



PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
SCHOOL OF ENGINEERING

ASSESSMENT AND MODELLING OF ORGANIC CARBON DYNAMIC IN SOILS OF CHILE.

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Thesis submitted to the Office of Graduate Studies in partial fulfillment of
the requirements for the Degree of Doctor in Engineering Sciences

Advisor:

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PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

ANÁLISIS Y MODELACIÓN DE LA DINÁMICA DEL CARBONO ORGÁNICO EN SUELOS DE CHILE.

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*To my husband Fernando, my parents
and friends for their love and support.*

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ABBREVIATION INDEX

ANCOM	Analysis of composition of microbiomes
BD	Bulk density
db-RDA	Distance-based redundancy analysis
ET	Evapotranspiration
GHG	Greenhouse gas
LFOM	Light fraction organic matter
MAP	Mean annual precipitation
MAT	Mean annual temperature
MidIR	Mid-infrared spectroscopy
NIR	Near-infrared spectroscopy
OTU	Operational taxonomic unit
PCA	Principal component analysis
PCNM	Principal coordinates of neighbor matrices
POXC	Permanganate-oxidizable C
RDA	Redundancy Analysis
SCG	Soil Crenarchaeotic Group
SOC	Soil organic carbon
SOM	Soil organic matter
WSA	Water stable aggregate

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ABSTRACT

An improved understanding and modelling of changes in soil organic carbon (SOC) stocks is critical for developing strategies that ensure effective climate change mitigation and the long-term productivity. Changes in SOC are likely to vary across different soil and climate conditions, however, this remains an active area of research as SOC content does not always respond predictably, due to its dependency on specific ecosystem properties. In this thesis we evaluate spatiotemporal variability in SOC across a gradient of climate conditions, soil orders, and land uses. Additionally, we study chemical, physical and microbiological properties associated with C dynamic in Chile. We sought to examine SOC changes in agricultural lands over the past three or four decades and to understand the main factors involved for improving predictions of SOC dynamics. Topsoil samples were analyzed for total C, N content, aggregate stability, texture, bulk density, pH, labile fraction, microbial attributes as well as spectral properties using Mid-infrared (MidIR), near-infrared spectroscopy. Our findings show that the largest SOC losses occurred in semiarid and subhumid areas during the time frame considered, decreasing their initial C stocks by 24.2% and 26.7%, respectively. Moreover, cultivated soils in semiarid regions were more vulnerable than those in arid regions to SOC losses, which is consistent with the highest labile fraction content found here. The results also indicated that in cooler and

more humid regions, SOC stocks were either stable or increased over time. Among soil orders, Mollisols showed the largest losses (29.9% reduction between sampling dates). The MidIR results indicate that the mineral bands for clays and silicates were associated with these sites demonstrating SOC conservation, suggesting that mineral protection played an important role in the long-term SOC storage in semiarid areas. Additionally, climate regimes played an important role in microbial community structure. Community composition was largely modulated by labile fractions, in which members of phyla Bacteroidetes, Proteobacteria and Archaea were associated with lower water availability and lower SOC conditions. RothC model, a widely used tool, was a suitable tool of reproducing the dynamics and historical trends of SOC stocks using annual cropping systems (wheat and corn), however, the model performed well under certain climatic and soil type conditions in Chile. Given limitations of the model used, we propose that mathematical models could be calculated considering microbial attributes and the site-specific mechanism of SOC protection. This thesis discusses the role of ecosystem properties as a limiting factor in carbon losses, examine the most important driver of SOC dynamic in Chile, and evaluate the use of a turnover of organic carbon model to address the restoration and adaptation for future trends in the context of global change.

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RESUMEN

La comprensión y predicción de los cambios en el contenido del carbono orgánico del suelo (SOC) es fundamental para desarrollar estrategias que aseguren una mitigación efectiva del cambio climático y la productividad agrícola a largo plazo. Los cambios en el SOC pueden variar según las diferentes condiciones del suelo y del clima, sin embargo, esto sigue siendo un área activa de investigación, ya que el contenido de SOC no siempre responde de manera predecible, debido a su dependencia de las propiedades específicas de cada ecosistema. En esta tesis, evaluamos los cambios espaciales y temporales de SOC a lo largo de diferentes condiciones climáticas, tipos y usos de suelo. Además, estudiamos las propiedades químicas, físicas y microbiológicas asociadas con la dinámica de SOC en Chile. En este trabajo buscamos encontrar evidencia de los cambios de SOC en suelos cultivables en las últimas tres o cuatro décadas y comprender los principales factores involucrados para el futuro mejoramiento o desarrollo de herramientas de simulación de la dinámica de SOC. Las muestras de suelo se analizaron en relación al contenido total de C, N, estabilidad de agregados, textura, densidad aparente, pH, fracción lábil, atributos microbianos y propiedades espectrales. Nuestros hallazgos muestran que las mayores pérdidas de SOC se produjeron en áreas semiáridas y subhúmedas durante el marco de tiempo considerado, disminuyendo sus reservas iniciales de C en un 24,2% y un 26,7%, respectivamente, lo que es consistente con el mayor contenido de fracción lábil encontrados en estas áreas. Los

resultados también indican que en las regiones más frías y más húmedas, los contenidos de SOC se mantuvieron estables o aumentaron con el tiempo. Entre los tipos de suelos, los Mollisoles mostraron las mayores pérdidas (29.9% de reducción entre las fechas de muestreo). Los resultados de espectroscopía indicaron que las bandas de minerales para arcillas y silicatos se asociaron con los sitios que muestran mayor almacenamiento de SOC, lo que sugiere que la protección mineral desempeñó un papel importante en la acumulación de SOC a largo plazo en áreas semiáridas. Los regímenes climáticos desempeñaron un papel importante en la estructura de la comunidad microbiana. La composición de la comunidad fue controlada en gran medida mediante las fracciones lábiles, en las que miembros de los phyla Bacteroidetes, Proteobacteria y Archaea se asociaron con una menor disponibilidad de agua y menores contenidos de SOC. El modelo RothC, el cual es ampliamente utilizado, fue una herramienta efectiva para reproducir la dinámica y las tendencias históricas de los contenidos de SOC en sitios con cultivo anuales (trigo y maíz), sin embargo, el modelo se desempeñó bien bajo ciertas condiciones climáticas y de tipo de suelo en Chile. Dadas las limitaciones del modelo, proponemos que los modelos matemáticos se pueden desarrollar teniendo en cuenta los atributos microbianos y el mecanismo de protección de SOC específico del sitio. Esta tesis analiza el papel de las propiedades de los ecosistemas como un factor limitante en las pérdidas de carbono, examina el impulsor más importante de la dinámica de SOC en Chile y evalúa el uso de un modelo del carbono orgánico para abordar la restauración y adaptación a las tendencias futuras en el contexto del cambio global.

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1 INTRODUCTION

1.1 What is Soil Organic Carbon?

The dynamics of terrestrial ecosystems depend on interactions between a number of biogeochemical, nutrients, and hydrological cycles, all of which may be modified by human actions. There are five principal global C pools, all these pools are inter-connected and C circulates among them (Lal, 2001). The oceanic C pool is the largest, followed by the geologic, pedologic, biotic and the atmospheric pool. The pedologic or soil C pool comprises two components: soil organic carbon (SOC) and the soil inorganic carbon (SIC) pool. The SIC, a dominant form of C in soil of arid and semi-arid regions (rainfall <500 mm/year), comprises of primary or lithogenic carbonates and secondary, or pedogenic carbonates (Lal, 2016, 2004a).

SOC is one part in the much larger global carbon cycle that involves the cycling of carbon through the soil, vegetation, ocean and the atmosphere (Figure 1). SOC is highly dynamic, its mean residence time depends on the degree of protection (physical, chemical, biological, and ecological) within the soil matrix. The main entry of C into the biosphere is through the process of photosynthesis or gross primary productivity (GPP), that is the uptake of C from the atmosphere by plants (FAO, 2004). Soil organic matter (SOM), is composed about 45–60% of SOC (Lal, 2016). SOM influences a variety of soil functions and properties, thus making it a key indicator not only relevant for agricultural productivity, but also for resilience against climate change and other perturbations (Table 1-1).

Table 1-1. Impact of soil organic carbon on soil quality. Adapted from (Lal, 2014)

Soil	Soil parameter
Physical quality	Aggregation and structural stability Tilth, resistance to crusting and compaction Aeration and gaseous composition in soil air Water retention and availability Water transmission (infiltration and percolation) Heat capacity Surface area Soil strenght/erodibility
Chemical quality	Cation exchange capacity Nutrient retention and availability Buffer capacity (against pH)
Biological quality	Soil biodiversity Food and Habitat for soil biota
Ecological quality	Net primary productivity Use efficiency of input Nutrient ciclyng and biogeochemical transformations Carbon sequestration Rate of new soil formation Water purification Denaturing of pollutant

1.2 SOM composition

SOM encompass all the organic component of soils: 1) plant and animal residues and living microbial biomass 2) active or labile SOM and 3) relatively stable SOM conformed by largely amorphous and colloidal mixture of complex organic such a humus, or substances no longer identifiable as a tissues (Lal, 2016). Humus was initially thought to be composed of heterogeneous molecules characterized by aromatic ring and supramolecular structure. Humus has similar, worldwide characteristics, but varies with abiotic controls, soil type, vegetation inputs and composition, and the soil biota. It contains carbohydrates, proteins, lipids, phenol-aromatics, protein derived and cyclic nitrogenous compounds, and some still unknown compounds that were the largest and most stable SOM fraction. However, now it is understood that, these components represent only a small fraction of total organic matter (Schmidt et al., 2011). Molecular structure does not only control long-term

decomposition of soil organic matter (SOM). Thus, ecosystem dynamics and perturbations can broadly affect C structure (Grandy and Neff, 2008). The interdependence such as compound chemistry, reactive mineral surfaces, climate, water availability, soil acidity, soil redox state and the presence of potential degraders in the immediate microenvironment might also exert a direct effect. Certain plant-derived molecules (classically, long chain alkanolic acids, n-alkanes, lignin and other structural tissues) often persist longer than others. In mineral soil, however, these relatively persistent components appear to turn over faster. Even components that appear chemically labile, including proteins and saccharides of plant and microbial origin (different biological source), seem to turn over (on average) at rates similar on the order of years or even decades (Schmidt et al., 2011).

1.3 Methods for SOC determination

Methods for the determination of organic carbon in the soil such as the Walkley and Black, (1934), and the dry combustion (DC) (Kalembasa and Jenkinson, 1973; Nelson and Sommers, 1996) have been the standard approaches for SOC determination. The in situ C techniques has been developed during the last decades, such as Inelastic Neutron Scattering (INS) method (Wielopolski et al., 2011). One of the most promising techniques to emerge recently is the remote sensing technology for environmental and public sector applications, given the availability of historical data, the reduction in data cost, and increased resolution from satellite platforms (De Paul Obade and Lal, 2013). The utility of remote sensing for soil carbon determination is demonstrated through the measurement of spectral reflectance of surface and sub-surface soils in the laboratory, and then developing models that relate SOC concentration at different depths with reflectance. In addition, other techniques involve thermal and spectroscopic methods, which has been developed to characterize quality and quantity of soil organic matter (Clemente et al., 2012; Derenne and Quéné, 2015; Mouazen et al., 2016). These techniques hold promise for soil quality assessments (Bünemann et al., 2018). A further discussion of the methods applied to measure SOC as indicator of soil quality are summarized in Table 1-2.

Table 1-2. Techniques for determination of soil organic carbon. Adapted from (De Paul Obade and Lal, 2013; Schumacher, 2002)

Method	Term	Advantages	Disadvantages	References
Chemical oxidation	Walkley–Black	Good for SOC single estimation within a region, variable C oxidation, min. equipment needs.	Destructive and polluting method, inaccurate for SOC monitoring (underestimate C).	Chatterjee et al. (2009); Matus et al. (2009); Walkley and Black (1934)
Direct heating	The loss-on-ignition 350°C and 440°C.	Semi-quantitative method, C highly oxidized, rough estimate of SOC.	Overestimation of SOC content by losses of clay structural water.	Nelson and Sommers, (1996).
Automated dry-combustion (DC)	Quantify the evolved CO ₂ using either thermal conductivity or infrared detectors.	Destruction of all C form (1350°C), min. sample preparation and short-time analysis, accuracy and comparable to wet techniques.	Initial expense to purchase the unit is high, leak-free gas flow path must be maintained or false positive	LECO (1996); Nelson and Sommers (1996)
Spectroscopy of gamma rays and neutrons interacting with soil	Inelastic Neutron Scattering (INS).	Field based method, non-destructive, analysis of large volumes of soil samples, non-multiple calibrations.	Expensive, complex sampled collection.	Chatterjee et al. (2009); Wielopolski et al., (2011)
Atomic emission, and unique spectra of C	Laser Induced Breakdown Spectroscopy (LIBS)	Portable, rapid on-site method, high resolution (1 mm).	Destructive, expensive, small volumes analyses and point measurement, numerous calibrations.	McCarty et al. (2002); Wielopolski et al. (2011)
Spectral reflectance	Remote sensing	Non-destructive, repetitive, detailed.	Attenuation of reflectance signals by atmosphere, mixed pixels problem, no 3D.	Mora-Vallejo et al. (2008)
Based on ancillary data	GIS model e.g GEMS, RothC and CENTURY	Non-destructive, cost effective	Error propagation depends on input data accuracy.	Del Grosso et al. (2001); Liu et al. (2004)

1.3.1 Methods for labile SOC carbon determination

The most useful approach to characterize soil organic matter quality is to recognize different portions or pools of organic carbon that vary in their susceptibility to microbial metabolism (Brady and Weil, 1999). Labile soil organic carbon pools have emerged as standardized indicators because of their potential to detect early SOC trends over time and the SOC dynamics induced by changes in soil management practices. The sensitivity of the labile SOM fractions to soil physical conditions and climate also have been reported (Duval et al., 2018, 2013; Galantini and Rosell, 2006). These labile fractions are characterized by being an organic material in transition between fresh plant residues and stabilized organic matter, with a turnover time of <10 years (Duval et al., 2018). Labile fractions of SOC are essential in terms of soil fertility (their mineralization provides nutrients to plants), soil physical condition (aggregate stability largely depends on labile C), and of soil biodiversity (labile organic matter being the trophic resource of organisms (Chenu et al., 2019; Cosentino et al., 2006). A number of labile SOC fractions has been suggested, largely based on specific chemical or physical properties of the fractions. Despite their sensitivity to management and strong correlations to other parameters that are more difficult to measure, their relationship with soil processes is not well understood, partly because it is not clear which part of the organic matter they represent. In the Table 1-3 are listed the methods for separation and/or evaluation of labile C fraction.

Table 1-3. Methods for labile soil organic fractions analysis. Adapted from Haynes (2005) and Strosser (2010).

Method	Term	Principle	Laboratory Method	Reference
Physical				
	Particulate organic carbon (POC)	Composed primarily of plant-derived remains with recognizable cell structure, fungal spores, hyphae, and inert charcoal.	Sand-sized fraction (>53 – $100\ \mu\text{m}$ or 53 – $250\ \mu\text{m}$) followed by density separation (1.4 – $2.2\ \text{g cm}^{-3}$)	Cambardella and Elliott, (1992); Gregorich and Ellert (1993).
	Light Fraction (LF)	Composed largely of incompletely decomposed organic residue, and thus may represent a large fraction of the available substrate for microorganisms in soil.	Air-dried soils are weighed and added with NaI solution at a density of $1.7\ \text{g cm}^{-3}$. SOC content in the LF are often determined using a CHN-Analyzer	Janzen et al. (1992)
	Dissolve organic carbon (DOC)	Dissolved form of organic material in soil solution. It originates as leachates from plant litter, exudates from microflora, and roots and hydrolysis of insoluble soil.	Field-moist soils are followed by a centrifugation. The extract is often passed through a membrane filter (e.g., $0.45\ \mu\text{m}$) to remove dispersed soil material.	Giesler et al. (1993); Jones and Willett (2006)
	Hot Water extractable organic carbon (HWEOM).	Important but not homogenous pool compose of more stable components that form the close reserve of nutrients and energy for plants and microorganisms	60 min gentle boiling in destilated water 16 h at 80°C shaking in destilled water (sequential after CWEOM extraction)	Körschens et al. (1990)
	Cold water extractable organic carbon (CWEOM).	It is very close to dissolved organic carbon measured directly in soil using different lysimeters and suction cups	30 min at 20°C shaking in destilled water	Ghani et al. (2003)

Chemical				
	Permanganate oxidizable C (POXC)	It accounts for 5–30% of SOC and encompasses all those organic components that can be readily oxidized by KMnO_4 , including labile humic material and polysaccharides.	C oxidizable by a solution of KMnO_4 333 mM per 1 h and then centrifuged for 5 min.	Blair et al (1995); Culman et al (2012)
	Acid Hidrolisys	Refluxing soil samples in acid removes chemically labile materials such as proteins, nucleic acids and polysaccharides, leaving behind more hydrolysis resistant compounds such as aromatic, humified components and wax-derived long-chain aliphatics.	Air-dried and root-free soil with 6M HCl mixture is boiled at 100°C for 18 hr using a heating plate. After boiling, the suspension is filtered. The residues are dried, weighed, and ground for C.	Silveira et al.(2008)
	Mineralizable C	It consists in CO_2 evolved from soil during incubation. It indicates the total metabolic activity of the heterotrophic microorganisms in the soil that are decomposing OM using substrate C as an energy source, and respiring CO_2 .	CO_2 is measured from rewetted soils during a aerobic incubation (typically 10–30 d) with 1 mol L^{-1} NaOH base trap. The CO_2 -C accumulated in the trap is measured by acid titration.	Öhlinger (1996)
Biochemical				
	Microbial Biomass Carbon (MBC)	It measures the fraction of the C or N biomass extractable to K_2SO_4 after microbial cells lysis by chloroform.	Chloroform fumigation-extraction for 24 h, followed by extraction with 0.5 M K_2SO_4 .	Vance et al. (1987)
	Water-soluble polysaccharides	It is caused by soil microorganisms and crop residues, which are actively involved in soil aggregation. It represents labile polymers such starch, hemicellulose, and pectins.	The carbohydrate fraction in soil samples is determined with dilute-acid soluble hydrolysis with a phenol sulfuric acid reagent	Liu et al. (2005); Duval et al. (2013)

1.4 SOC sequestration

SOC is linked to metabolic capacity of soils and play a crucial role in increasing food production and soil resilience to climate change (Lefèvre et al., 2017). Soil carbon sequestration is defined as the process of transferring CO₂ from the atmosphere into the soil of a land unit, through plants, plant residues and other organic solids which are stored or retained in the unit as part of the soil organic matter (humus) (Olson, 2013). In addition, carbon storage is broader as it is defined as the increase in SOC stocks over time in the soils of a given land unit, not necessarily associated with a net removal of CO₂ from the atmosphere (Chenu et al., 2019).

1.4.1 Processes affecting SOC sequestration: Saturation

After each disturbance and change of residue input to an agroecosystem in steady state, a period of constant management is required to reach a new steady state. The SOM content in this new steady state scenario will be at a level that is characteristic of the soil, the vegetation, and the management system used. The new stabilization levels may be lower, the same as, or greater than the steady state level prior to the disturbance (Figure 1-1). However, very substantial amounts of plant residue would need to be returned to the soil to maintain SOM at levels above the original steady-state under native vegetation. An important consideration in the sequestration or reaccumulation of C by cropped soils is that they are managed very actively. This provides the opportunity to implement changes in management systems or to take up new technologies reasonably quickly. It can take up to 100 years before the new equilibrium is established when the SOM has been degraded to the steady state level. The recovery is strongly influenced by temperature (Swift, 2001). Liski et al. (1998) have reported that about 2000 years are needed to restore the SOC after forest fires in boreal forests (Swift, 2001). The only limitation for SOC stock increase is the amount of C inputs that can be provided. Several authors challenged the concept that an extra C input would always produce a similar SOC stock increase (Stewart et al., 2007). However, yet it remains controversial from many perspectives. For instance, Hassink, (1997) suggested that soils have a finite capacity to protect organic matter from

mineralization, i.e., a finite capacity to store carbon for decades or more, which was termed the carbon saturation capacity. In this concept, which was expanded by Six et al. (2002) and then by Beare et al. (2014), the long residence times of SOM are essentially due to fine sized minerals ($< 20 \mu\text{m}$), clay minerals and sesquioxides (oxides, hydroxides, and oxyhydroxides of Al and Fe), that provide protection by organo-mineral interactions (physico-chemical protection) or reduced accessibility of organic compounds to decomposers or oxygen to decomposers (physical protection). The abundance of the $< 20 \mu\text{m}$ size fraction would determine the amount of C that can be stored for decades to centuries, in association with this fraction. This alternative view allows explaining the results of the few studies that showed little or no increase of SOC stocks following increased OC inputs. The second concept is the so-called “priming effect”, which has been evidenced in deep soil layers: when SOC mineralization is limited by the energy supply to microorganisms, increased C inputs of fresh material may accelerate old SOC mineralization (Chenu et al., 2019; Fontaine et al., 2007).

