, invertebrate and microbial communities were more developed in the forest. Consequently, organic C, N and P availability in the forest were also higher than in the roadside tephra, which remained similar to the original material deposited in 2011. We consider that surviving canopy and understory play a key role in succession

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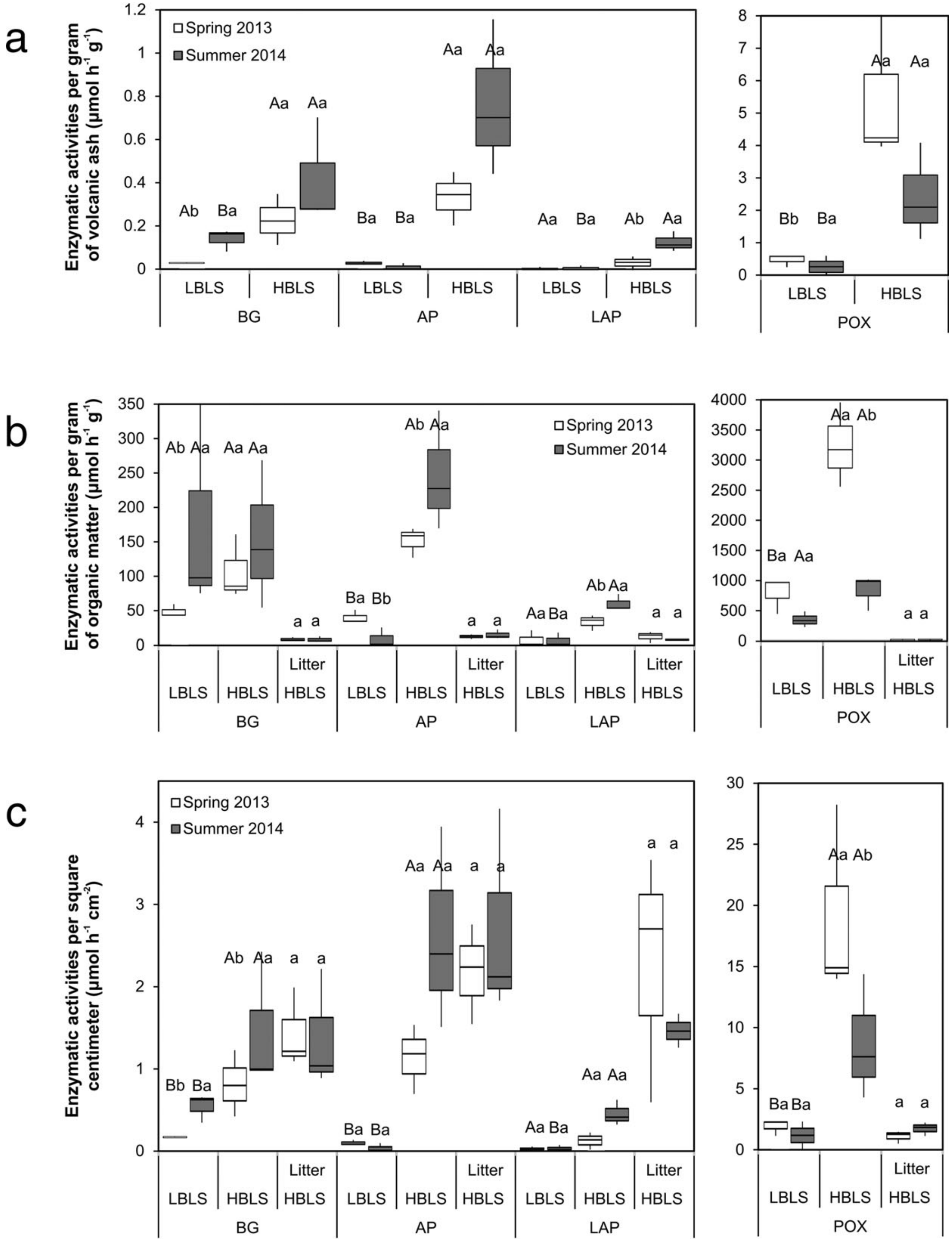

Fig. 3. (a) Enzymatic activities per gram of volcanic ash (µmol h^{-1} g^{-1}), (b) enzymatic activities per gram of organic matter (µmol h^{-1} g^{-1}), (c) enzymatic activities per square centimetre (µmol h^{-1} cm^{-2}). The boxplot displays the minimum, first quartile, median, third quartile and maximum. Different capital letters indicate significant differences between biological legacy levels, while different small letters indicate significant differences between seasons ($P < 0.05$). AP, acid phosphomonoesterase; BG, *β*-Glucosidase; LAP, leucine-aminopeptidase; POX, phenol oxidase activity.

after tephra deposition, providing organic matter, seeds, and probably acting as refugees for microorganisms and invertebrates that colonize the substrate and promote soil formation.

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