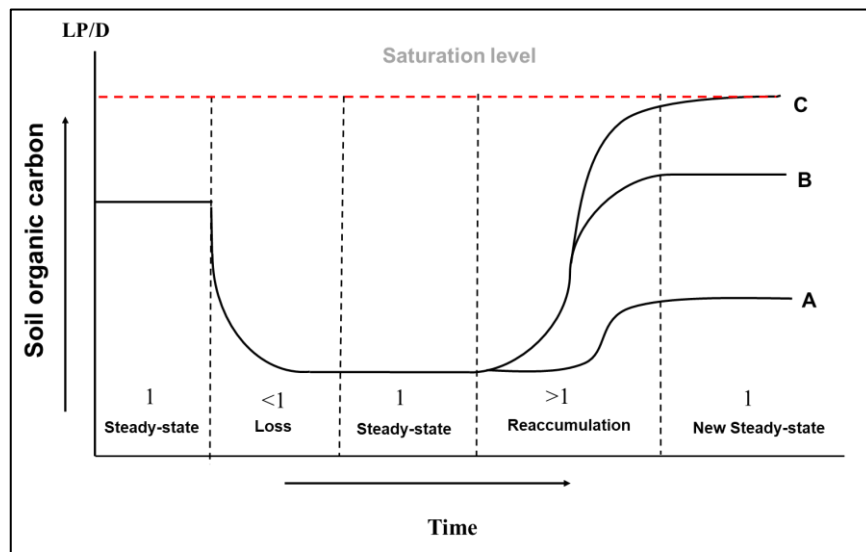


Figure 1-1. Schematic changes in soil organic matter levels caused by the disturbance of an existing steady-state. This is followed by a reaccumulation phase resulting from improved management practices and increased residue or litter returns. LP represents litter production and D represents the amount of decomposition. When $LP/D < 1$, SOM levels will decline, and for accumulation and sequestration to occur, $LP/D > 1$.

1.4.2 Drivers of soil organic carbon storage

The rate at which soil organic matter either increases or decreases is determined by the balance between gains and losses of carbon. The gains are influenced by land management practices such as cropping frequency, tillage, fertilizer application and manure. The losses are mainly due to plant removal, erosion and land uses conversion, which cause the disturbance of aggregate structure leading to mineralization SOC and the release of atmospheric CO₂ concentrations (Barancikova et al., 2010; Sleutel et al., 2003; Wei et al., 2014). Climate, and factors such as topographical features, parent material, soil type and Soil aggregation are relevant for SOC accumulation. Climatic conditions, namely temperature and precipitation, are key drivers of SOC storage globally as well as at broad (sub-)regional scales, affecting both C input into the soil and SOC decomposition. Precipitation determines net primary productivity (NPP) in many terrestrial environments and thus the input of C into the soil (Wiesmeier et al., 2019). Furthermore, humid conditions promote the formation of SOC stabilizing mineral surfaces by intensified weathering of the parent material and often cause soil acidification leading to reduced decomposition of soil organic matter (SOM) (Martínez et al., 2008). Temperature largely affects the microbial decomposition of SOM as its complex molecular attributes have a high intrinsic temperature sensitivity. Although this relationship is governed by multiple constraints, numerous studies have indicated a decrease of SOC with increasing temperatures (Allison et al., 2010; Gottschalk et al., 2012; Kirschbaum, 1995; Lal, 2001; Setia et al., 2013).

1.4.3 Carbon stabilization

We generally use the comprehensive term stabilization for processes or mechanisms that lead to prolonged turnover times in soil. Stabilization is defined as protection of OM from mineralization. Stability is the integrated effect of recalcitrance, organo-mineral interactions and accessibility. Generally, stabilized OM is older than unstabilized OM, a fact that is demonstrated in longer turnover and mean residence times. Turnover times of stabilized OM cannot be given as absolute values because they are dependent on

environmental conditions such as climate and soil properties (Lützow et al., 2006). The vulnerability of SOM to degradation will depend on the nature of the disturbance as well as the stabilization and destabilization mechanisms at play in a given ecosystem (Schmidt et al., 2011). Newly added carbon can be stabilized in the soil by a number of mechanisms (Jastrow et al., 2007; Kane, 2015; Six et al., 2002). Physically, carbon may be stabilized via its isolation inside soil micro- and macro aggregates where it is inaccessible to soil organisms. Chemically, carbon may be strongly adsorbed to clays via chemical bonds which prevents the consumption of carbon by organisms. Biochemically, carbon may be re-synthesized into complex molecule structures that may hinder decomposition. The three mechanisms depend on a number of biotic, abiotic and management factors that shape their soil carbon stabilization efficacy (Kane, 2015; Lal, 2018). However, proposals to return the carbon lost through agricultural activities in previous decades often emphasize the need to build or augment a ‘stable humus’ pool, drawing on the outdated concept of ‘humification’. Such a pool has been suggested to increase soil organic matter resistance to decomposition through *in situ* synthesis of macromolecules or hydrophobic protection by ‘humic substances’. This goal seems counterproductive given that soil organic matter is most beneficial when it decays and releases energy and nutrients. Thus, the management of soil organic matter turnover seem to be more important than the accrual of non-productive organic matter deposits. This requires a mechanistic understanding of interactions with minerals, movement into areas of lower mineralization and mediation of microbial activity (Lehmann and Kleber, 2015).

1.4.4 Soil functionality, health and soil resilience

Among scientists, the concept of ecosystem services is often used in connection with the soil functions. The concept function is variably used as a synonym for 1) process, 2) functioning, 3) role and 4) service (Bünemann et al., 2018). SOC concentration, along with its quality and dynamics, is essential to diverse soil functions and ecosystem services (Lal, 2016). In the next few decades, an increase in SOC has the potential to improve the five essential ecosystem services (Figure 1-2). (1) growing food through plants and animals by

storage and availability of plant nutrients, cycling, and transformation of elements and delivering macro-and micronutrients when needed; 2) storing water in the root zone to increase plant-available water capacity, denaturing, and filtering of pollutants, and appropriately using blue and gray/black water for mitigating drought stress and recycling nutrients; (3) moderating of climate through sequestration of C in the soil and biota, buffering against sudden/abrupt fluctuations in moisture and temperature regimes, and regulation of gaseous emissions (CO_2 , CH_4 , N_2O) into the atmosphere; (4, 5) providing habitat and energy source to support soil biodiversity. Other benefits provided by soils include industrial raw materials (e.g., clay, peat, minerals), and of antibiotics for human and animals and of other pharmaceuticals, and of organisms which create disease-suppressive soils. Indeed, soil health is the engine of economic development. It impacts quality and magnitude of renewable water resources, adaptation/mitigation and stabilization of climate, production of biomass and net and ecosystem productivity, and the above and below-ground productivity. (Banwart et al., 2014; Lal, 2016). This potential is dependent on time and is constrained by varying factors. It is known that under given climatic, substrate, relief and hydrological conditions there are biophysical limits to how much carbon a soil can store. However, there is little information on the inherent capacity of many soils to sequester carbon since native reference soils no longer exist (Banwart et al., 2014).

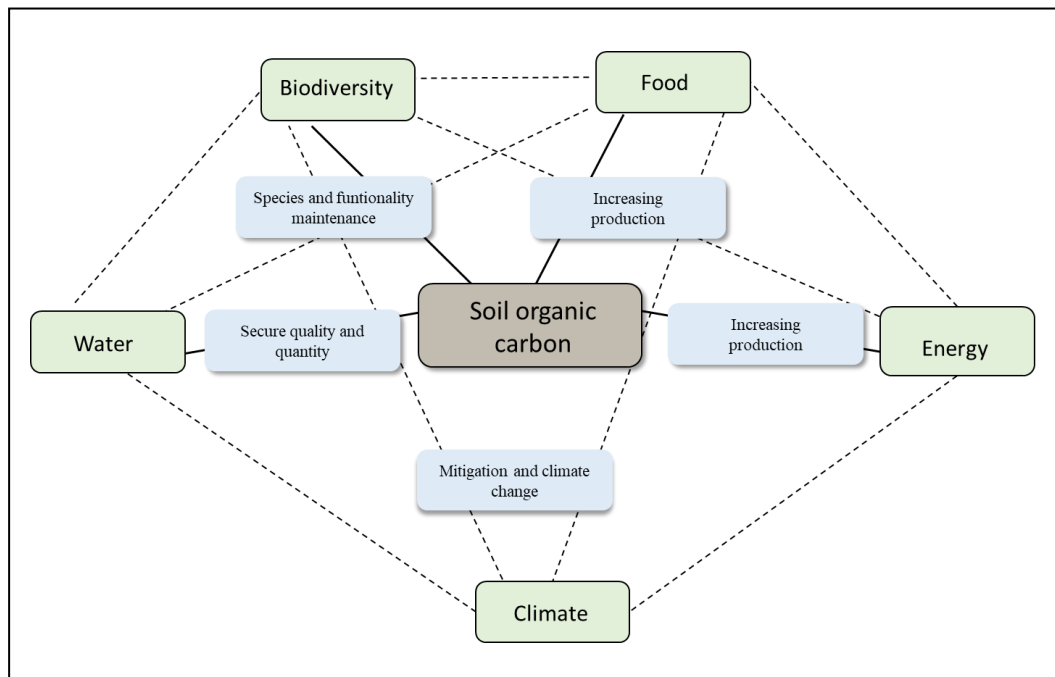


Figure 1-2. Interactions between soil organic carbon and the five essential services.

1.4.5 Soil carbon sequestration for mitigating climate change

Increasing SOC has been proposed to mitigate climate change with an additional benefit of improving soil structure and conditions (Lal, 2016), by converting CO₂ into biotic or abiotic carbon sequestered in terrestrial ecosystems. As soil stores two to three times more carbon than the atmosphere, a relatively small increase in the stocks could exert a significant role in mitigating greenhouse gases emissions. The annual greenhouse gas emissions from fossil carbon are estimated at 8.9 giga ton C (8.9×10^{15} g), and a global estimates of soil C emissions to 2 m depth are 2400 giga ton (2400×10^{15} g) (Batjes, 2014).

Three basic strategies to enhance SOC sequestration are those which (i) increase input of biomass C, (ii) decrease losses of SOC by erosion and decomposition and (iii) increase the mean residence time (MRT) by stabilization of the sequestered SOC. The amount of biomass-C input needed to attain soil C saturation or fill total C sink capacity varies among soils, but saturation does occur. Therefore, the strategy of mitigating climate change through SOC sequestration is to identify those soils which are farther from C saturation

(Stewart et al., 2007) as global hotspots. These global hotspots would include the eroded, degraded, and depleted soils. The saturation C sink capacity may also change with the change in atmospheric concentration of CO₂ because it can enhance SOC turnover and potentially limit long-term C sequestration in ecosystems depending on the plant community composition (Hofmockel et al., 2011; Lal, 2018)

Some general limitations to the effectiveness of C sequestration in soil or vegetation in mitigating climate change include the following (Powlson et al., 2011). 1) The amount of C locked up is finite: the increase in SOC content ceases as a new equilibrium value is approached. This principle is clear from long-term studies, which show that SOC does not accumulate indefinitely (Johnston et al., 2009), 2) The process is reversible: the change in land management leading to increased C in soil or vegetation must be continued indefinitely to maintain the increased stock of SOC (Freibauer et al., 2004), and 3) Land management changes leading to increased soil C may either increase or decrease fluxes of powerful greenhouse gases such as N₂O or methane. In some, perhaps many, situations these changes may be far more significant than changes in SOC stock because the very large global warming potentials (GWP) of these two gases, N₂O and methane, have, respectively, 298 and 25 times the GWP of CO₂ when considered on a 100- year time scale (IPCC, 2007).

1.4.5.1 International framework to address SOC in climate change mitigation

Within the Framework of the United Nations Framework Convention on Climate Change (UNFCCC), international agreements such as the Kyoto Protocol and the Paris Agreement have set the rules for greenhouse gas (GHG) emissions targets, as well as the necessity to regularly report on anthropogenic GHG emissions. As part of these efforts, accurate inventories on emissions due to SOC stock changes should be reported.

The Intergovernmental Panel on Climate Change (IPCC, 2007) provides guidelines for measuring, reporting and verifying national SOC stock inventories following the Monitoring, Reporting and Verifying (MRV) Framework, which ensures that these inventories fulfill the criteria of completeness, transparency, consistency, accuracy and

thus comparability. To achieve greater specificity and accuracy, improved methods are required to measure, account, monitor and report on this specific carbon pool.

Under the Paris Agreement (2015), all ratifying countries have committed to make nationally determined contributions (NDC) to mitigate climate change, to report on their anthropogenic emissions and removals, and to track progress of their contributions to climate change mitigation. The MRV framework tracks and assesses the implementation of mitigation contributions. Article 13 of the Paris Agreement introduced a new Enhanced Transparency Framework for reporting, allowing for better transparency in reporting on GHG emissions.

The international initiative 4 per 1000 was launched by UNFCCC at COP 2 in 2015 and supported by almost 150 signatories (countries, regions, international agencies, private sectors and NGOs). Stakeholders commit in a voluntary action plan to increasing the organic matter content of soils, with a special focus on agricultural land, in order to contribute to food security, adaptation to climate change, and mitigation of climate change (<http://4p1000.org>) (Chenu et al., 2019). According to this initiative, if the carbon level was increased by 0.4% per year, in the top 30-40 cm of soils, the annual increase in carbon dioxide (CO₂) in the atmosphere would be stopped. This 4 per 1000 initiative values cannot be applied everywhere as soil varies widely in terms of C storage, which includes desert, peatlands, mountains (Minasny et al., 2017).

1.4.6 Technological options for Soil Carbon Sequestration

Managing agroecosystems is an important strategy for SOC/terrestrial sequestration. Agriculture is defined as an anthropogenic manipulation of C through uptake, fixation, emission and transfer of C among different pools. Whereas land misuse and soil mismanagement have caused depletion of SOC with an attendant emission of CO₂ and other GHGs into the atmosphere, there is a strong evidence that SOC pool has the potential to offset fossil fuel emissions. However, the SOC sink capacity depends on the antecedent level of SOM, climate, profile characteristics and management. Conventional tillage and erosion deplete SOC pools in agricultural soils. Thus, soils can store C upon conversion

from plow till to no till or conservation tillage, by reducing soil disturbance, decreasing the fallow period and incorporation of cover crops in the rotation cycle. For instance, leguminous cover crops enhance biodiversity, the quality of residue input and SOC pool. It is well established that ecosystems with high biodiversity absorb and sequester more C than those with low or reduced biodiversity (Chenu et al., 2019; Lal, 2004a). Eliminating summer fallowing in arid and semi-arid regions and adopting no till with residue mulching improves soil structure, lowers bulk density and increases infiltration capacity. However, the benefits of no till on SOC sequestration may be soil/site specific, and the improvement in SOC may be inconsistent in fine textured and poorly drained soils (Wander et al., 1998). Beside no-tillage and conservation agriculture, other practices that increase C inputs include irrigation, increasing below-ground inputs, organic amendments, managing soil nitrogen mineral amendments, manipulating microbial physiology, managing soil biodiversity, a change from annual to perennial crops typically increases belowground, C inputs (and soil disturbance is reduced) (Paustian et al., 2016). Converting degraded soils under agriculture and other land uses into forests and perennial land use can enhance the SOC pool. The magnitude and rate of SOC sequestration with afforestation depends on climate, soil type, species and nutrient management. A summary of the decisions recommended for management practices in relation to soil organic carbon sequestration are given in Table 1-4.

Table 1-4. Comparison between traditional and recommended management practices in relation to carbon sequestration. Adapted from Lal (2004) and Schmidt et al., (2011).

Traditional methods	Recommended management practices
Biomass burning and residue removal	Residue returned as surface mulch
Conventional tillage and clean cultivation	Conservation tillage, no till and mulch farming
Bare/idle fallow	Growing cover crops during off-season
Continuous monoculture	Crop rotations with high diversity
Low input subsistence farming and soil fertility mining	Judicious use of off-farm input
Intensive use of chemical fertilizers	Integrated nutrient management with compost, biosolids and nutrient cycling, precision farming
Intensive cropping	Rotate perennials; use agroforestry and high C input species, grow cover crops
Surface flood irrigation	Drip, furrow or sub-irrigation
Indiscriminate use of pesticides	Integrated pest management
Cultivating marginal soils	Conservation reserve program, restoration of degraded soils through land use change.

1.5 Global SOC stocks

The distribution of SOC is very heterogeneous and is strongly dependent on soil type, land use and climatic conditions. On certain soil types and under certain land uses, SOC storage is highly effective. Although they cover proportionally little of the global land surface, these areas require special attention: they are hot-spots of SOC. These hot-spots are very sensitive to climate change and can easily become sources of GHG emissions due to their high SOC content. Finally, large land areas with low SOC stocks per km² represent a potential for further carbon sequestration: they are bright spots of SOC (Lefèvre et al., 2017).

1.6 Hot-spots of SOC: Major areas for consideration

1.6.1.1 Black soils

Black soils, broadly defined here as soils that contain a mollic horizon, cover about 7% of the ice-free land surface (916 million ha). Most of them occur in three regions in the northern hemisphere and one region south of the equator. The natural areas from which black soils developed are the prairies and steppes that experience summer-dry and freezing conditions (Altermann et al., 2005; Liu et al., 2012). These soils are dark brown to black in color due to their enrichment of high-quality humus down to a depth of more than 40 cm - mostly 60 to 80 cm. This high-quality humus is the result of a high base saturation (i.e. a high percentage of the cation exchange capacity is occupied by the basic cations Ca^{2+} , Mg^{2+} and K^{+}), stabile aggregate structure, and intensive biological mixing (bioturbation, e.g. by earthworms). Due to their high productivity, most of these soils are intensively used for agriculture. However, they are very sensitive to soil degradation (e.g. erosion, crusting and nutrient mining) and SOC losses and need to be managed carefully to maintain their productive potential (Liu et al., 2012). In Chile, mostly agricultural productivity zones are distributed in Mollisols and Andisols soils, these resources are very important for maintaining the food security. In Chile, these soils have largely been managed or burned, except in protected areas where pristine ecosystems can still be found. Selective clearing and biomass removal are common forest practices in Chile that have been shown to affect the status of soil nutrients in surface horizons (Alvear et al., 2005; Panichini et al., 2017).

1.6.1.2 Permafrost

Low temperatures and waterlogging in permafrost terrain reduce decomposition rates and increases cryoturbation as a result of freeze-thaw processes. SOC accumulation in these soils make them important for the global climate system because of their potential to thaw, and thus decompose organic matter accumulated over a long period. Climate, especially low temperatures, is one of the most influential factors affecting the formation of northern soils. In the case of Gelisols (soils underlain by permafrost), soil formation is greatly shaped by unique cryopedogenic processes driven by the combination of low temperatures,

water movement, and freeze-thaw cycles. Although permafrost distribution is globally known, SOC estimates in the permafrost region remain variable. However, it is considered that about 30% of the total SOC stock to 2 m depth is held in the Northern Circumpolar Region and that the permafrost region contains twice as much carbon as there is currently in the atmosphere (Lefèvre et al., 2017; Matamala et al., 2017).

1.6.1.3 Drylands

Drylands cover approximately 430 million ha, which comprise 40% of the Earth's surface (FAO, 2004). In Chile, arid and semiarid regions comprise 41% of the continental area of Chile (Perez-Quezada et al., 2011). Although there is no clear boundary, drylands are considered to be areas where average rainfall is less than the potential moisture losses through evaporation and transpiration (FAO, 2004). The soils of drylands are characterized by frequent water stress, low organic matter content and low nutrient content. However, their carbon storage accounts for more than one third of the global stock, mainly due to their large surface area and long-term SOC storage (when the soil is not degraded), rather than due to vegetation cover. Drylands have the potential to sequester more carbon than as they are far from saturated, but carbon storage in drylands is affected and limited by bioclimatic elements and is slow.

1.6.1.4 Forest soils

Forests cover 4.03 billion ha globally, approximately 30% of Earth's total land area. The majority of soil carbon is concentrated in peatlands within the boreal forests and the tropical forests in Southeast Asia (Pan et al., 2013). Forest vegetation and soils contain about 1240 PgC (1 PgC = 10¹⁵ g of carbon), and the carbon stock varies widely among latitudes. Of the total terrestrial C stock in forest biomes, 37% is in low latitude forests, 14% in mid latitudes and 49% in high latitudes. The SOC stock may comprise as much as 85% of the terrestrial C stock in the boreal forest, 60% in temperate forests and 50% in tropical rainforest. A large part of the total SOC stock occurs in soils of tundra, pre-tundra and taiga regions. The SOC content in forest soils may range from 0% in very young soils to as much as 50% in some organic or wetland soils, with most soils containing between 0.3 and 11.5%

in the surface 20 cm of mineral soil (Lal, 2005). Around the world, deforestation causes about 25% of the total loss of SOC.

1.6.1.5 Peatlands

Peatlands are wetland ecosystems characterized by the accumulation of organic matter (peat) derived from dead and decaying plant material under conditions of permanent water saturation. Peatlands and organic soils contain 30% of the world's soil carbon but only cover 3% of the Earth's land area occurring across 180 countries. Most of them occur in regions of permafrost and in the tropics. There, high plant productivity combines with slow decomposition as a result of high rainfall and humidity. In Chilean southernmost area, it is possible to find a large area of peatlands of about 4.5Mha (Yu et al., 2010). The current legislation fails to protect this fragile ecosystem, and many areas are being drained to establish forest plantations or being extracted for peat.

1.6.1.6 Grasslands

Grasslands, which include rangelands, shrublands, pasturelands, and croplands sown with pasture and fodder crops, cover approximately 40% of the earth's land surface. It represents 70% of the global agricultural area, and contain about 20% of the world's SOC stocks. Due to their high natural soil fertility, around 20% of the world's native grasslands have been converted to cultivated crops and lands for livestock production.

1.7 Chile and carbon stock

Due to its geography, Chile presents a clear north-south gradient of precipitation and temperature, and consequently SOC distribution, which divides the territory into areas prone to different management and SOC capture potentials. Chile may be roughly divided into soils with low SOC stocks ($\text{SOC} < 2.5 \%$) in the hyper-arid to semiarid and northern Mediterranean zones, and soils with medium-high ($\text{SOC} > 2.5 \%$) stocks in the southern Mediterranean and Rainy and Patagonian zones (Casanova et al., 2013). The top 0.3 m SOC stock estimate for Chile is 5.52 Gt and 9.8 Gt for the top 1 m (Padarian et al., 2017). A 4 per mile increase per year would translate to the capture of 39.2 Mt of carbon across the country, which is enough to offset its CO_2 emissions (19.11Mt C, excluding Land Use

Change and Forestry (LUCF), or offset 21.8% of the total GHG emissions (42.3 Mt of CO₂ equivalent, excluding (LUCF). However, if we consider agricultural area, which only occupies 4.6% of the land area, the C stock for the top 1 m is 0.25 Gt. The northern most part of the territory is dominated by arid and hyper-arid conditions (Atacama Desert), with scarce vegetation and a capture potential close to zero, except for small amounts from atmospheric deposition. In peatlands, the composition of the vegetation communities of this land type varies across the landscape but, on average, it accumulates around 0.16 t C ha⁻¹ yr⁻¹, which translates to 6.3 Mt per year for the whole area. The area of cultivated lands is mostly under traditional management. In this area, it is possible to extend the use of management practices like zero tillage or crop rotation, with a potential increase of 0.5 t C ha⁻¹ yr⁻¹ where water is not limiting (Martinez et al., 2013), leading to 1.05 Mt C sequestered per year. In Patagonia, a soil C sequestration rate of 0.87, 0.34, and 1.09 t C ha⁻¹ yr⁻¹ was calculated for silvopasture, plantation and prairie system respectively (Dube et al., 2011).

Chile presents several governmental programs to enhance soil conditions, which finance managements that may lead to an increase of SOC stocks. For example, reforestation and implementation of zero tillage. Additionally, Chile have also adopted international commitments like the implementation of Nationally Appropriate Mitigation Actions (NAMAs), which include a strong forest component, with plans to afforest about 2.29 million ha (Minasny et al., 2017; Padarian et al., 2017).

1.8 Carbon modeling and new perspectives

A number of soil model have been developed to monitor soil carbon changes and the subsequent effect when soil management practices are changed. The first were developed in the 1970's and 1980's based on long-term experiments in either temperate or tropical agricultural environments (Chenu et al., 2019). The conceptual diversity of SOM models is split into two categories. The first, traditional pseudo first-order decay approaches with a range of SOM pools and controls on turnover times and decomposition pathways. The most widely known are Century (Parton et al., 1987) and RothC (Coleman and Jenkinson,

1999). The second, more recently developed, explicit microbial models, some with representations of mineral–surface interactions, vertical transport, nutrient controls and plant interactions (Smith et al., 2018).

First-order category are widely used, the mineralization and transformation of plant litter and soil organic matter has mainly been modeled using schemas of conceptual pools that turn over at different rates. For example, the Roth-C model (Coleman et al., 1997) splits litter into decomposable plant material (DPM) and resistant plant material (RPM). Then, both pools decompose to form microbial biomass (BIO), humified organic matter (HUM) and evolved CO₂. The model also includes an inert pool of organic matter (IOM). With the exception of IOM, each compartment decomposes by first order kinetics, and each has an intrinsic maximum decomposition rate. The actual rate of decomposition is determined using modifiers for soil moisture, temperature and plant cover, operating on the maximum rate. Falloon and Smith (2006) Similar schemas are used in CENTURY (Parton et al., 1988), DAISY (Hansen et al., 1991), and ECOSSE (Smith et al., 1997) (Ranatunga et al., 2001).

Although simple conceptually, the problem of these models is that they require information on the size and turnover rate of each compartment, which is difficult to obtain from field studies. Moreover, these pools have no analogues in nature. According to latest biogeochemical insights into SOM dynamics, classical conceptual pool-based models are obsolete. Contemporary analytical approaches suggest that SOC turnover is governed by physical accessibility rather than chemical recalcitrance (SOM, 2017). Additionally, other of the major weaknesses of current models is the lack of representation of edaphic characteristics (i.e. those physical and chemical features that are intrinsic to the soil), and the fact that the major stabilization mechanisms will vary spatially with soil type and topographic positions (Schmidt et al., 2011).

Although the debate about whether more explicit process representations lead to more accurate predictions remains unresolved. Next generation SOM models should integrate process from microbial level to global scales. Thus, ecosystem models are beginning to incorporate information on microbial abundance, composition, and diversity, which is

needed to improve predictions of soil C stocks and their dynamics, used to refine and validate them (Maestre et al., 2015). Other challenges for next generation SOM models are detailed in Table 1-5.

Table 1-5. Key challenges to modeling soil supporting and degrading processes. Adapted from Schmidt et al. (2011), Smith et al. (2018) and Vereecken et al. (2016).

Key challenges	
Challenge I	To include soil structural dynamic; represent mineral association, soil heterogeneity across a broad range of space and time scales
Challenge II	To better link nutrient dynamics and availability in soils with hydrological and biogeochemical processes.
Challenge III	To model soil formation processes over long-time scale
Challenge IV	To integrate new understanding of carbon dynamic, chemical and biological processes (dissolved organic carbon)
Challenge V	Modelling microbial functional types, analogous to plant functional types. Introduce soil nitrogen cycle couple to carbon cycle, priming effects, leaching, stabilization
Challenge VI	Add O ₂ limitation and freezing effects on CO ₂ and CH ₄ production. Develop soil columns to represent inundation; Permafrost soils warming and melting.
Challenge VII	To improve water, erosion and sediment routing modeling in complex landscapes.

1.9 Hypothesis and objectives

Due to low vegetation cover and relatively poor soil structure, soils in drier climates require special attention because of land degradation and desertification, which is exacerbated by erosion, salinization of irrigated lands, and the impact of climate change (FAO, 2017; Lal, 2004b, 2001). Because of this, we hypothesize Agricultural soils in arid and semiarid regions are more vulnerable to SOC losses. During the last few decades, these soils may have experienced a greater reduction of SOC content when compared to soils found in more humid regions, as a result of climate conditions rather than land-use decisions. To explore this hypothesis, the general objective of this thesis is to understand spatial and temporal changes of SOC under distinct soil ecosystems in order to further evaluate alternative strategies that minimize carbon stock losses in Chile.

In this context, three specific objectives were established:

- 1) To assess spatial and temporal changes in SOC content among different climates, land uses and soil types.
- 2) To elucidate key environmental, physical-chemical and biological factors and/or mechanisms associated with soil organic carbon storage.
- 3) Evaluate the effects of management practices decisions in agroecosystems under different climates, as well as soil types conditions.

This work comprises a broader effort to study SOC dynamics in Chile, a topic that remains largely unexplored. Hence, an interdisciplinary approach was needed to achieve the objectives of this work. Chapter I provides a general introduction to address the following research questions: i) How much have carbon levels changed in Chile? ii) What has been the main cause of these changes?. These questions are responded to in chapter II, which additionally provides a baseline for comparison with future SOC measurements, and identifies the most vulnerable areas that require our attention. With these considerations in mind, it is highly important to develop knowledge in order to build soil resilience. In this context, chapters III and IV contribute to our understanding of carbon dynamics at a micro

and macro scale, and determine the key factors controlling SOC changes. In particular, chapters III and IV aim to study microbial patterns and labile carbon fractions across various environmental conditions, which may lead to an improvement of predictive ecosystem models, as well as the determination of early indicators of SOC trends. The empirical evidence collected in chapters II, III and IV is our basis for responding to the following research question: Is it possible to restore SOC levels by increasing carbon inputs from plant litter in areas more vulnerable to C losses? A growing interest in predicting SOC trends has led to a need for assessment tools and procedures. Hence, the availability of reliable predictive models will have played an important role in predicting the effects of agriculture and climate on SOC stock and in designing policies for soil protection. Thus, chapter V concludes the thesis with the validation of a widely used model for SOC prediction and its improvement with data collected in the previous chapters. Detailed information regarding the chapters I, II, III, IV and V is discussed below. The steps that will be undertaken to address the hypotheses and the research questions are shown in Figure 3-1.

Chapter I

This chapter reviews the literature of most important aspects of soil organic carbon (SOC), more specifically, it addresses the concepts for the next chapter of thesis, such as SOC quality, SOC sequestration, SOC vulnerability and analytical methods. Also, this chapter includes the main challenges for SOC modeling. The hypothesis and objectives of this thesis can also be found in this chapter.

Chapter II

In this chapter, we focus specifically on temporal changes in soil organic carbon, which involves an integrated study of C dynamics to diagnose the soil C status in distinct agroecosystems and different climates across the country. This chapter provides empirical data and scientific evidence for more vulnerable agroecosystems.

Chapter III

Chapter III explores the main factors influencing microbial communities in soils, which is necessary to predict changes in SOC stocks and the development of new management strategies to identify declining organic matter content trends and soil fertility losses.

Chapter IV

The overall purpose of this chapter is to study the dynamics of soil organic carbon associated with chemical and physically separated labile fractions, which are widely used as indicators of soil quality. Here, we evaluate labile SOC fractions across different land uses, climates and soil types and relate these concepts with the persistence of the soil organic matter as an ecosystem property.

Chapter V

The objective of this chapter is to test the RothC model, in order to evaluate temporal variability of SOC stocks between the years 1980 and 2016 as a function of the climate condition and plant input under the typical annual cropping system in Chile.

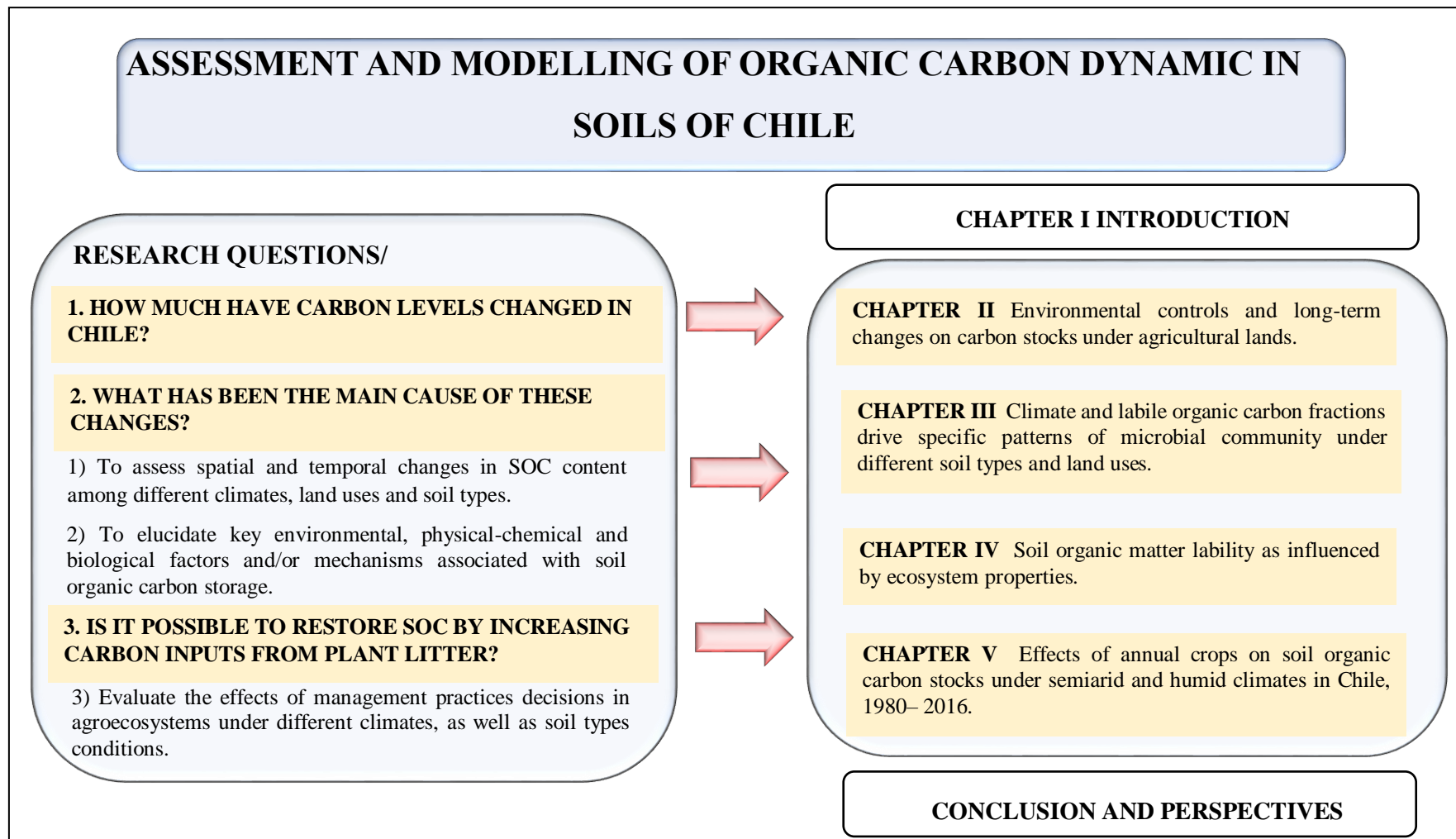


Figure 1-3. Schematic outline illustrating the relationship between thesis structure and scientific articles.

2 ENVIRONMENTAL CONTROLS AND LONG-TERM CHANGES ON CARBON STOCKS UNDER AGRICULTURAL LANDS

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2.1 Introduction

Maintaining SOC stocks is fundamental for supporting long-term agricultural productivity, as well as for mitigating the effects of, and adapting agricultural systems to climate change (Lal, 2015, 2004a; Powlson et al., 2011). Several studies have examined the effects of temperature, precipitation, land use, and geochemistry as drivers of SOC storage (Conant et al., 2011; Hobbey et al., 2017; Kirschbaum, 1995; Kramer and Chadwick, 2016; Post and Kwon, 2000). However, these effects are still the focus of many studies as the SOC content is not easy to predictably, mainly because of it is highly dependent on the site characteristics (Chenu et al., 2019; Giardina and Ryan, 2000; Schmidt et al., 2011).

Chile exhibits numerous advantages for studying soil C changes due to a large north-south gradient of climate and geology, and consequently, displays wide variations in SOC stocks and rates of turnover (Bonilla and Johnson, 2012). Arid and semiarid regions comprise 41% of the continental zone (Perez-Quezada et al., 2011), but precipitation ranges widely, from areas with almost no precipitation in the north, up to 3,000 mm yr⁻¹ in southern Chile (Casanova et al., 2013; Lobo et al., 2015). Due to low vegetation cover and relatively poor soil structure, soils in dry climates are often more prone to SOC depletion because of land degradation and desertification, which is exacerbated by the erosion, salinization of irrigated lands, and the impacts of climate changes (Lal, 2004b, 2001; Lefèvre et al., 2017). Because of this, during that last several decades we might expect arid and semiarid regions to have relatively greater losses of SOC and associated changes in chemical properties compared to soils under more humid conditions. Conversely, due to their low initial SOC content (usually less than 0.5% by weight) and longer SOC residence time, arid and

semiarid soils may have considerable potential for C sequestration (FAO, 2004; Farina et al., 2013; Lal, 2009).

The recent advances in Mid-infrared (MidIR) and Near-Infrared (NIR) spectroscopy techniques provide a suitable method to characterize soil chemical composition (Bellon-Maurel and McBratney, 2011; Viscarra Rossel and Behrens, 2010). The infrared spectroscopy technique characterizes bands for organic and mineral functional groups including amides, aliphatics, aromatics, and carbonyls, as well as silicate and lattice clay Si-O bonds providing information about soil organic matter (SOM) composition and possible changes in future scenarios (Calderón et al., 2013; Leifeld, 2006; Parikh et al., 2014; Viscarra Rossel et al., 2006). Because of this, MidIR spectroscopy has been typically used to predict diverse soil characteristics through advanced chemometric methods such as partial least squares regression (Leifeld, 2006).

On the other hand, recent studies have focused on the spatial distribution and future changes in SOC stocks in Chilean soils (Bonilla and Vidal, 2011; Minasny et al., 2017; Padarian et al., 2017; Reyes Rojas et al., 2018). The soil descriptions were obtained through soil survey studies performed by the Natural Resources Information Center (CIREN), three or four decades ago. Considering the effects of time, agricultural practices, and climate change, some limitations could be expected in these studies as they do not consider the changes and/or SOC sequestration/loss in recent years. In addition, there is a limited knowledge on the role of the soil of type, climate and land use as drivers for SOC variations in agricultural soils in Chile, and the mechanisms involved in the SOC sequestration. Thus, in this study we compute the SOC losses under contrasting soil types, climates, and land use. SOC changes were evaluated in a series of sites from arid to hyper humid climates, different soil physical and chemical properties, and land use. The main objectives were to: 1) Evaluate and compare SOC losses under different soil types, climate, and agricultural land use over the last decades, 2) identify which agroecosystems have been more affected by SOC losses and, 3) examine the linkage between soil spectral differences and changes in SOC content.

We used a dataset comprised of soils from 51 different sites that were initially sampled between 1968 and 1994 within different soil types and climate conditions across the agricultural lands. These same sites were then sampled again at least 20 years later and characterized in terms of soil physicochemical parameters and spectral differences determined by Fourier-transform MidIR spectroscopy. In addition, this study provides updated information for improving geospatial modeling and SOC projections in Chile or when building SOC global maps.

2.2 Materials and methods

2.2.1 Study site

Sampling sites were located in central Chile, from Valparaíso in the north to the Araucanía Region in the south, which includes most of the cultivated and productive land in the country. The sites extend across a 1000 km transect, from 32°S to 40°S in the central valley between the Andes mountains and the coastal range (Figure 2-1A). All sites were on slopes of < 6% located at elevations between 30 and 580 m. In general, the climate associated with the sites is characterized by rainfall during the southern hemisphere's winter season (May to September) and a drier summer season (Figure 2-2) (Bonilla and Vidal, 2011). In the arid systems, mean annual temperature (MAT) was about 15°C, mean annual evapotranspiration (ET) was 1250 mm, and mean annual precipitation (MAP) was 200 mm. In general, sites that received more precipitation also had lower mean annual temperatures. In hyper humid zones, MAT reached 12°C approximately, with an ET of 750 mm and MAP of 2000 mm.

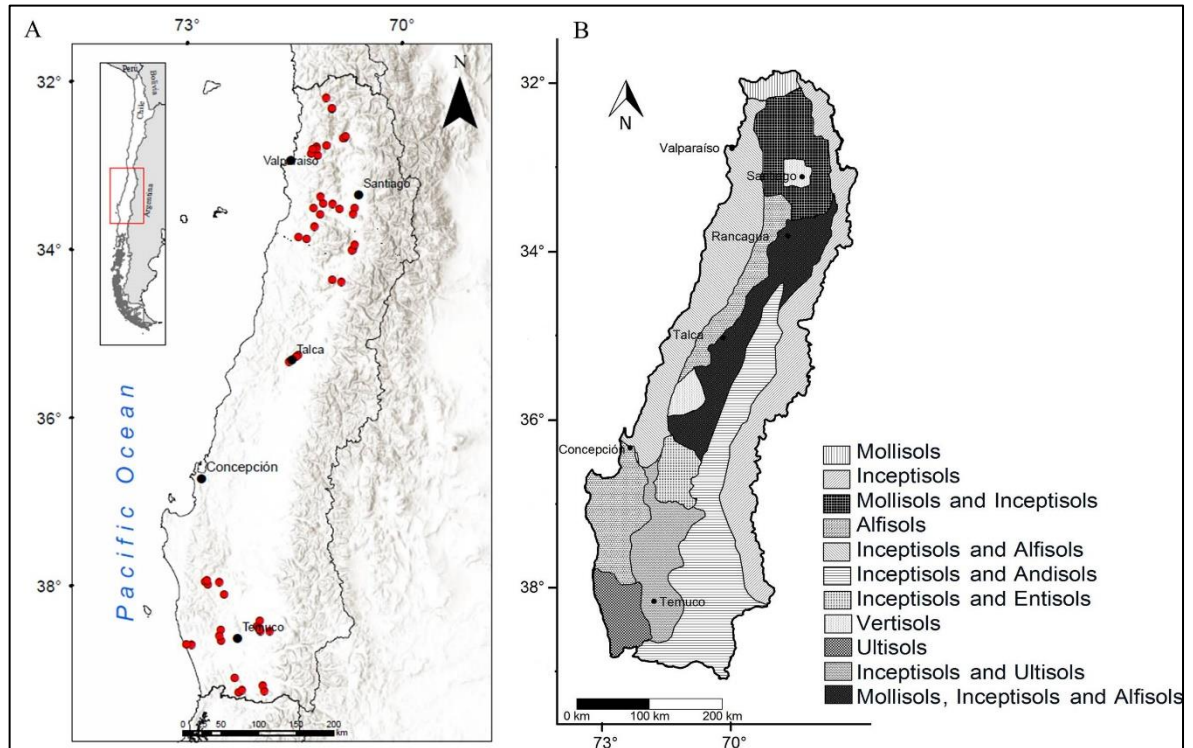


Figure 2-1. Sampling sites. (A) Sampling sites location in the central Chile, and (B) soil order distribution in the study area.

2.2.2 Soil sampling

The soils were initially sampled in 1968 and later in 1994 for various field surveys conducted by CIREN (CIREN, 2002, 1997a, 1997b, 1996a, 1996b). At that time, sites were georeferenced and local landmarks surrounding the sites were noted. In the second sampling, a total of 51 sites were relocated with GPS and sample in the summers of 2014, 2016, and 2017. In order to evaluate the spatial variability in soil properties, two additional samples were collected in each site in addition to the GPS sampling point. Soils were collected from topsoil horizon described by CIREN and plant residue and O horizon were removed before sampling. Depending on the site, first horizon depths ranged from 8-29 cm (Table 2-1).

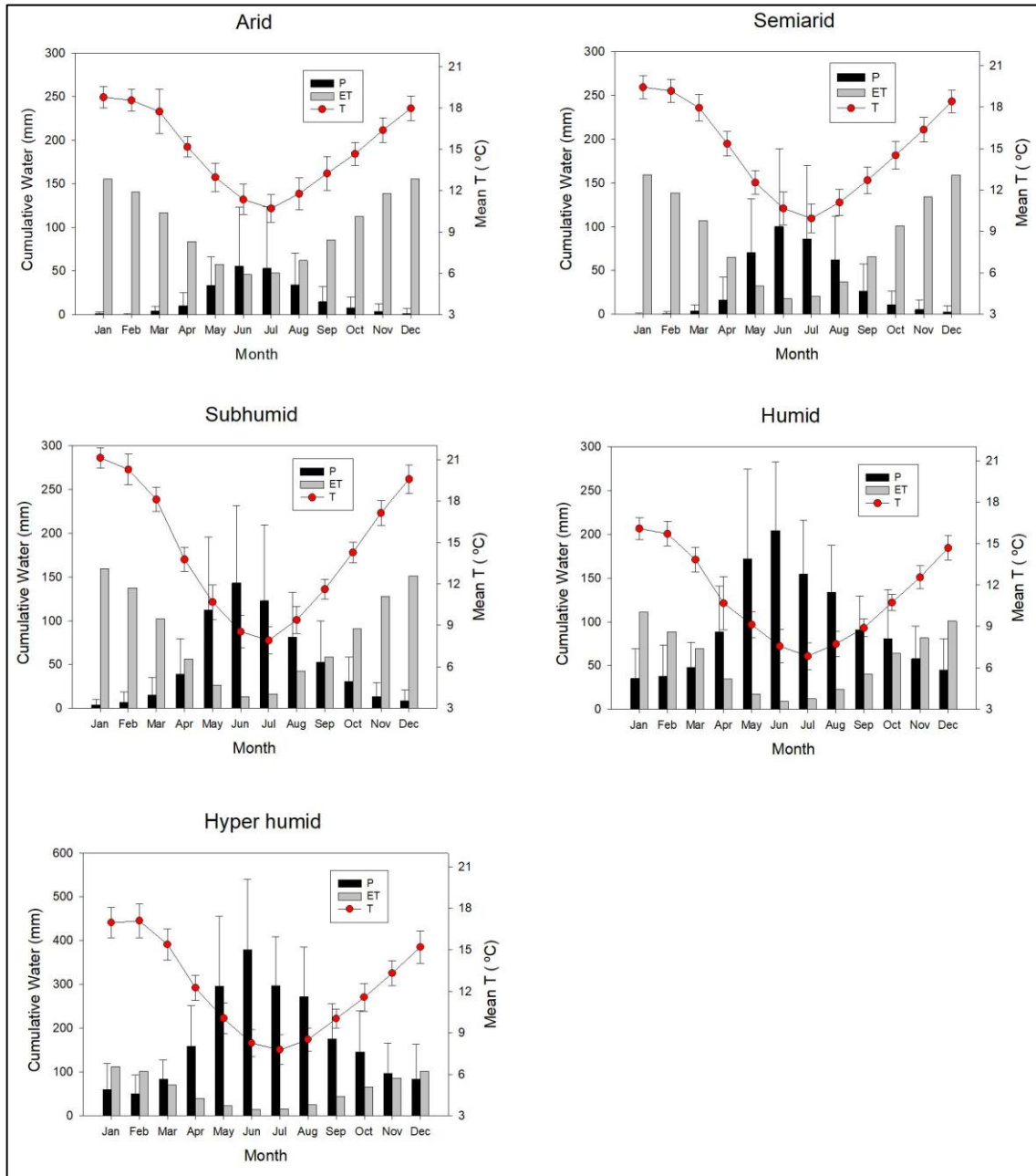


Figure 2-2. Average monthly of temperature (T), cumulative precipitation (P), and evapotranspiration (ET) in different aridity regimes during the approximate time period considered in this study (1980-2015).

2.2.3 Land use

The land use and management records were compiled based on interviews with local farmers and satellite imagery interpretation. High-resolution images were obtained from Google Earth Pro, which are available since year 2004. Initially, all sites were used for agricultural purposes, however, some of them changed the land use over the last decades. Some sites were continuously cultivated, while other sites had a short fallow rotation (1–2 years), followed by a period of orchard plantation. According to all this information, sites were classified into three groups based on the land use over the last 10 years: 1) Non-cultivated sites (NC), as those sites abandonment or without agricultural practices, 2) woody perennial (WP) as those sites with forest plantations, vineyards, and orchards, and 3) cultivated sites (C), typically planted with cereal crops and/or vegetables.

In the study, arid, semiarid and subhumid areas are located between 31.0° S to 35.0° S. This area is dominated by Mollisols, Inceptisols followed by Alfisols and Entisols (north in Figure 2-1B). Intensive agriculture in this region (crops and grazing) began in the 16th century and is largely dependent on irrigation (Ovalle et al., 1999). The humid and hyper humid sites are located in the southern portion of this region (36° to 40° S), a region where agriculture began to expand at the beginning of the 20th century. Here the Andean foothills are dominated by Andisols with high SOC content (about 110 g C kg⁻¹ soil). The cultivated soils are mainly rainfed and largely used for cereal production (Casanova et al., 2013; Rouanet et al., 2005; Valle et al., 2018).

2.2.4 Climate data

We classified the CIREN sites according to the aridity regime (UNESCO, 2010), by computing the ratio between MAP and ET (UNEP, 1997, 1992). Continuous monthly temperature and precipitation series from 1980 to 2015 were obtained from stations managed by the Dirección General de Aguas (DGA), Instituto de Investigaciones Agropecuarias (INIA), and Dirección General de Aeronáutica Civil (DGAC). Monthly ET was computed using Hargreaves and Samani (1985) method, after calibrating with ET data from INIA stations.

Table 2-1. Location and main characteristics of the sampling sites.

Site	Depth (cm)	Lat. (S)	Long. (W)	Soil order*	Aridity regime**	Land use	Sampling date
1	0-19	32.79°	70.83°	Mollisol	Arid	C	1979, 2014
2	0-18	32.79°	70.84°	Histosol	Arid	WP	1979, 2014
3	0-15	32.44°	71.00°	Mollisol	Arid	C	1979, 2014
4	0-18	32.31°	71.08°	Inceptisol	Arid	WP	1979, 2014
5	0-23	32.43°	71.00°	Mollisol	Arid	C	1979, 2014
6	0-16	32.77°	70.81°	Histosol	Arid	NC	1979, 2014
7	0-18	34.12°	70.73°	Mollisol	Semiarid	WP	1972, 2014
8	0-8	32.99°	71.21°	Mollisol	Semiarid	C	1980, 2014
9	0-14	33.70°	71.20°	Mollisol	Semiarid	NC	1994, 2014
10	0-18	34.51°	70.91°	Alfisol	Semiarid	C	1972, 2014
11	0-14	32.89°	71.23°	Inceptisol	Semiarid	C	1994, 2014
12	0-29	33.62°	71.29°	Mollisol	Semiarid	C	1994, 2014
13	0-16	33.84°	71.28°	Inceptisol	Semiarid	C	1994, 2017
14	0-22	33.96°	71.52°	Alfisol	Semiarid	NC	1994, 2017
15	0-14	33.98°	71.40°	Mollisol	Semiarid	WP	1994, 2017
16	0-23	33.49°	71.19°	Mollisol	Semiarid	C	1994, 2017
17	0-19	33.57°	71.15°	Mollisol	Semiarid	WP	1994, 2017
18	0-25	33.58°	71.01°	Mollisol	Semiarid	C	1994, 2017
19	0-16	33.64°	70.92°	Mollisol	Semiarid	NC	1981, 2014
20	0-16	33.63°	70.71°	Mollisol	Semiarid	NC	1981, 2014
21	0-15	33.70°	70.73°	Alfisol	Semiarid	C	1981, 2014
22	0-17	34.48°	71.04°	Mollisol	Semiarid	C	1981, 2014
23	0-19	34.13°	70.75°	Mollisol	Semiarid	C	1983, 2014
24	0-20	34.07°	70.71°	Mollisol	Semiarid	NC	1983, 2014
25	0-20	32.88°	71.10°	Mollisol	Semiarid	NC	1980, 2014
26	0-14	32.97°	71.31°	Mollisol	Semiarid	NC	1980, 2014
27	0-18	32.91°	71.23°	Mollisol	Semiarid	NC	1994, 2014
28	0-15	32.92°	71.28°	Inceptisol	Semiarid	C	1994, 2014
29	0-16	35.37°	71.56°	Alfisol	Subhumid	C	1968, 2017
30	0-23	35.45°	71.70°	Inceptisol	Subhumid	C	1968, 2017
31	0-15	35.39°	71.60°	Inceptisol	Subhumid	C	1968, 2017
32	0-20	38.04°	73.06°	Inceptisol	Humid	NC	1970, 2016
33	0-17	38.20°	72.78°	Alfisol	Humid	C	1974, 2016
34	0-12	38.62°	72.85°	Inceptisol	Humid	NC	1974, 2016
35	0-19	38.07°	73.02°	Inceptisol	Humid	C	1974, 2016
36	0-16	38.06°	72.85°	Inceptisol	Humid	C	1974, 2016
37	0-16	38.02°	73.03°	Inceptisol	Humid	C	1974, 2016
38	0-12	38.79°	73.31°	Inceptisol	Hyper humid	NC	1970, 2016
39	0-12	39.31°	72.24°	Andisol	Hyper humid	NC	1970, 2016
40	0-17	39.38°	72.62°	Andisol	Hyper humid	WP	1970, 2016
41	0-10	37.93°	72.69°	Ultisol	Hyper humid	C	1970, 2016
42	0-16	38.66°	72.11°	Andisol	Hyper humid	C	1970, 2016
43	0-16	38.66°	72.25°	Andisol	Hyper humid	C	1970, 2016
44	0-15	39.20°	72.67°	Andisol	Hyper humid	C	1970, 2016
45	0-16	38.60°	72.30°	Inceptisol	Hyper humid	C	1970, 2016
46	0-13	38.64°	72.26°	Andisol	Hyper humid	C	1970, 2016
47	0-17	39.35°	72.57°	Andisol	Hyper humid	C	1970, 2016
48	0-13	38.69°	72.88°	Inceptisol	Hyper humid	C	1970, 2016
49	0-20	38.77°	73.39°	Inceptisol	Hyper humid	NC	1970, 2016
50	0-16	38.53°	72.25°	Inceptisol	Hyper humid	NC	1970, 2016
51	0-15	39.38°	72.22°	Andisol	Hyper humid	C	1970, 2016

2.2.5 Laboratory and Analytical methods

The data for the initial sampling date corresponds to that reported by CIREN and consists of SOC, bulk density, pH, and texture. No archived soil samples exist from the initial CIREN sampling. For the 2014 and 2017 sampling, all soils were air-dried and passed through a 2-mm sieve. As with the initial CIREN survey, the more recent soil samples were analyzed for SOC using the Walkley Black method (Walkley and Black, 1934). The SOC content (g C kg^{-1}) in the topsoil horizon were converted into SOC stock (Mg ha^{-1}) as follows:

$$SOC_{stock} = BD \times SOC \times D \times 0.1 \quad (1)$$

where, BD = bulk density (g cm^{-3}), SOC = SOC content (g C kg^{-1} soil) and D = thickness of the top horizon (cm). The average annual change rate of SOC stocks (ΔCT^{-1}) ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$), was calculated using the following equation (Zhao et al., 2013):

$$\Delta CT^{-1} = \frac{SOC_{end} - SOC_{ini}}{T_{end} - T_{ini}} \quad (2)$$

where SOC_{ini} , SOC_{end} represents the SOC stocks at each sampling period (Mg C ha^{-1}). T_{ini} , T_{end} represents the initial and final sampling years, respectively.

Soil bulk density (BD) was measured by the core method (Blake and Hartge, 1986). The cores were usually easy to collect without roots and stones in the surface soils of the agricultural plots. Additionally, to ensure the SOC stock were correctly estimated, we collected a minimum of three repetitions per site using cylinders of 5 cm in height and 8.5 cm in diameter, but the BD information was incomplete for 17 of the 51 sites accounting the initial and last sampling. To solve these problems, pedotransfer functions were used to predict BD in those instances (Heuscher et al., 2005). Particle size distribution (clay, silt, sand) was measured by the hydrometer method (Gee and Bauder, 1986). Total N was measured using Kjeldahl digestion and pH was measured in a 1:2.5 soil/water suspension

(Sadsawka et al., 2006). Finally, the water-stable aggregates (WSA) were measured using a wet-sieving apparatus (Eijkelkamp, Giesbeek, Netherlands) and calculated as the proportional mass of stable aggregates in the 1–2 mm range relative to the whole soil, as presented by Kemper and Rosenau (1986).

2.2.6 Near-infrared and Mid-infrared spectroscopy

In the absence of archived soils, spectroscopic analysis was only applied to the new soil samples. Prior to scanning with the infrared spectrometer, air-dried soils were dried further at 40°C, and the dry samples were then ground to a fine powder using a ball mill. These samples were then further homogenized and mixed by hand with an agate mortar and pestle before scanning. All samples were scanned at MidIR (4000 to 400 cm^{-1}) and NIR (10,000 to 4000 cm^{-1}) spectral ranges. The NIR has a combination of bands and overtones of spectral bands observable in the MidIR region, which can provide us with unique and complementary information. For example, the NIR is closer to the visible spectrum and can potentially encompass information related to soil color, which is not detected in the MidIR. For this reason, calibrations developed from NIR data often differ in quality from those developed with MidIR absorbances, making it worthwhile to explore calibrations using both spectral ranges. The MidIR does have the advantage that band assignments for specific mineral and organic features are more straightforward than the NIR (Parikh et al. 2014). The MidIR and NIR spectra of the soils were obtained with a Digilab FTS 7000 Fourier-transform spectrometer (Varian, Inc., Palo Alto, CA, now Agilent Technologies) with a Pike AutoDIFF auto-sampler (Pike Technologies, Madison, WI). KBr and Sulfur were used as background samples for the MidIR and NIR, respectively. For the MidIR, a deuterated triglycine sulfate detector and KBr beam splitter were used. A lead selenide detector and a quartz beam splitter were used for the NIR. Each spectrum consisted of 64 co-added scans at 4 cm^{-1} resolution. Spectra were obtained in pseudoabsorbance ($\log [1/\text{Reflectance}]$). Two replicate subsamples from each soil were scanned separately and averaged to obtain the spectrum for each CIREN soil. The main spectral bands were selected according to prominent peaks and shoulders observed in the spectra. The

assignments for the organic matter and mineral components were carried out using the absorbances reported by Parikh et al. (2014).

2.2.7 Statistical analyses

Correlations between soil properties and climate variables with SOC data were tested for significance using Pearson's correlations in Sigma Plot software. In order to test for significant differences in the percent change in SOC storage between the different soil types and aridity regimes, one-way ANOVA (SigmaStat software) was conducted, with Holm-Sidak multiple comparisons used for testing differences between multiple groups. The data passed a Shapiro-Wilk test for normality and assumptions of homoscedasticity. To determine which site/soil factors best explained variations in the MidIR and NIR spectral datasets, we performed a Principal Component Analysis (PCA) of all spectra ($n = 51$) using Unscrambler 10.3 software (Camo Software, Norway). The spectra were mean centered prior to executing the PCA, which included the entire NIR or MidIR spectra. We used the PCA loadings to identify spectral bands that best explained the distribution of sample scores.

Redundancy Analysis (RDA) was performed with Canoco software v5.0 (Microcomputer Power, USA). This was used to visualize the relationships between the nine response variables (rate of change in SOC, and soil physicochemical properties), and the explanatory data set (climate, land use, and soil type). The RDA is a linear, direct gradient ordination method by which the response variables are constrained to be a linear combinations of explanatory variables (ter Braak and Smilauer, 2004). All response variables were centered and standardized before performing the RDA. Interactive forward stepwise selection and Monte Carlo permutation test (499 permutations) were performed to determine the environmental variables influencing the response variables. In the RDA diagrams, arrows that point in the same direction indicate strong positive correlations, while those pointing in opposite directions suggest negative correlations, with arrow length indicating the magnitude of the association.

Chemometric calibrations for WSA ($\% > 0.25$ mm), SOC content (g C kg^{-1} soil) and the difference in SOC contents between sampling times (ΔSOC , g C kg^{-1} soil), were conducted with Partial Least Squares (PLS) regressions using Unscrambler 10.3 (Camo Software, Norway). The spectral data was mean centered, and the validation method was a random cross validation with 20 segments, with 51 calibration samples. The performance of the PLS calibrations and validations were determined by computing the coefficient of determination (R^2). When using PLS regression to develop a predictive model, it is important to use the proper number of factors or loading vectors. The first few factors tend to be more related to the soil attributes to be predicted, while the higher number factors tend to increase the model spectral noise. When few factors are included, the accuracy of the PLS model declines because not all the spectral differences related to the constituent are included. Predictive calibrations where a large portion of total variance is explained using a minimal number of factors are desirable. In this study, the optimal number of PLS factors used was determined from the relationship of the explained variance and the number of factors, and this was done automatically by the Unscrambler 10.3 software (Camo Software, Norway). For the MidIR there were 1868 absorbance points used as variables, and five factors were suggested by the model. For the NIR there were 3113 absorbance points used as variables, and five factors were suggested by model.

2.3 Results

2.3.1 Descriptive soil and climate parameters

The average SOC content for the different soil types ranged from 15.1 to 102.4 g C kg^{-1} soil (Table 2-2). The SOC content, aggregate stability and clay content were high in Andisols with an average 102.4 g C kg^{-1} soil, 86.5% > 0.25 mm, and 14 $\text{g } 100 \text{ g}^{-1}$ soil, respectively. The sites with Mollisols showed the lowest SOC concentrations with an average of 15.1 g C kg^{-1} soil. In general, soil pH ranged from 5.4 to 7.5, with the most acid conditions in the Andisols, and the more alkaline conditions in the Mollisols and Histosols. The C:N ratio varied between 15 and 21, with the lowest values occurring in the Mollisols and Inceptisols. Bulk density had an inverse relationship with SOC, and the Andisols

reached the lowest BD values. The SOC concentrations increased from arid to hyper humid conditions (Table 2-3). In addition, as conditions became more humid, the aggregate stability increased, reaching 88.9% > 0.25 mm in the hyper humid regime. Conversely, bulk density and soil pH were lower in the hyper humid sites, with values of 0.8 g cm⁻³ and 5.8 respectively.

The correlation analysis conducted between climate and soil parameters (Table 2-4) shows 45 attribute pairs with 12 positives and 15 negatives significant ($P < 0.05$) correlated variables. SOC was positive and strongly correlated with MAP ($r = 0.88$), WSA ($r = 0.50$) and silt ($r = 0.35$). Negative correlations were observed between SOC and pH ($r = -0.52$), MAT ($r = -0.68$) and BD ($r = -0.88$). Clay was correlated negatively with SOC content ($r = -0.30$). However, a significant positive relationship ($P < 0.05$) was observed when SOC and clay contents were grouped into arid-semiarid conditions ($r = 0.68$). Soil C:N ratio did not vary significantly with SOC content, but showed a significant correlations with WSA ($r = -0.53$) and MAP ($r = -0.31$).

The historical meteorological data trends indicated that the mean annual temperatures have slightly increased in subhumid and humid zones by 0.011 and 0.019 °C yr⁻¹ respectively, and slightly decreased in hyper humid zones by -0.006 °C yr⁻¹ (Table 2-5). Annual precipitation has generally decreased with time across all sites, but this trend was most pronounced on semiarid, subhumid and hyper humid sites, with rates of change of -4.86, -5.48 and -8.23 mm yr⁻¹, respectively.

Table 2-2. Soil properties of different soils orders samples across a climatic gradient in Central Chile.

Order (*)	n	Depth (cm)	WSA (%>0.25 mm)	BD (g cm ⁻³)	SOC (g C kg ⁻¹ soil)	Sand (g 100 g ⁻¹ soil)	Clay (g 100 g ⁻¹ soil)	C:N	pH
Andisols	8	15 ± 2	86.5 ± 3.0	0.6 ± 0.1	102.4 ± 22.5	44 ± 12	14 ± 5	18 ± 3	5.8 ± 3.2
Alfisols	5	18 ± 2	75.6 ± 12.2	1.4 ± 0.3	17.3 ± 8.9	38 ± 11	28 ± 5	20 ± 4	6.5 ± 0.7
Histosol	2	17	69.5	1.4	20.4	31	30	20	7.5
Inceptisols	16	16 ± 3	81.0 ± 14.8	1.2 ± 0.1	25.3 ± 11.0	45 ± 16	22 ± 10	16 ± 4	6.2 ± 0.9
Mollisols	19	19 ± 5	63.0 ± 10.6	1.5 ± 0.3	15.1 ± 6.2	42 ± 18	26 ± 9	21 ± 7	7.5 ± 0.5
Ultisol	1	10	92	1.1	34	21	45	17	5.4

Values are mean ± standard deviation of the n indicated in the second column. WAS: Water stable aggregates; BD: Bulk density; SOC: Soil organic carbon.

*: The Soil Order Taxonomy was reported by CIREN according to Soil Survey Staff (2006)

Table 2-3. Mean values for soil properties of different soils across a climatic gradient in Central Chile.

Aridity regime [*]	n	Depth (cm)	WSA (%>0.25 mm)	BD (g cm ⁻³)	SOC (g C kg ⁻¹ soil)	Sand (g 100 g ⁻¹ soil)	Clay (g 100 g ⁻¹ soil)	C:N	pH
Arid	6	18 ± 3	64.0 ± 8.9	1.5 ± 0.2	14.9 ± 4.6	42 ± 16	24 ± 8	18 ± 3	7.7 ± 0.2
Semiarid	22	18 ± 4	64.1 ± 11.8	1.4 ± 0.3	15.7 ± 7.0	41 ± 19	28 ± 10	21 ± 7	7.3 ± 0.6
Subhumid	3	18 ± 4	73.0 ± 5.1	1.3 ± 0.1	16.3 ± 3.3	32 ± 10	27 ± 1	18 ± 0	6.5 ± 0.1
Humid	6	17 ± 3	88.5 ± 8.8	1.1 ± 0.1	26.5 ± 6.1	49 ± 14	19 ± 6	16 ± 1	5.5 ± 0.3
Hyper humid	14	15 ± 3	88.9 ± 4.2	0.8 ± 0.2	73.6 ± 37.9	43 ± 12	19 ± 10	16 ± 5	5.8 ± 0.3

Values are mean ± standard deviation of the n indicated in the second column. WAS: Water stable aggregates; BD: Bulk density; SOC: Soil organic carbon.

*: According to UNESCO, 2010.

Table 2-4. Pearson's correlation coefficient (r) among soil and climates attributes used in the study for soil sampled between 2014 and 2016 in central Chile.

	MAT	WSA	SOC	Sand	Silt	Clay	C:N	pH	BD
MAP	-0.90	0.67	0.88	n.s	n.s	-0.44	n.s	-0.76	-0.93
MAT		-0.75	-0.68	n.s	n.s	0.40	0.31	0.83	0.81
WSA			0.50	n.s	n.s	n.s	-0.53	-0.79	-0.60
SOC				n.s	0.36	-0.35	n.s	-0.52	-0.88
Sand					-0.80	-0.78	n.s	n.s	n.s
Silt						n.s	n.s	n.s	-0.37
Clay							n.s	0.35	0.43
C:N								0.34	n.s
pH									0.63

MAP: Mean annual precipitation (mm); MAT: Mean annual temperature (°C); WAS: Water stable aggregates (%>0.25 mm); SOC: Soil organic carbon (g C kg⁻¹ soil); BD: Bulk density (g cm⁻³).

BD values (17 of 51 soils) computed with pedotransfer functions were excluded from the analysis.

n.s: Non-significant relationship between the two variables, $P > 0.05$.

Table 2-5. Linear regression for mean annual temperature and precipitation across different aridity zones from 1980 to 2015.

Aridity regime	n^(a)	MAT^(b) (°C)	Rate of change (°C yr⁻¹)	MAP^(b) (mm)	Rate of change (mm yr⁻¹)
Arid	6	15.2 ± 0.5	0.004	277 ± 146	-3.94
Semiarid	4	14.6 ± 0.5	0.005	436 ± 191	-4.86
Subhumid	3	14.9 ± 0.4	0.011	626 ± 227	-5.48
Humid	4	12.0 ± 0.2	0.019	1122 ± 226	-3.84
Hyper humid	3	12.2 ± 0.4	-0.006	1742 ± 368	-3.23

^a Number of meteorological stations used for the analysis.

^b Standard deviation in 35 years of data.

MAT: Mean annual temperature.

MAP: Mean annual precipitation.

2.3.2 Carbon storage and climate, soil types and agricultural effects

Table 2-6 shows the SOC storage changes in the surface horizon between the baseline and most recent samplings. The Mollisols, which decreased by 29.9% with an average SOC loss rate of $0.99 \text{ Mg C yr}^{-1}$ were significantly higher than the Inceptisols and Andisols ($P = 0.002$). The average change in SOC storage grouped by aridity regime ranged from $20.4 \text{ Mg C ha}^{-1}$ for semiarid to 2.8 Mg C ha^{-1} for hyper humid. Semiarid soils demonstrated a significant decrease ($P = 0.008$) in SOC storage compared to hyper humid areas. Semiarid soils lost 24.7% of their total C stocks with an average change of $-0.80 \text{ Mg C yr}^{-1}$ during the time period considered, while the subhumid sites lost 26.1% with an average rate of $-0.31 \text{ Mg C yr}^{-1}$. Soils in arid climates decreased by 8.8% with rate of $-0.26 \text{ Mg C yr}^{-1}$. Relatively small gains were observed in humid and hyper humid conditions, with increases of 5.4% and 10.5% and average rates of SOC accumulation at 0.04 and $0.06 \text{ Mg C yr}^{-1}$, respectively.

The RDA analysis was carried out using the site characteristics, which included aridity regime, land use, and soil properties (Figure 2-3). The explanatory variables (climate, land use and soil types) accounted for 54.3% of the total variation. A total of 46.3% of the variation was explained by the first two constrained axes of the RDA, while the first axis explained 38.4% and the second 8.0%. The RDA shows clear differences related to SOC storage and soil physical properties between the dry and humid zones. These differences were explained ($P=0.002$) by the soil types and climate conditions. Andisols and Mollisols accounted for 47.5% and 39.2%, respectively. Additionally, hyper humid and semiarid climates accounted for 48.1% and 39.0% of total variation, respectively. Non-significant land use effects were found to explaining the total variation.

Table 2-6. Change in soil organic carbon storage and average annual change rate in different soil orders and aridity regime over the last decades.

Soil order				Aridity regime			
	Change in SOC storage		ΔCT^{-1}		Change in SOC storage		ΔCT^{-1}
	(Mg C ha ⁻¹)	(%)	(Mg C ha ⁻¹ yr ⁻¹)		(Mg C ha ⁻¹)	(%)	(Mg C ha ⁻¹ yr ⁻¹)
Andisols	4.8 (17.8)	10.3 ^a	0.10	Arid	-8.9 (36.1)	-8.8 ^{ab}	-0.26
Alfisols	-4.1 (7.8)	-8.23 ^a	-0.10	Semiarid	-20.4 (25.9)	-24.7 ^b	-0.80
Histosols	9.2	29.4 [*]	0.26	Subhumid	-15.1 (9.6)	-26.1 ^{ab}	-0.31
Inceptisols	-1.7 (15.1)	-5.7 ^a	-0.05	Humid	1.8 (8.9)	5.4 ^{ab}	0.04
Mollisols	-26.5 (29.8)	-29.9 ^b	-0.99	Hyper Humid	2.8 (17.8)	10.5 ^a	0.06
Ultisols	-1.1	-3.0 [*]	-0.02				

SOC: Soil organic carbon; ΔCT^{-1} : Average annual change rate. SOC storage are the mean values calculated from the data of (1972–1994) to (2014–2016). Values in parentheses are the standard deviation. Means not sharing a letter suffix are significantly different according to one-way ANOVA ($P < 0.05$).

*: Standard deviations and ANOVA results are not provided for the Ultisols and Histosols due to insufficient replication

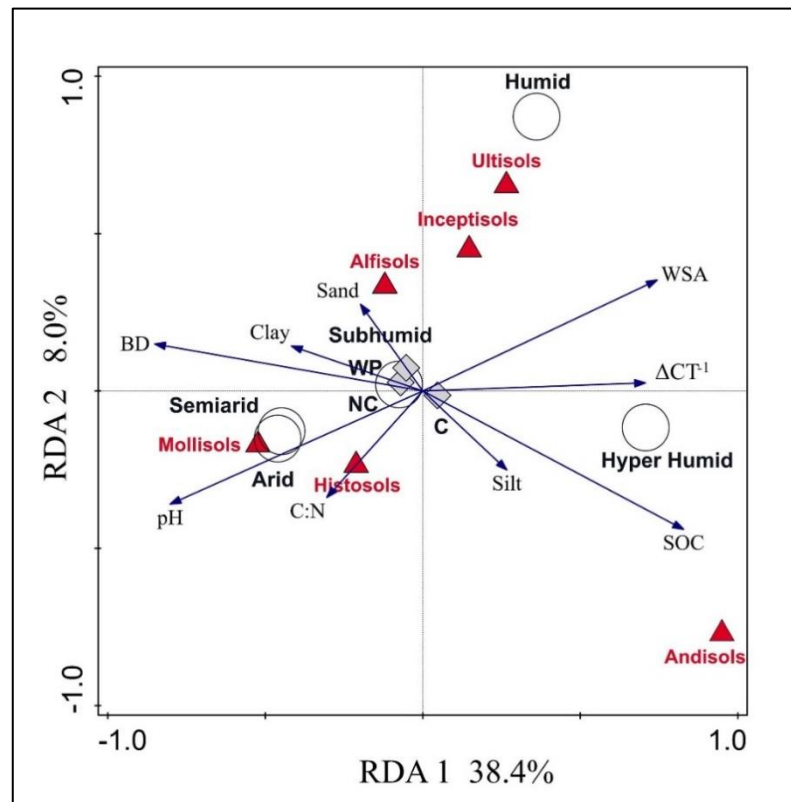


Figure 2-3. Redundancy analysis (RDA) showing the relationships between SOC content and environmental factors including aridity regime, soil orders and average annual change in SOC (ΔCT^{-1}). Soil order is shown in red triangles, Land uses in gray diamonds and aridity regimes in white circles. The percent of the variation in the data explained by each axis is shown on the axis label.

Gains in SOC were represented by positive values of annual change rate (ΔCT^{-1}) of SOC stocks, which was positively associated with WSA humid conditions (Figure 2-3). Mollisols and Histosols had greater net SOC losses under semiarid/ arid areas, which were represented by an inverse relationship with ΔCT^{-1} . All land use changes types did not seem to affect distinctly the decrease in SOC under semiarid conditions. The negative relationships of ΔCT^{-1} with pH is explained by the acidity of the Andisols, which prevail on the more humid climates that also tended to accumulate SOC. Soil textural properties such as clay and sand content, and C:N ratio were negatively associated with ΔCT^{-1} .

2.3.3 Spectroscopic analysis

The PCA of the MidIR absorbance data shows that hyper humid samples have different spectral properties compared to soils from the rest of the climate classifications (Figure 2-4A). Soils from arid and semiarid regions tend to cluster separately from the humid, subhumid, and hyper humid samples. The Andisols, which generally occur in hyper humid environments, are different from the rest of the soil types. The sample distributions in the PCA analyses are indicative of chemical and mineralogical differences between the soils. PCA loadings are useful because they indicate which spectral bands have more influence over the separation of the samples along each component. Loadings for component 3 indicate that samples from the more humid climates (as well as the high SOC Andisols) have pronounced absorbance at the lattice clay band at 3690 cm^{-1} , the aliphatic CH bands between $2930\text{-}2850\text{ cm}^{-1}$, and at the 1210 cm^{-1} band assigned to C-O stretch and/or OH deformation (Figure 2-4B). Furthermore, component 1 loadings indicate that the Andisols have high absorbance at the broad OH/NH band around 3400 cm^{-1} , the aliphatic CH bands between $2930\text{-}2850\text{ cm}^{-1}$, the amide I band at 1660 cm^{-1} , as well as the polysaccharide C-O-C, C-OH stretch bands at 1140 and 1040 cm^{-1} . Conversely, loadings for component 3 indicate that the semiarid soils, which had the highest SOC losses between sampling times, have relatively high absorbance at $2000\text{-}1770$ (silicates), 1480 (near the band for aliphatic C-H bend), and 1310 cm^{-1} (near the band for ester C-O bend).

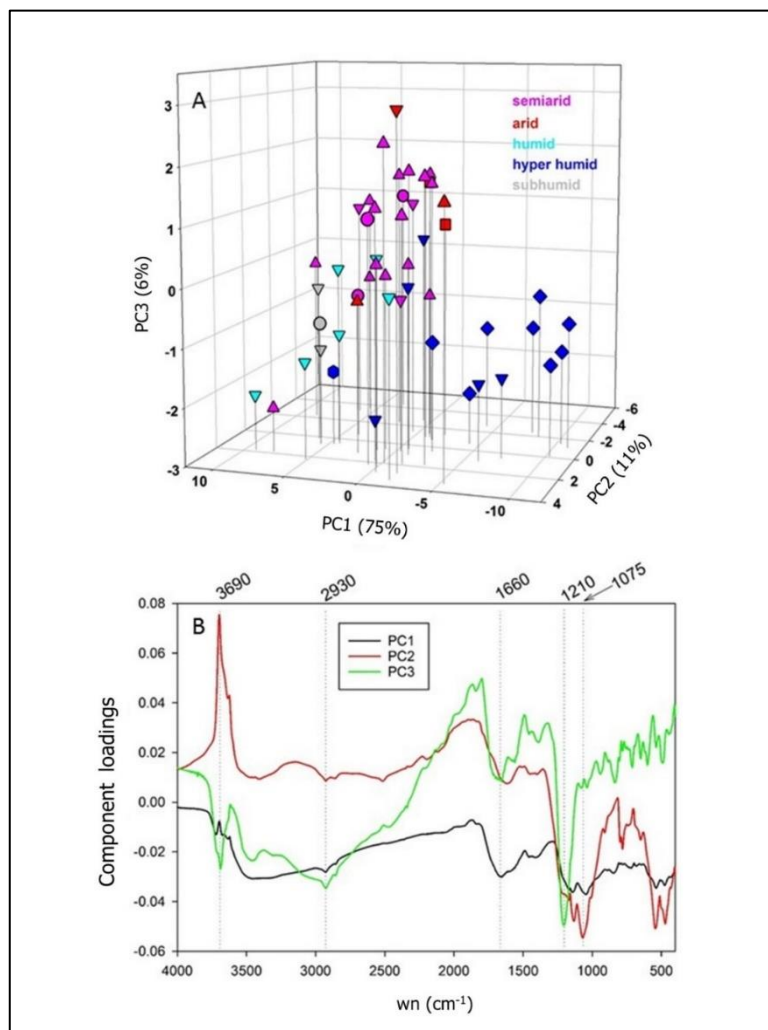


Figure 2-4. Principal components analysis of the mid infrared spectral data of Chilean soils. Color coded by climate (A). Component 1 explains 75% of the variation, component 2 explains 11% of the variation, and component 3 explains 6% of the variation in the absorbance data. Mollisols are shown in triangles, Alfisols in circles, Inceptisols in downward triangles, Andisols in diamonds, Histosols in squares, and Ultisols in hexagons. Panel (B) shows the component loadings for the principal components analysis shown in (A).

A PCA analysis of the MidIR absorbance data from the semiarid soils exclusively shows a separation according to the net change in SOC, indicating that soils that had been susceptible to high C losses also have specific spectral features (Figure 2-5A). Component 3 loadings indicate that the arid and semiarid soils that lost the most C were characterized by absorbance at 1200 cm^{-1} (Figure 2-5B), attributed to C-O stretch, and/or OH deformation. At the same time, the soils, which had smaller declines in SOC had high absorbance between $1770\text{-}2000\text{ cm}^{-1}$, consistent with higher sand Si-O content, and also high absorbance at the lattice clay band at 3700 cm^{-1} .

The NIR spectral region contains absorbance related to overtones and combination bands of the MidIR region. Besides that, the NIR also encompasses absorbances close to the visible region. Because of this the NIR is sometimes a useful complement to MidIR data, shedding light on chemical differences between the soil samples beyond those detected with MidIR. The multivariate analysis of the NIR data had similar separations according to climate regime to those obtained with the MidIR data (Figure 2-6). The Andisols tended to group together, and completely separate from the Alfisols. However, the NIR absorbance data was not as useful as the MidIR to tell apart the Mollisols samples according to SOC change values (data not shown).

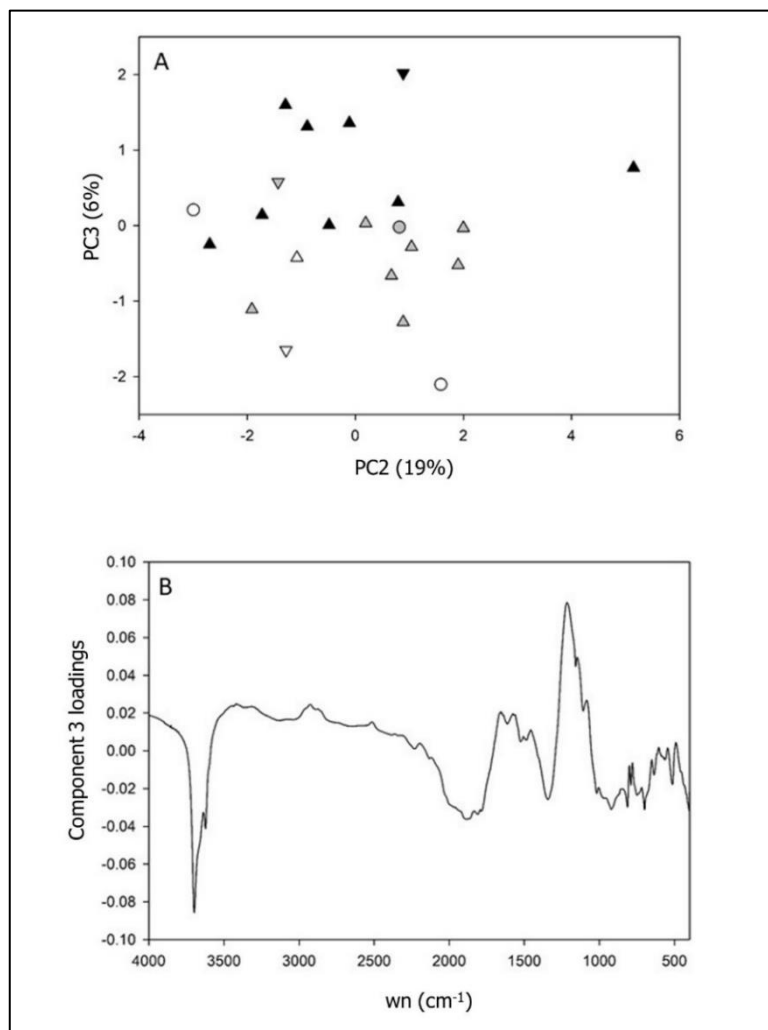


Figure 2-5. (A) Principal components analysis of the Mid-infrared spectral data from Chilean semiarid soils. Symbol colors are according to SOC change (g C kg⁻¹ soil): Black are < -8 , grey are between -8 and 0, and white are > 0 . Mollisols are shown in triangles, Alfisols in circles, Inceptisols in downward triangles. Component 2 explains 19 % of the variation, and component 3 explains 6% of the variation in the absorbance data. (B) Component 3 loadings for the principal components analysis shown in Figure (A).

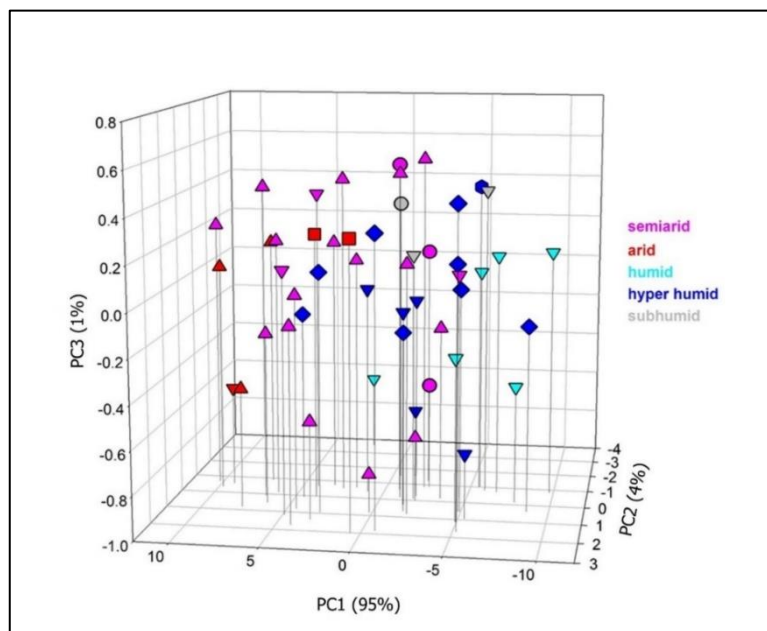


Figure 2-6. Principal component analysis of the Near-infrared spectral data of Chilean soils color coded by climate. Component 1 explains 95% of the variation, component 2 explains 4% of the variation, and component 3 explains 1% of the variation in the absorbance data. Mollisols are shown in triangles, Alfisols in circles, Inceptisols in downward triangles, Andisols in diamonds, Histosols in squares, and Ultisols in hexagons.

2.3.4 Chemometric calibrations for WSA, SOC content and Δ SOC

Chemometric calibrations can often be used to predict soil properties using absorbance data. In this study, we set out to determine if NIR and/or MidIR data could be used as an indicator of past change in soil C (as well as other soil properties). Partial least squares regressions were performed in order to develop calibrations for WSA, SOC content and Δ SOC. The calibrations were done with the full NIR or MidIR spectra, with mean centering as the only pre-treatment. The PLS with the MidIR data showed that calibration R^2 were moderately good for the prediction of SOC content, but failed to calibrate for Δ SOC and WSA. The MidIR calibration/validation R^2 were 0.53/0.47, 0.89/0.60, and 0.55/0.39 for WSA, SOC content, and Δ SOC, respectively. Likewise, the NIR calibration/validation R^2 were 0.40/0.26, 0.73/0.58, and 0.46/0.28 for the WSA, SOC content, and Δ SOC, respectively.

2.4 Discussion

2.4.1 Climate controls on soil C storage

The higher SOC stocks observed under more humid and cooler environments is comparable to previous research showing an increase in SOC with higher latitude in Chilean soils (Bonilla and Johnson, 2012; Padarian et al., 2017). Overall, our field data demonstrates that SOC content has changed over the last decades. Our analyses suggest that climate had a significant influence on soil chemical and physical properties. Furthermore, there is a boundary between the subhumid and humid climates in which the trend for ΔCT^{-1} reverses, shifting from a net loss of SOC in the drier climates to an increase in SOC in humid and hyper humid areas. High water availability are able to sustain higher primary productivity, and consequently have greater plant residue inputs contributing to a net increase in soil organic matter (Chenu et al., 2019). Hence, the results indicate that climate and initial soil organic carbon content appear to strongly influence SOC stocks, which could accommodate periods of declining precipitation without the moisture becoming a limiting a factor to plant growth and associated C sequestration.

In arid systems, SOC levels may be promoted by the long residence time of SOM as a result of lower soil water availability and lower levels of microbial activity (Austin et al., 2004; Barnard et al., 2013; Stark and Firestone, 1995) and thus may have significant potential for atmospheric C sequestration as (FAO, 2004; Lal, 2009; Stockmann et al., 2013). In general, organic matter decomposition and nutrients release are accelerated in warmer climates, as long as water availability is not a limiting factor (Jobbágy and Jackson, 2000). Here, we found that arid areas have been less impacted by SOC losses compared to semiarid areas. Although these findings may be supported by the fact that agroecosystems in arid region have higher C inputs than natural ecosystems, a meta-analysis performed by Guo and Gifford (2002) evidenced that the effects of land use conversion on soil C stock losses can be strongly influenced by precipitation. For example, after conversion from pasture to crop, areas with 400-500 mm of precipitation lost about 75% of the original SOC, and those with 300-400 mm and more than 500 mm, lost 54% and 50%, respectively.

SOC decomposition rates clearly vary with temperature and precipitation but can also be determined by land use and management practices (Bruce et al., 1999; Paustian et al., 2016; Six et al., 1998). Although a non-significant fraction of the variation was accounted for by land use, we cannot simply ignore the effect of land use because it seems to affect the SOC stock differently across climates. We observed that more humid climates exhibited less SOC losses in surface soils compared with drier conditions. Although during the last 20 years the adoption of reduced/non-tillage practices have become more common in southern Chile, conventional tillage is a common practice, which includes the burning of stubble and disk-harrowing operations (Alvear et al., 2005). There is evidence that the effects of tillage on SOC can be relatively minor in moist, cold-temperate soils (Hermle et al., 2008). Furthermore, recent studies have demonstrated that the response of SOC to non-tillage is dependent on climate conditions, in particular precipitation, with a pronounced effect in drier climates (Cosentino et al., 2006; Dimassi et al., 2014).

The abandonment of agricultural lands and subsequent recolonization by natural vegetation is associated with increases in SOC (Novara et al., 2013, 2014), but our results showed different a trend. Non-cultivated soils do not necessarily increase SOC content compared to cultivated soils in semiarid areas. This is explained by the increasing trend to land abandonment in these areas due to water scarcity, resulting in lower carbon inputs with little or no vegetation. Additionally, soils with woody perennial species were also affected by SOC losses under drier climates. However, it has been shown a soil carbon stock increment in forest soils in deeper soil layers (Lal, 2005), condition that can support the reverse trends observed in arid/semiarid sites. Similarly, a recent study reported that the transition from agriculture to agroforestry land increased by 40% the SOC stock between 0–30 cm depth (De Stefano and Jacobson, 2017).

2.4.2 Soil type controls on soil C storage

The results in this study indicate that soil type was the main factor explaining the soil physicochemical patterns across different areas. The results exhibited similar patterns to previous studies. For instance, Doetterl et al. (2015) found that geochemistry was the most

important controlling factor for soil carbon storage in Chilean soils from grasslands and shrublands. Additionally, Reyes Rojas et al. (2018) indicated the soil type at suborder level was the best predictor for SOC stock. Arable Mollisols in this study were located in semiarid conditions and were the most prone soil type to SOC losses. In contrast, Andisols, located in more humid areas, had a net increase in SOC during the study period. Mollisols have been considered naturally high in soil fertile and play a significant role as a regional hotspot of carbon (Lefèvre et al., 2017). However, they are very sensitive to soil degradation and SOC losses (Liu et al., 2012). Similar results have been reported in China, where a loss of 22.3% of SOC has occurred in Mollisols over the past three decades, mainly attributed to high erosion and low input of organic matter (Xu et al., 2017; Yan et al., 2011). Our results confirm that the response of agroecosystems to the temporal SOC variations are site-dependent and site-specific.

2.4.3 Soil physicochemical protection

The RDA analysis indicates that soil aggregate stability tends to be higher in soils that retain SOC, most of them Andisols. These soils, derived from volcanic materials, have been shown to have some of the largest and persistent SOC pools (Huygens et al., 2005; Kramer and Chadwick, 2016). However, Asano and Wagai (2014) demonstrated that the aggregate hierarchy in Andisols is achieved on a micro scale (Oades and Waters, 1991), which limits the comparison of Andisols with other soils in this study. Amorphous inorganic materials, smaller or less-crystalline phyllosilicate clays, and pyrophosphate-soluble metals contribute significantly to Andisols aggregation and organic matter stabilization (Asano and Wagai, 2014; Matus et al., 2014).

Some studies have reported that clay sized minerals tend to be the most important mineral constituents of organo-mineral associations because of their high specific surface area and hydroxylated reactive surfaces (Doetterl et al., 2015). In this study, the clay contents were negatively correlated to SOC content. However, a significant positive relationship was found in arid and semiarid soils, which indicate that physicochemical stabilization mechanisms caused by carbon-clay interactions are predominant in drier areas. This

discrepancy across the areas might be related to multiple mechanisms of SOC protection. For instance, the distinctive soil mineralogy in Andisols associated with Al-Fe-Mn oxyhydroxides, as a factor controlling SOC accumulation, is considered to be more relevant than clay content influence (Doetterl et al., 2015; Matus et al., 2006; Panichini et al., 2017; Takahashi and Dahlgren, 2016).

2.4.4 Use of MidIR to characterize soil the chemical composition

The MidIR spectral properties of soils measured in this study provide insight on their underlying chemical and mineral differences and suggest that samples from different climate regimes and soil types had distinguishing spectral features. Absorbance at 3400 cm^{-1} and 2930-2850 cm^{-1} have been associated with high SOM and C rich fractions in samples from a variety of environments (Calderón et al., 2011a; Matamala et al., 2017). Furthermore, the aliphatic CH absorbance bands between 2930-2850 cm^{-1} have been correlated to permanganate-oxidizable C (POXC), which is a widely used metric of labile soil C (Calderón et al., 2017). Absorbance at 1660 cm^{-1} has been correlated with microbial biomass N (Calderón et al., 2011b), and 1140 and 1040 cm^{-1} have been assigned to labile polysaccharides by others (Parikh et al., 2014). All of these bands had higher absorbance in Andisols, as well as the soils under more humid climates with higher SOC content. This may indicate that the stability of the SOC stocks in Andisols is not due to the inherent recalcitrance of its soil C chemistry, but to other factors such as temperature and mineral protection.

A large body of previous work has demonstrated that it is possible to obtain predictive calibrations for a variety of soil analytes using MidIR and NIR data from diverse soil samples (Parikh et al., 2014). Although our multivariate calibration work shows that spectroscopy and chemometrics hold promise to predict SOC content, predicting past C gains or losses remains elusive. The assumption for this analysis was that there are spectroscopic properties in soils that are related to whether a soil tends to lose or gain SOC over time. Both the NIR and MidIR failed to achieve good calibration quality for aggregation and ΔSOC over time. We suggest a larger set of samples might have been

needed to resolve SOC dynamics and soil composition and that spectral characteristics cannot be extrapolated to different soil types, which was consistent with previous studies (Bellon-Maurel and McBratney, 2011; Malley et al., 2004; Stevens et al., 2013).

The spectroscopic data sheds light on the soil characteristics that typify resilient soils. However, it should be noted that these analyses were carried out after the fact, so initial soil characteristics that might have governed SOC protection could have changed since the baseline sampling. Bands associated with soils that lost the most SOC within the arid and semiarid regimes had high absorbance at 1200 cm^{-1} . This suggests that moieties that absorb at 1200 cm^{-1} are labile. Oxygen containing functional groups, such as polysaccharide or ether C-O, are preferentially oxidized by soil microbes. As anticipated, clay particles were not associated with SOC retention when all soil types and climates were included in the analysis. However, our spectroscopic results evidence that in semiarid Mollisols, clay protection does have a role in the prevention of SOC losses. Within the arid and semiarid soils (largely Mollisols) lattice clay absorbance at 3700 cm^{-1} and quartz(-like) Si-O absorbance at $1770\text{--}2000\text{ cm}^{-1}$, was associated with diminished SOC losses. Several studies have shown that layered silicates (also called phyllosilicates) play an important role stabilizing and protecting SOM (Barré et al., 2014; Feng et al., 2013; Six et al., 2002) by adsorption onto phyllosilicate surfaces. Additionally, it has also been reported that charged phyllosilicate clays, and the relatively high SOM content can determine aggregate hierarchy in Mollisols (Asano and Wagai, 2014). In addition, considering the importance of protection and stabilization of SOC, further work is needed to elucidate adsorption mechanisms or physical protection through clay-sized aggregates.

2.5 Conclusions

This research examines the potential effect of climate, soil types and land use on SOC storage under agricultural land. Our findings indicate that SOC content have change over the last three or four decades in agricultural lands, which suggests that these trends must be considered in future studies. The RDA analysis revealed that soil types and climate elicited the most important responses in SOC storage over time. Although, land use does

not seem contribute significantly to explain the variance of the soil physicochemical parameter and the rate of SOC changes, the trends show that Mollisols under semiarid conditions in all land use change in our case, are more susceptible to C losses. Additionally, the results indicate that the mechanisms of SOC protection vary with the climate. In semiarid region, our observations suggest that phyllosilicate might play an important role controlling the preservation of SOC. Nevertheless, further studies are needed, given that these soils were not subject to an experimental manipulation, and climate, soil types, and land uses factors are confounded. As Mollisols in semiarid conditions are among the most fertile and productive soils, a better understanding regarding the influence of climate, topography, and management practices would be helpful for future soil restoration efforts.

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3 CLIMATE AND LABILE ORGANIC CARBON FRACTIONS DRIVE SPECIFIC PATTERNS OF MICROBIAL COMMUNITY UNDER DIFFERENT SOIL TYPES AND LAND USE

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3.1 Introduction

Microbes play a key role in soil organic matter (SOM) decomposition and processes associated with SOM stabilization. Therefore, changes to the microbial communities in response to ecosystem alterations might represent large net changes in the labile soil organic carbon (SOC) stocks. For example, microbial responses to aridity levels indicate that soil carbon in humid regions may be highly susceptible to future drought scenarios (Ren et al., 2018b). In the last several years, there has been a surge in the use of labile SOC fractions in soils as early indicators of SOC changes and sensitive indicators of soil productivity in response to alterations in soil management practices or environmental conditions (Belay-Tedla et al., 2009; Biederbeck et al., 1994; Gregorich et al., 1994; Song et al., 2012). However, there are still important gaps in our understanding about how such microbial communities are associated with the labile soil fractions. This is an important issue to address since studies have not accounted for the intrinsic role of soil microorganisms as important factors controlling soil organic matter lability, accessibility, and turnover rates.

SOM is not chemically uniform, and the availability of the labile components and long-term accessibility of the stored carbon to microbes affects carbon turnover rates and carbon sequestration in soils (Janzen, 2006). Consequently, a wide range of labile SOC fractions have been suggested based on chemical or physical separation methods (Cambardella and Elliott, 1992; Haynes, 2005; Sparling, 1992; Strosser, 2010). The permanganate oxidizable carbon (POXC) fraction determined by the chemical SOM fractionation method, is one of the most reliable indicators for evaluating the short and long-term soil management impact

and soil health assessment (Culman et al., 2012; Hurisso et al., 2016; Morrow et al., 2016; Skjemstad et al., 2006). The light fraction organic matter (LFOM) separated from the soil by density fractionation techniques, is composed of more recently deposited organic matter particles (plant residues), and responds more rapidly to the effects of management practices (Gregorich and Ellert, 1993; Janzen et al., 1992; Spycher et al., 1983).

In the SOM decomposition process, microorganisms involved at each step, have a life strategy adapted to their preferred substrates (Razanamalala et al., 2018). For example, copiotrophs such as Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria are expected to have higher respiration rates compared to oligotrophs including Actinobacteria, Acidobacteria, and Deltaproteobacteria (Liu et al., 2018). Despite this, contradicting results have been reported because of variations in bacterial community structures are not always entirely controlled by changes in the SOC content when they are evaluated from local to regional scales (Delgado-Baquerizo et al., 2018; Maestre et al., 2015; Tian et al., 2017). Accordingly, we hypothesized that shifts in the microbial (bacterial-archaeal) diversity and composition are primarily explained by labile SOC fractions rather than SOC content under different climatic regimes and land uses. In that sense, microbial communities associated with the labile fractions might be more suitable as ecological indicators to evaluate SOC changes in response to global warming and agricultural intensification and this will improve the ability to predict soil degradation as a consequence of the SOC decline.

To test our hypothesis, we studied microbial communities across soils with contrasting SOC contents and labile SOC fractions under different climates and land use conditions. Sequencing of the 16S rRNA genes together with physicochemical analyses, LFOM, and POXC measurements were used to discern the dynamics of the soil bacterial and archaeal communities. Therefore, the objectives of this study were: (1) to assess the relationships between soil microbial community diversity and the physicochemical factors, (2) to evaluate the relative importance of the labile SOC fraction as a driver of the soil microbial communities in terms of their structure and composition, and (3) to identify the specific taxa that may be linked to the increased labile SOC fraction.

3.2 Materials and methods

3.2.1 Study area description

The study sites were located in central Chile from the Metropolitana to Araucanía regions (33°00' S to 40°00' S), at elevations below 500 m on nearly flat slopes (less than 6%) (Fig. 3-1). A total of 28 sites were sampled over two years during the summer seasons of 2016 and 2017 (January to February). Cropland in semiarid and sub-humid regions enclose the most productive agriculture areas. In these areas the farmers usually irrigate and practice intensive agriculture that results in erosion and depletion of the soil fertility, along with the degradation of the natural vegetation (Muñoz et al., 2007; Ovalle et al., 1999). In addition, these soils suffer significant periods of water shortages, and are inherently low in SOC (Álvaro-Fuentes et al., 2008). Here, the mean annual temperature (MAT) reaches 15° C, the annual evapotranspiration (ET) about 1200 mm, and the mean annual precipitation (MAP) ranges from 400 mm to 635 mm. In the southern area, the climate is typically rainy cool temperate with an ET of 650 mm, a MAT of 12 °C and a MAP > 1200 mm. Here, the Andean foothills are dominated by volcanic ash soils (Andisols) characterized by high SOC contents and the presence of both secondary and evergreen forests dominated by *Nothofagus* species (Panichini et al., 2017). Additionally, the soils are commonly used for small-grain crops, permanent pasture, or forest plantations (Stolpe, 2005).

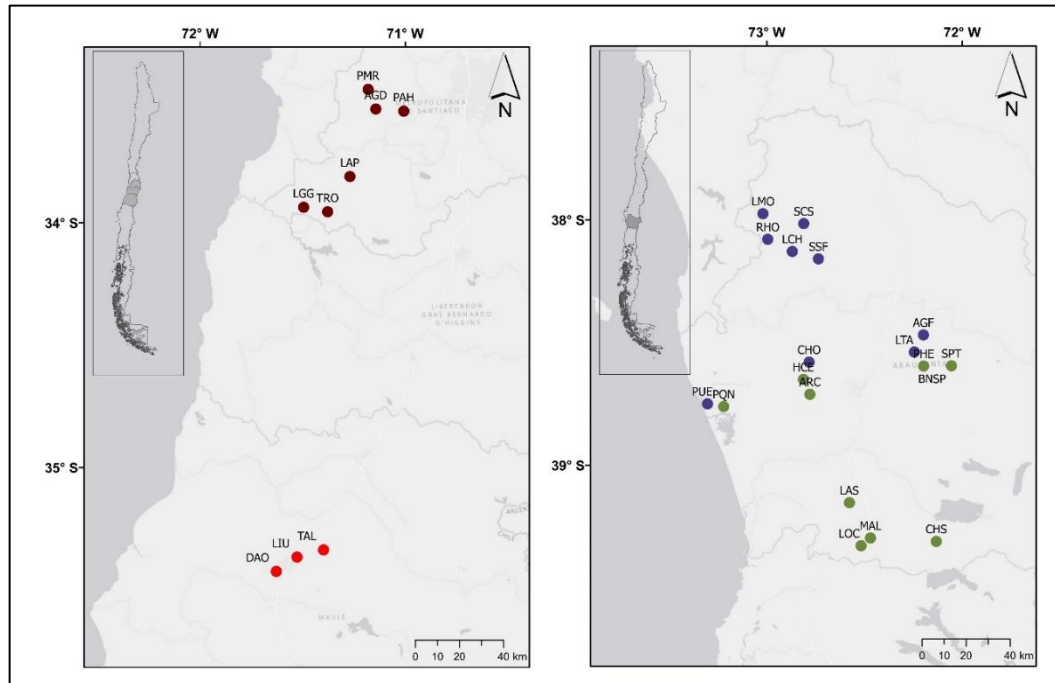


Figure 3-1. Soil sampling areas in central-south Chile. Semiarid sites are shown in brown circles, subhumid in red circles, humid in blue circles and hyper humid in green circles.

3.2.2 Soil sampling and experimental design

The sites were selected to represent a large range of different soil types and C contents (Table 3-1). The sites were identified according to data sources reported by the official repository institution of the Chilean Natural Resources Information Center (CIREN). The sampling sites were classified in four categories according the land use: 1) cultivated (C), sites planted with annual crop systems and/or vegetables, 2) non-cultivated (NC) agricultural sites without recent activity or abandoned with natural vegetation due to secondary vegetation succession, 3) native forest (NF, mainly with *Nothofagus obliqua* (Mirb.) Blume, a type of native oak), and 4) woody perennial (WP, fruit orchard and forest tree).

Each site was sampled up to a 30 cm average depth using a 20 cm diameter auger. At each site, two points were randomly assigned at a distance of 100 m, and just prior to sampling, plant residues and the O horizon were removed. For the microbiological analyses, two soil

samples were scraped from the topsoil (0–15 cm) on the inner walls of the core with 15 ml polypropylene tubes that were transported in an ice-box and stored at -80 °C until the DNA extractions.

3.2.3 Climate and soil physicochemical properties

The climate data were obtained from two Chilean government agencies, Dirección General de Aguas (DGA) and Instituto de Investigaciones Agropecuarias (INIA). The aridity at each site was obtained by computing the aridity index (AI) based on the UNEP (1992) as the ratio of the mean annual evapotranspiration to precipitation (E/P). Thus, lower AI values in extremely arid regions imply that all precipitation was essentially converted into evapotranspiration. Considering the AI and the dry season duration (UNESCO, 2010), the climate regimes were categorized into four types: semiarid (AI = 0.20–0.5), sub-humid (AI = 0.5–0.65), humid (AI = > 1) with a dry period of 3–4 months, and hyper humid (AI = > 1) with a dry period of 1–2 months.

For the physicochemical analyses, air-dried soil samples were sieved at 2 mm. The SOC was determined using the Walkley Black method (Walkley and Black, 1934). The soil textures (clay, silt, and sand) were measured using the hydrometer method (Gee and Bauder, 1986). The nitrogen was measured using the Kjeldahl digestion, and the pH was measured in a 1:2.5 soil/water suspension (Sadsawka et al., 2006). The bulk soil density was measured using the gravimetric method with the soil cores oven-dried at 105 °C for 48 h (Blake and Hartge, 1986). The water stable aggregates (WSA) were measured by using a wet-sieving apparatus (Eijkelpamp, Giesbeek, Netherlands) and calculated as the proportional mass of stable aggregates in the 1–2 mm range relative to the overall soil as described by Kemper and Rosenau (1986).

Table 3-1. Location, soil type and land use across 28 study sites. Soil Taxonomy reported by CIREN (Soil Survey Staff, 2006)

Site	Soil type	Climate	Elevation (m)	Lat. (S)	Long. (W)	Land use ^a
AGD	Mollisol	Semiarid	168	33.57°	71.15°	Orchard (WP)
LAP	Inceptisol	Semiarid	105	33.84°	71.28°	Cereal crop (C)
LGG	Alfisol	Semiarid	186	33.96°	71.52°	Grassland recolonization/tree shrub (NC)
PAH	Mollisol	Semiarid	221	33.58°	71.01°	Cereal crop (C)
PMR	Mollisol	Semiarid	186	33.49°	71.20°	Crop (C)
TRO	Mollisol	Semiarid	143	33.98°	71.40°	Orchard (WP)
DAO	Inceptisol	Subhumid	92	35.45°	71.70°	Cereal crop (C)
LIU	Inceptisol	Subhumid	108	35.39°	71.59°	Cereal crop (C)
TAL	Alfisol	Subhumid	141	35.37°	71.56°	Cereal crop (C)
CHO	Inceptisol	Humid	34	38.62°	72.85°	Grassland recolonization (NC)
LCH	Inceptisol	Humid	125	38.07°	73.02°	Cereal crop (C)
LMO	Inceptisol	Humid	111	38.04°	73.06°	Grassland recolonization (NC)
LTA	Inceptisol	Humid	288	38.60°	72.30°	Grassland recolonization (NC)
RHO	Inceptisol	Humid	83	38.02°	73.03°	Crop (C)
SCS	Inceptisol	Humid	71	38.06°	72.85°	Grassland recolonization/shrub (NC)
SSF	Alfisol	Humid	293	38.20°	72.78°	Cereal crop (C)
AGF	Inceptisol	Hyper humid	308	38.53°	72.25°	Grassland recolonization (NC)
ARC	Ultisol	Hyper humid	56	38.75°	72.85°	Cereal crop (C)
CHS	Andisol	Hyper humid	457	39.38°	72.22°	Grassland recolonization (NC)
HCE	Inceptisol	Hyper humid	33	38.69°	72.88°	Grassland recolonization(C)
LAS	Andisol	Hyper humid	97	39.20°	72.67°	Grassland recolonization (NC)
LOC	Andisol	Hyper humid	115	39.38°	72.62°	Pine forest (WP)
MAL	Andisol	Hyper humid	150	39.35°	72.57°	Cereal crop (C)
PHE	Andisol	Hyper humid	321	38.66°	72.25°	Grassland recolonization/shrub (NC)
PQN	Inceptisol	Hyper humid	63	38.79°	73.31°	Grassland recolonization (NC)
PUE	Inceptisol	Hyper humid	4	38.77°	73.39°	Grassland recolonization (NC)
SPT	Andisol	Hyper humid	400	38.66°	72.11°	Cereal crop (C)
SPTN	Andisol	Hyper humid	398	38.65°	72.11°	Native forest (NF)

^a Soil types were categorized by C = Cultivated; NC = Non-cultivated; NF =Native forest; WP=Woody perennial

3.2.4 Labile SOC fractions

The POXC was determined following the method described by (Culman et al., 2012). The soil samples were weighed in duplicate and contained 2.5 g of air-dried soil. Deionized water and 0.2 M KMnO_4 were added and the mixtures were shaken vigorously for 2 min at 240 oscillations/min. The tubes were removed from the shaker and allowed to settle for 10 min. Following dilution with deionized water, the absorbance of the supernatants was measured at 550 nm. The LFOM was measured using a modified Janzen et al. (1992) method. Specifically, 10 g of soil were weighed and 40 ml NaI was added with a density of 1.7 g cm^{-3} . The tubes were shaken and then allowed to settle for 48 h before removing the floating material using a fiberglass filter in a Buchner funnel. The material was washed twice with demineralized water, transferred onto filter papers, dried at 60°C for 24 h, and weighed. The LFOM was reported as the mass in g kg^{-1} soil because on degraded soils, mostly under semiarid conditions, the amount of LFOM was negligible and not sufficient for the elemental C analysis of LFOM.

3.2.5 Soil DNA extraction, 16S rRNA gene amplification and sequencing

The total microbial community DNA was extracted from 0.25 g of dry soil using the Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. The nucleic acids were quantified using the Qubit DNA probe (Invitrogen) and the quality was assessed by spectrophotometry (A_{260}/A_{280} ratio).

Amplification and sequencing of the 16S rRNA gene was performed according to the Earth Microbiome Project protocols (www.earthmicrobiome.org/protocols-and-standards). The V4-V5 region (16S rRNA) was amplified by PCR with 515F (GTGYCAGCMGCCGCGGTAA) and 926R (CCGYCAATTYMTTTRAGTTT) bacterial-archaeal primer pairs (Walters et al., 2016). The amplification products were multiplexed and sequenced in the Illumina MiSeq platform (250 bp x 2) at Argonne National Laboratory (Lemont, IL, USA) as described previously by (Caporaso et al. (2012)). The sequences were submitted to the NCBI BioProject database with the project

identification number PRJNA509899 and sample accession numbers SAMN10589514 to SAMN10589621.

3.2.6 Sequenced data processing and taxonomic assessment

Raw sequence processing for the operational taxonomic unit (OTU) tables were performed using the QIIME2 software package v.2017.10 (Caporaso et al., 2010). The sequences were quality-checked, assembled, and chimera-filtered using the DADA2 algorithm (100% identity) (Callahan et al., 2016). The OTU taxonomy was assigned using the SILVA 128 database (Quast et al., 2013). The 0.98% of the reads that corresponded to chloroplasts and mitochondria were discarded. The OTU table was composed of 1,635,321 reads distributed into 17,541 OTUs across 108 samples (4 samples were discarded for low depth sequencing). The OTU table was rarefied to 6,808 reads per sample to compute the diversity metrics. The α -diversity measures included the OTU richness, Shannon, Pielou's evenness, and Faith's phylogenetic diversity. The soil β -diversity was determined using the Bray Curtis dissimilarity (1 - similarity) and weighted UniFrac distance. For the phylogenetic metrics, the sequences were aligned with MAFFT (Katoh and Standley, 2013) and the tree was constructed using FastTree (Price et al., 2010).

3.2.7 Statistical analyses

Correlations between the soil properties and climate variables with the SOC data were tested for significance using the Pearson's correlations in Sigma Plot software (Systat Software, Inc, Point Richmond, CA). The data were tested by analysis of the one-way variance (ANOVA) and the pairwise comparisons were performed using the Fisher's LSD (least significant difference) method at 95% confidence using Statgraphics plus 5.1 (Manugistics, Inc., Rockville, MD, USA). The spatial and environmental analyses of the communities were performed in the R vegan package (Oksanen et al., 2013). The geographical coordinates were transformed into Principal Coordinates of Neighbor Matrices (PCNM) that represent all the different spatial patterns that can be distinguished

in a given sampling scheme (Borcard and Legendre, 2002). The environmental metadata were first checked for collinearity and the descriptors with a variance inflation factor > 10 were removed. A forward selection procedure was performed to remove the non-significant descriptors of the environmental metadata and the PCNM (Blanchet et al., 2008). The significant descriptors were used as the explanatory matrix for the distance-based redundancy analysis (db-RDA). The contribution of each variable explaining the model was computed using the variation partitioning approach implemented in the R vegan package.

The identification of the microbial genera that were differentially abundant across the POXC/SOC levels was performed by analyzing the community composition (ANCOM, Mandal et al., 2015) implemented in QIIME2. The non-rarefied OTU table was collapsed to the genus level. Low abundance genera ($< 0.01\%$ of the total reads and present in < 3 of the 108 samples) were filtered out because no comparisons among the groups could be computed with these values. ANCOM was applied to find the differences in the mean abundances for each genus across 5 groups defined by increasing the POXC/SOC ratio levels (%) to 0.5–1.0, 1.0–1.5, 1.5–2.0, 2.0–3.0, and > 3.0 . The 62 genera with significant different mean abundances were filtered into a new table and each one was correlated with the three main environmental descriptors in the metadata, namely, the POXC/SOC, LFOM, and pH. After the multiple Spearman correlations, genera passing the following four criteria were kept: 1) $\rho < -0.6$ (strong negative correlation), 2) $\rho > 0.6$ (strong positive correlation), 3) $p\text{-value} < 0.0001$ (significant correlation), and 4) $|\rho_{\text{POXC/SOC}}| > |\rho_{\text{LightFraction}}|$, $|\rho_{\text{POXC/SOC}}| > |\rho_{\text{pH}}|$ (the correlation with the POXC/SOC was stronger than the correlation with the other two variables). The same procedure was applied for the LFOM, where the 5 groups were defined by the increased LFOM content (g kg^{-1} soil): 2–5, 5–10, 10–25, 30–60, and > 100 . The 25 genera with significant different mean abundances were filtered using the same criteria, except for the fourth that was: $|\rho_{\text{LightFraction}}| > |\rho_{\text{POXC/SOC}}|$, $|\rho_{\text{LightFraction}}| > |\rho_{\text{pH}}|$.

3.3 Results

3.3.1 Soil Physicochemical properties

The results showed that changes in the climate regimes had significant effects ($P < 0.05$) on the soil properties. The aridity index strongly correlated with the bulk density ($r = -0.92$), water stable aggregates ($r = 0.61$), and the pH ($r = 0.57$). As conditions became cooler and more humid, the analyzed soil samples were typically more acidic ($\text{pH} > 6.0$), and the aggregate stability increased, reaching 89.8% in the hyper humid climate (Table 3-2). The SOC was also significantly affected by the climate regimes ($r = 0.89$) with the highest content observed under the hyper humid (71.4 g kg^{-1}) and lowest under the semiarid conditions (10.9 g kg^{-1}) (Table 3-3). Significant differences in the POXC and LFOM contents were found in the different climate regimes. The POXC accounted for a high proportion of the SOC (POCX/SOC) in drier climates, from 3.0% for the semiarid conditions to 1.2% in the hyper humid conditions. The LFOM increased in the range of $3.3\text{--}4.4 \text{ g kg}^{-1}$ of soil in the drier areas to 41.0 g kg^{-1} of soil for the hyper humid areas (Table 3-3).

Table 3-2. Soil physicochemical properties categorized according to climatic regimes. Average values and standard deviations for the studied soils are shown.

Aridity regime	Sand ($\text{g } 100\text{g}^{-1}$)	Clay ($\text{g } 100\text{g}^{-1}$)	BD (g cm^{-3})	WSA ($\%>0.25 \text{ mm}$)	C/N	pH
Semiarid	50.3 ± 24.3^a	24.5 ± 11.4^a	1.5 ± 0.1^c	66.5 ± 11.6^a	19.5 ± 3.9^a	7.2 ± 0.6^c
Subhumid	32.4 ± 10.7^a	26.8 ± 0.7^a	1.3 ± 0.1^b	73.0 ± 5.3^a	18.0 ± 0.0^a	6.5 ± 0.1^b
Humid	49.3 ± 13.7^a	18.9 ± 6.2^a	1.1 ± 0.1^b	88.5 ± 9.0^b	16.2 ± 0.4^a	5.5 ± 0.3^a
Hyperhumid	37.2 ± 15.6^a	24.3 ± 11.8^a	0.8 ± 0.3^a	89.8 ± 3.3^b	16.2 ± 4.4^a	5.7 ± 0.3^a

Significant pairwise differences ($P < 0.05$) are denoted with different letters.

BD: bulk density; WSA: water stable aggregates. Significant pairwise differences ($P < 0.05$) are denoted with different letters.

Table 3-3. Mean values \pm standard deviation for soil organic carbon and labile fractions observed in different climate regimes.

Aridity regime	SOC (g kg⁻¹ soil)	POXC (g kg⁻¹ soil)	POXC/SOC (%)	LFOM (g kg⁻¹ soil)
Semiarid	10.7 \pm 3.8 ^a	0.32 \pm 0.12 ^a	3.0 \pm 0.5 ^a	4.0 \pm 1.1 ^a
Subhumid	16.3 \pm 3.2 ^a	0.46 \pm 0.10 ^a	2.8 \pm 0.1 ^a	3.3 \pm 0.5 ^{ab}
Humid	26.5 \pm 6.1 ^a	0.40 \pm 0.08 ^a	1.5 \pm 0.2 ^b	8.2 \pm 1.9 ^{ab}
Hyperhumid	71.4 \pm 35.4 ^b	0.77 \pm 0.27 ^b	1.2 \pm 0.3 ^b	41.0 \pm 45.0 ^b

Significant pairwise differences ($P < 0.05$) are denoted with different letters.

SOC: soil organic carbon; POXC: permanganate oxidizable carbon; POXC/SOC: POXC to SOC ratio; LFOM: light fraction organic matter.

3.3.2 Bacterial-archaeal diversity associated with environmental factors

The relationships between the common α -diversity indices were also explored (Table 3-4). Analysis of the correlations among the diversity measures showed that the Shannon index was significantly correlated with each of the other diversity metrics. In addition, the Shannon diversity showed significant correlations ($P < 0.05$) with most of the soil physicochemical properties. There was a significant ($P < 0.05$) and positive relationship with the aridity regime ($r = 0.57$), SOC ($r = 0.50$), POXC ($r = 0.51$), and silt ($r = 0.48$). In addition, the Shannon diversity index showed a slight but significant decrease with pH ($r = 0.38$) and the cumulative POXC as a fraction of the SOC ($r = -0.43$). The OTU richness showed a significant ($P < 0.05$) increase with the silt and aridity regime and the SOC content ($r = 0.50$), and a decrease with BD ($r = -0.49$) and the POXC/SOC ratio ($r = -0.43$). The phylogenetic diversity did not show significant correlations with most of the soil parameters, with exception of the WSA ($r = 0.51$) and C/N ($r = 0.41$). The LFOM content was not significantly correlated with any of the diversity metrics.

The microbial community clustering based on the Bray-Curtis dissimilarity placed the samples into two main groups. The first cluster was composed of the 73 samples from the south (i.e. colder/wetter climates), low POXC/SOC, and low pH. The second group was composed of the 35 samples from the north, where the climate is warmer/drier (Fig. 3-2). Since the community similarities decreased with geographical distance (Fig. 3-S1), we investigated whether the community differences were correlated to the geographical

distance or the environment. Mantel tests showed that the Bray-Curtis community distance matrix was significantly correlated with both the geographic (Mantel's $r = 0.6487$, $p = 0.001$) and environmental (Mantel's $r = 0.237$, $p = 0.001$) distance matrices. On the other hand, the matrix of the pairwise phylogenetic distances (weighted UniFrac) was significantly correlated (Mantel's $r = 0.6469$, $p = 0.001$) with only the geographic distance matrix, but not with the environmental distance matrix (Mantel's $r = 0.0497$, $p = 0.178$).

Table 3-4. Pearson's correlation coefficient (r) between soil physicochemical properties, climate and α -diversity indices.

	PD	Richness	Evenness	SOC	POXC	POXC/ SOC	WSA	LFOM	Sand	Silt	Clay	C/N	pH	BD	AI
Shannon	0.41	0.89	0.80	0.50	0.51	-0.43	NS	NS	-0.44	0.48	NS	NS	-0.38	-0.58	0.57
PD		0.61	NS	NS	NS	NS	-0.51	NS	NS	NS	NS	0.41	NS	NS	NS
Richness			0.45	0.47	0.45	-0.38	NS	NS	NS	NS	NS	NS	NS	-0.49	0.54
Evenness				NS	0.41	NS	NS	NS	-0.41	0.51	NS	NS	-0.49	-0.48	0.43
SOC					0.95	-0.73	0.43	0.70	NS	0.46	NS	NS	-0.43	-0.91	0.89
POXC						-0.58	NS	0.74	NS	0.54	NS	NS	NS	-0.90	0.83
POXC/SOC							-0.81	-0.44	NS	NS	NS	NS	0.70	0.79	-0.85
WSA								NS	NS	NS	NS	-0.41	-0.72	-0.58	0.61
LFOM									NS	NS	NS	NS	NS	NS	0.58
Sand										-0.85	-0.77	NS	NS	NS	NS
Silt											NS	NS	NS	-0.42	0.38
Clay												NS	NS	NS	NS
C/N													NS	NS	NS
pH														0.63	-0.61
BD															-0.92

N.S: $P > 0.05$, non-significant relationship between the two variables.

PD: phylogenetic diversity; SOC: soil organic carbon, POXC: permanganate oxidizable carbon; POXC/SOC: POXC to SOC ratio; WSA: water stable aggregates; LFOM: light fraction organic matter; BD: bulk density; AI: aridity index.

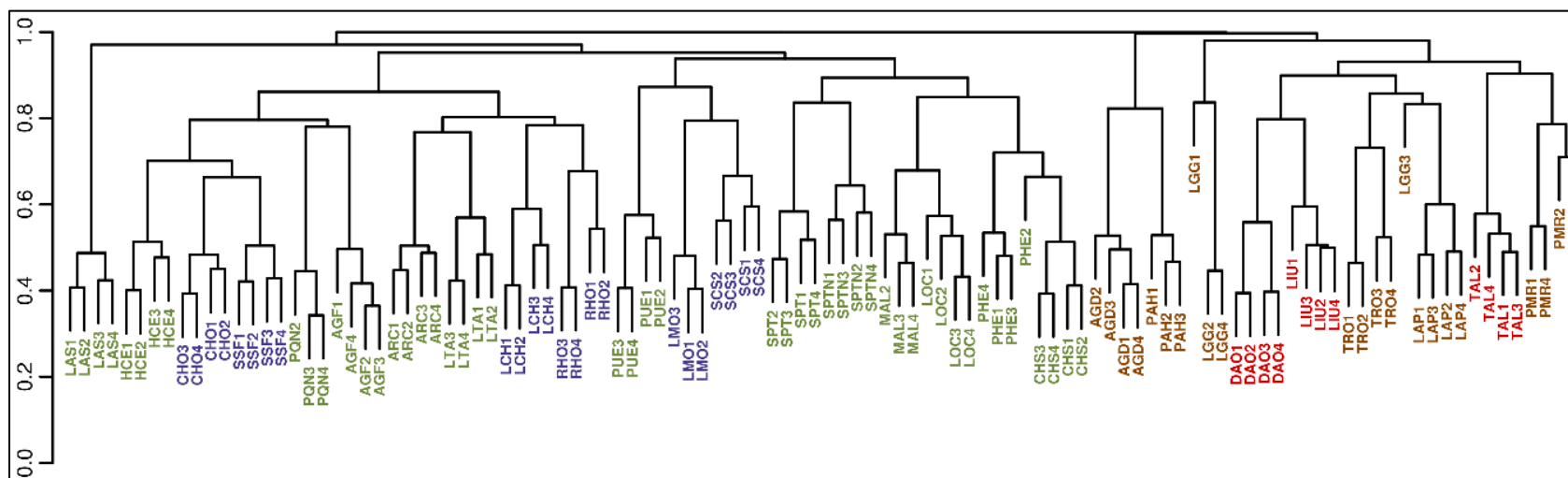


Figure 3-2. Cluster analysis of microbial communities based on Bray–Curtis dissimilarity matrix.

Table 3-5. Proportion of variation explained by environmental (soil variables) and spatial variables (PCNM variables) alone and combination based on Bray Curtis.

Explanatory variables	Bray-Curtis	
	Total	Exclusive
pH	21.1	8.0
POXC/SOC	20.9	6.1
LFOM	7.5	6.7
C/N	4.7	2.6
Environment	37.9	9.5
Space	39.8	11.5
Environment + Space	28.4	-
Total explained	49.4	-
Unexplained	50.6	-

POXC/SOC: POXC to SOC ratio; LFOM: light Fraction organic matter; WSA: water stable aggregates.

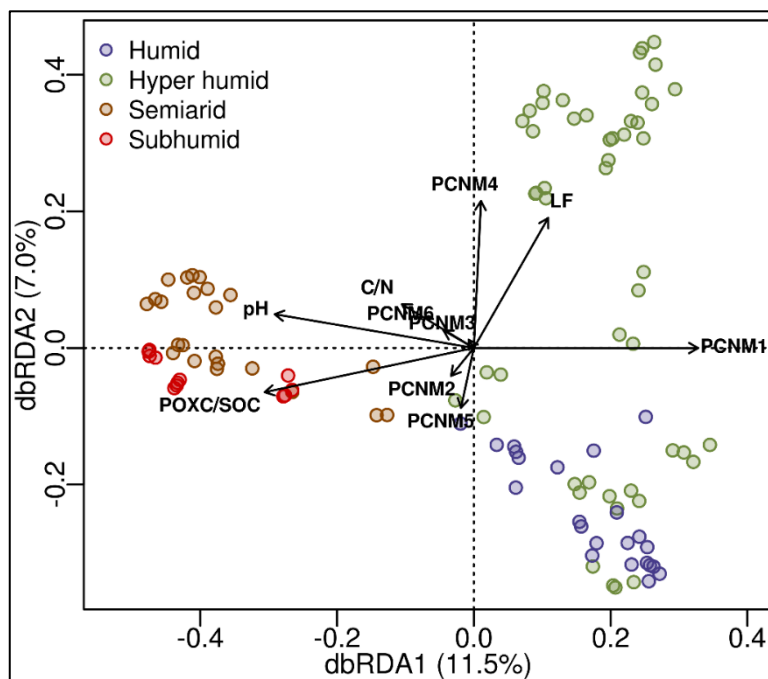


Figure 3-3. Distance-based redundancy analysis (db-RDA) of the community beta-diversity (Bray-Curtis dissimilarity), soil physicochemical properties and spatial variables. Variables and physicochemical vector correlation plots showing strengths and directions of relationships between physicochemical variables.

3.3.3 Linking soil bacterial-archaeal structure to labile SOC fractions

After determining the importance of both the POXC/SOC ratio and the LFOM for explaining the microbial community, the samples were grouped into five POXC/SOC ratio ranges (%): 0.5–1.0, 1.0–1.5, 1.5–2.0, 2.0–3.0, and > 3.0. Genera that correlated with these different labile carbon content ranges were identified across the samples. The soil pH was excluded from further taxa analyses because it has been widely described in the literature. A total of 62 genera were significantly different in abundance between the five POXC/SOC ratio ranges. Consistent shifts in the microbial community composition were observed across the larger POXC/SOC ratios and the relative abundance of specific bacterial genera became more dominant when the POXC/SOC values were higher than 2%. Three genera, *Adhaeribacter* (Bacteroidetes), *Skermanella* (Alphaproteobacteria), and a genus belonging to Acidobacteria subgroup 7 increased with the POXC/SOC (Spearman's $\rho > 0.6$, $P < 0.0001$), accounting for 0.14–0.20% of the total sequences. Five other unidentified genera were found to decrease with the POXC/SOC (Spearman's $\rho < -0.6$, $P < 0.0001$), namely, one each assigned to the Acidobacteria subgroup 2, the Chloroflexi JG37-AG-4 group, the Planctomyceteceae family, and two members of the Solirubrobacterales (Actinobacteria), accounting for 0.4–1.5% of the total sequences (Fig. 3-4a).

For the LFOM content (g kg^{-1} soil), the same analysis was performed by grouping the samples into five ranges: 2–5, 5–10, 10–25, 30–60, and > 100. A total of 25 bacterial genera were found to have significantly different abundances across the five LFOM content ranges. Two genera, *Edaphobacter* (Acidobacteria) and Candidate *Xiphinematobacter* (Verrucomicrobia) were found to increase with the LFOM (Spearman's $\rho > 0.6$, $P < 0.0001$), accounting for roughly 0.2% of the total sequences. Five genera (0.3–1.5% of the total), *Flavisolibacter* (Bacteroidetes), *Niastella* (Bacteroidetes), *Ramlibacter* (Betaproteobacteria), *Sphingomonas* (Alphaproteobacteria) and an uncultured soil Thaumarchaeota, decreased with the LFOM content (Spearman's $\rho < -0.6$, $P < 0.0001$).

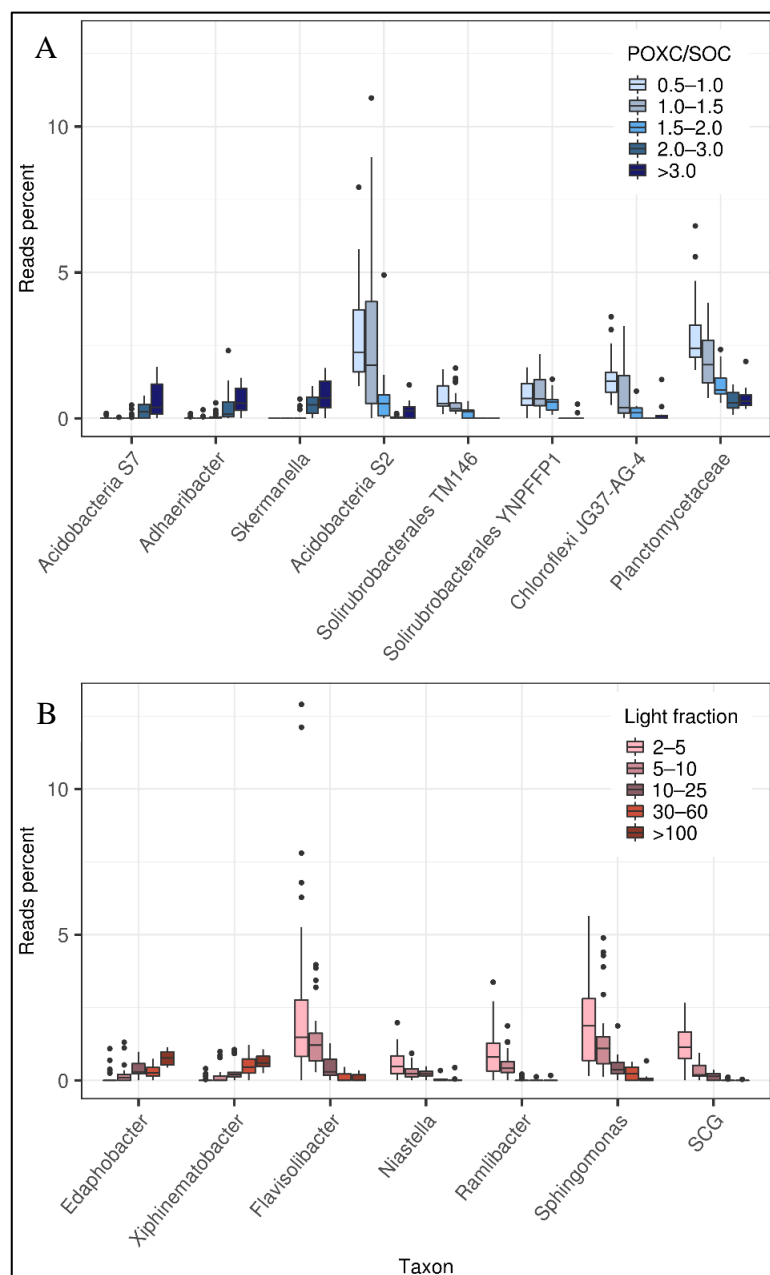


Figure 3-4. Changes in the relative abundances of bacterial-archaeal genera at different intervals of A) POXC/SOC ratio, and B) LFOM. Only those genera significantly correlated are shown, genera with Spearman's $\rho > 0.60$ were positively correlated, and $\rho < -0.6$ were negatively correlated. SCG: Soil Crenarchaeotic Group.

3.4 Discussion

3.4.1 Effects of physicochemical properties in soil bacterial-archaeal diversity

Although our findings provide evidence that the SOC content was not a significant predictor of the community β -diversity, a positive correlation was found between SOC and certain α -diversity metrics (OTU richness and the Shannon index) (Table 3-4). Similarly, previous studies have also identified a significant relationship between the α -diversity and the SOC content (Maestre et al., 2015; Ren et al., 2018b). In addition, previous studies (Delgado-Baquerizo et al., 2016; Maestre et al., 2015; Wieder et al., 2014) have reported SOC as the strongest bacterial community predictor in SOC contents under 40 g kg⁻¹ soil. In the present study, the C content ranged from 0.6 to 12.1%. Thus, the results appear to indicate that the SOC has a greater influence in the communities when the SOC contents are lower. The soil POXC/SOC ratio was negatively correlated with the richness and the Shannon index (Table 3-4). Moreover, POXC/SOC and LFOM were important variables for predicting β -diversity, indicating that the labile SOC fraction content is a greater contributor to variations in the community features compared to the SOC (Table 3-5). The non-significant effects of SOC on microbial diversity may be largely associated with the soil physical protection mechanisms (i.e. clays particles, soil aggregation) which create a physical barrier between SOC and decomposer (Wiesmeier et al., 2019), whereas the LFOM and POXC contents are characterized by rapid mineralization due to the labile nature of its constituents and to the lack of protection from the soil colloids (Turchenek and Oades, 1979).

The relationships between the environmental variables and the microbial communities were performed by db-RDA, that included both the physicochemical features and the spatial variation of all the sites studied (Fig. 3-3). Here, the forward selection of the significant descriptors discarded most of the physicochemical and spatial variables. Besides the POXC/SOC and LFOM, variables such as soil pH and C/N were determined as the key environmental drivers of the community β -diversity (Table 3-5). The soil pH was the main descriptor together with the POXC/SOC and LFOM. Soil pH has been widely described as a main driver of soil dynamics (Ren et al., 2018b; Shen et al., 2013; Siciliano

et al., 2014; Zhou et al., 2015). Moreover, field investigations in soils from the southern hemisphere demonstrated that pH, spatial influence, and organic matter are the most important drivers of bacterial diversity and composition (Delgado-Baquerizo et al., 2016). Previous studies have demonstrated that the soil C/N ratio is an important factor for predicting the microbial community (Chu et al., 2010; Shen et al., 2013). Similarly, in this study, C/N was one of the main contributors to variation in the microbial communities based on the Bray Curtis (Table 3-5). Additionally, phylogenetic α -diversity was positively correlated with the soil C/N ratio, which is inconsistent with findings from an earlier work by Shen et al. (2013), who observed that phylogenetic diversity was inversely correlated with the soil C/N ratio. Different resource qualities are preferred by microorganisms, for example, high C/N plant-derived leaf litter is related to higher fungi decomposition, and low C/N favors SOM bacterial decomposition (Lange et al., 2014). Thus, the discrepancy from previous data could be due to the frequent disturbance experienced by some agricultural practices (fertilization, cultivation, litter types) that can contribute to site-specific variability in the microbial diversity (Cools et al., 2014; Post and Mann, 1990).

Low BD values reflect improvement in the soil structure, and it is generally correlated with high water infiltration and low rates of surface runoff and erosion (Xiao et al., 2017). Our analysis revealed a negative correlation between the BD and the Shannon diversity, richness, and evenness (Table 3-4). It has already been shown that BD affects soil microbial activity and microbial biomass (D. Liu et al., 2018; Torbert and Wood, 1992). Moreover, high bulk density is typically associated with soil compaction, decreased soil porosity, and a decrease in the bacterial abundance resulting in restrictions in the soil O₂ concentration and microbial respiration (Chao et al., 2019; Li et al., 2002) that can explain the lower α -diversity found in higher BD samples. Additionally, physical protection of the SOM by aggregates is considered to be an important mechanism for C stabilization (Wiesmeier et al., 2019). Phylogenetic diversity was positively correlated with the WSA (Table 3-4), indicating that the soil aggregation may be supported by a wide range of phylogenetically distant community members. Macroaggregates (> 2 mm) contain high organic carbon contents, greater concentrations of less chemically complex and newer organic matter

inputs, resulting in diverse niches that harbor different types of microorganisms (Bach et al., 2018).

3.4.2 Effects of climate in soil bacterial-archaeal diversity

The aridity indices applied to our data exhibited a high correlation with the microbial community α -diversity (richness and Shannon index) (Table 3-4). Some studies have reported that aridity is a major factor that decreases the abundance of soil bacteria (Delgado-Baquerizo et al., 2018; Maestre et al., 2015; Ren et al., 2018a). This is because access to nutrients becomes more limited as the water film thickness is reduced by drought (Barnard et al., 2013; Fierer et al., 2003; Stark and Firestone, 1995). In this study, climate regimes were not significant predictors of the microbial community β -diversity. However, the cluster analysis clearly divided the microbial community structure into two different groups according to the level of the POXC/SOC ratio under warmer and colder climates (Fig. 3-2). In warmer climate conditions, as long as the water availability is not a limiting factor, the organic matter decomposition and the release of nutrients is accelerated (Jobbágy and Jackson, 2000), and thus, these areas are especially susceptible to C depletion (FAO, 2004; Lal, 2001; Ramírez et al., 2019). Consistently, a community structure divergence was observed indicating the role of climate regimes as relevant environmental filters in the decomposition.

3.4.3 POXC/SOC ratio as a driver of soil bacterial-archaeal communities

POXC represents the loss of organic matter as an oxidative process by a mild oxidizing agent based on the premise that KMnO_4 oxidation is comparable to microbial oxidation (Blair et al., 1995; Conteh et al., 1999; Loginow et al., 1987). Two genera categorized as copiotrophic and as initial metabolizers of labile C, *Adhaeribacter* (Bacteroidetes; Cytophagaceae) and *Skermanella* (Alphaproteobacteria; Rhodospirillaceae), increased their relative abundances under higher POXC/SOC ratios (Fig. 3-4a) (Fierer et al., 2007; Padmanabhan et al., 2003; Tian et al., 2017). Previous studies have documented that POXC

reflects a stabilized pool of active soil C that can lead to organic matter formation (Hurisso et al., 2016; Tirol-Padre and Ladha, 2004). Arable farming and warming condition cause biological impacts and C depletion resulting in soil physical degradation (i.e. erosion, compaction) (Bradford et al., 2016; Girvan et al., 2003). Therefore, the presence of associated taxa may be related to sites where SOM formation is markedly lower, which is reflected by higher ratio POXC/SOC. Particularly, Proteobacteria and Bacteroidetes have been primarily related to soil respiration rates under climate change and land use intensification scenarios through shifting soil microbial community composition rates (Kuramae et al., 2012; Y. Liu et al., 2018; Xiao et al., 2017). Altogether, these findings reveal that these genera could potentially have a stronger role in the degradation of soil organic matter, and are indicators of the soil health and degradation in agroecosystems.

In the soils studied here, Acidobacteria subdivision 7 increased with the POXC/SOC ratio, whereas an inverse trend was observed between Acidobacteria subdivision 2 with the same ratio. Nevertheless, caution should be taken when interpreting these relationships because Acidobacteria members are highly susceptible to changes in soil pH (Sait et al., 2006). For example, members of subdivision 7 may respond to increases in the soil pH containing high levels of Ca, Mg, Mn, and B (Kielak et al., 2016; Rousk et al., 2010), and this is consistent with the calcareous and alkaline properties of northern central Chilean soils (Casanova et al., 2013). Previous studies have indicated that POXC reflects a more processed and degraded fraction of soil C highly influenced by climate and soil conditions (Duval et al., 2018). This seems to have affected the relative abundance of two unidentified genera belonging to *Solirubrobacterales* (Actinobacteria; Class Thermoleophilia), one belonging to Chloroflexi (JG37-AG-4), and the other belonging to the Planctomycetaceae family that were found to decrease with the POXC/SOC (Fig. 3-4a). These results also are in agreement with the study of Grządziel and Gałązka (2018) that noted a negative effect in *Solirubrobacter* in degraded soils in comparison to healthy ones. Furthermore, Trivedi et al. (2016) observed a higher relative abundance of Chloroflexi in agricultural soils as compared to non-cultivated soils, agreeing with our results where most of studied soils were previously disturbed by agriculture. These results suggest that higher POXC/SOC

levels in drier ecosystems might lead to greater differences in the soil microbial composition responses.

3.4.4 LFOM as a driver of soil bacterial-archaeal communities

Influenced by the quantity and the characteristics of plant C inputs, agricultural practices, and climate, the LFOM accumulation is a useful predictor of soil respiration and N mineralization (Cambardella and Elliott, 1992; Hassink, 1995; Janzen et al., 1992). Indeed, a higher LFOM content should reflect the slower decomposition of crop residues (Rui et al., 2016). However, we could not find a significant relationship between the LFOM content and the α -diversity metric (Table 3-4), and less than 10% of the β -diversity variation was accounted for by the LFOM content (Table 3-5). Consistent effects were found by Cookson et al., (2005), who used soil phospholipid fatty acid patterns (PLFA) and found no significant relationships between the LFOM and the microbial community structure. These results suggest that the accumulation of LFOM might selectively increase or decrease the abundance of specific bacterial and fungal taxa while having a small impact on the microbial species diversity. Thus, we identified some specific taxa in soils with higher LFOM content (Fig. 3-4b) that included *Edaphobacter* (Acidobacteria; Acidobacteriaceae) and the Candidatus *Xiphinematobacter* (Verrucomicrobia; Chthoniobacterales). Precipitation typically stimulates soil LFOM (Song et al., 2012), and this may induce the growth of specific taxa in decomposed freshly-added organic matter. Acidobacteria and Verrucomicrobia have been described as opportunistic bacterial towards short-term changes in water availability, with rapid declines in ribosomal synthesis during drought (Barnard et al., 2013; Maestre et al., 2015). Razanamalala et al. (2018), found a clear relationship between LFOC (Light Fraction Organic C) and the occurrence of Acidobacteria and Verrucomicrobia members ($R^2 = 0.88$, both cases). Additionally, some of the genera found in our study have been previously described as ecologically important indicators used to assess soil quality. For example, strictly aerobic *Edaphobacter* (Dedysh et al., 2012), has been associated with acidic soils with good quality (Grządziel and Gałazka, 2018). Further, Sun et al. (2017) determined that during long-term restoration of

reclaimed mine soils, an increase in the Candidatus *Xiphinematobacter* was also related to the nematode populations and the potential development of higher trophic levels in the soil. In contrast, as the LFOM content decreased, the microbial community appeared to shift from strictly oligotrophic to oligotrophic/copiotrophic, with an increase in Bacteroidetes (*Flavisolibacter* and *Niastella*), and members of Proteobacteria such as *Ramlibacter* and *Sphingomonas*. These shifts can be explained by a decrease in the labile C and N substrates and the increase of nutrient-poor and recalcitrant C compounds (Schimel and Schaeffer, 2012). Members of the family *Sphingomonadaceae* and the genus *Ramlibacter* (Burkholderiales) have been recommended as sensitive indicators of arable soil fatigue (Wolińska et al., 2018). Furthermore, it has been reported that some members of the genus *Ramlibacter* have the ability to divide using desiccation-tolerant mechanisms in arid environments (de Luca et al., 2011). Additionally, our results showed that decreases in the LFOM induce Thaumarchaeota growth (Fig. 3-4b) that result as a consequence of lower water availability starvation conditions (Meisner et al., 2018). Consistently, Thaumarchaeota have been described as ammonia-oxidizing archaea abundant in terrestrial high-temperature habitats with a history of drought and desert soils (Daebeler et al., 2018; Meisner et al., 2018; Tripathi et al., 2015).

3.5 Conclusions

This study successfully detailed factors controlling major shifts in the soil microbial community and identified taxa that might respond to changes in the labile SOC fraction. The microbial α -diversity is primarily controlled by edaphic variables, climate regimes, and the labile SOC fraction in semiarid to hyper humid areas under disturbed and natural soil conditions. Variations in the community structure (β -diversity) were largely modulated by pH and the labile SOC fractions. Thus, the POXC/SOC ratio and the LFOM were found to be better descriptors of the community structure than other commonly used descriptors such as SOC, C/N, and land use. Climate regimes and labile C availability appear to have strong effects on the microbial community structure and composition. In addition, this

study showed that the taxa associated with the POXC and LFOM content could have important implications as early biological indicators in response to global warming and agricultural intensification. Thus, these findings provide evidence that supports the relevant role of bacterial-archaeal in the SOC dynamic that are critical for our comprehension and predictions of soil C stocks.

3.6 Acknowledgements

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4 SOIL ORGANIC MATTER LABILITY AS INFLUENCED BY ECOSYSTEM PROPERTIES

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4.1 Introduction

The dynamics and quality of soil organic matter (SOM) are essential to a variety of important soil functions and ecosystem services (Lal, 2016; Lefèvre et al., 2017). In general, soil organic carbon (SOC) responds slowly to land use or disturbances, and it is, therefore, not an ideal indicator of short term impacts on soil quality and potential C losses (Mirsky et al., 2008). SOM contains a range of compounds varying in decomposability, and SOM chemistry is an ecosystem property that responds to edaphic, environmental, and management factors (Schmidt et al., 2011).

Different SOC fractions are thought to be responsible for different soil functions (Baldock and Skjemstad, 1999). These fractions can be divided into recalcitrant C, which does not change markedly over decades (Stevenson, 1994), and more labile SOC fractions that account for a small, but vulnerable, proportion of SOM (Poirier et al., 2005; Verma et al., 2013; Yan et al., 2007). Different SOC fractions have been suggested as early indicators of the effects of soil management and cropping systems on SOM quality (Denef et al., 2007; Gregorich and Ellert, 1993; Haynes, 2005). For example, light fraction organic matter (LFOM) and permanganate oxidizable C (POXC) have been suggested as early indicators of SOC stock changes (Culman et al., 2012; Duval et al., 2018; Gregorich et al., 1994; Hurisso et al., 2016). Both fractions respond to land use change in the short-term and have been used to detect the effects of different land management practices. POXC is a rapid and inexpensive chemical method for soil health assessment (Culman et al., 2012; Hurisso et al., 2016; Morrow et al., 2016). Comparatively, LFOM consists largely of undecomposed or partly decomposed root and plant fragments (Biederbeck et al., 1994; Cambardella and Elliott, 1992; Golchin et al., 1994; Gregorich and Janzen, 1996), and

hence, is largely influenced by plant litter and its chemical composition (Gosling et al., 2013).

A wide variety of analytical methods have been used to measure labile SOM, most of which have been based on its physical, chemical, and biochemical principles (Strosser, 2010). Fourier-transformed mid-infrared (MidIR) spectroscopy has been widely applied to characterize organic matter in soils and predict the distribution of SOC fractions (Leifeld, 2006; Madhavan et al., 2017; Viscarra Rossel et al., 2006). Specific MidIR absorbance bands typically associated with aliphatics, methyls, amides III, and polysaccharides in specific soils can be ascribed to labile SOC (Calderón et al., 2017; Peltre et al., 2014). Thus, MidIR could be used to help generate hypotheses on the regional distribution of SOC fractions, and the factors that determine their distribution and composition.

Differences in SOC storage have often been attributed to vegetation chemistry and quality (Kane, 2015; Lützow et al., 2006). However, according to the chemical convergence hypothesis, all changes in chemistry during decomposition result from the preferential loss of easily degradable compounds (e.g. starch and proteins) and the relative accumulation of compounds that are more resistant to decay (Wickings et al., 2012). Thus, chemically distinct litter types pass through the decomposer ‘funnel’ and can converge to have similar chemistries (Bradford et al., 2016; Moore et al., 2011; Wickings et al., 2012). Although it is well understood that such changes in the chemical complexity of SOM enables changes in functional group chemistry (Hsu and Lo, 1999; Lützow et al., 2006), little is known about how climate and edaphic properties may control LFOM decomposition and its predictable sequence of chemical changes where O-alkyl loss is followed by aromatic breakdown and stabilization of alkyl-C.

Although labile SOC fractions have emerged as standardized indicators of SOC changes, their chemical composition across different ecosystems has not been extensively studied. The impact of environmental conditions on labile SOM pools and soil properties has largely been determined by separate studies with only local-scale differences. It is possible that SOC prevalence is largely controlled by the inherent chemical composition of labile fractions or that it follows a common sequence of chemical changes mainly controlled by

environmental factors, but few studies have considered such aspects of SOM composition across multiple soil and environmental contexts. Therefore, the objectives of this study were to: i) evaluate whether POXC and LFOM are sensitive to climate and land uses; ii) examine the relationship between SOC content and LFOM and POXC; iii) assess the functional group composition of organic matter associated with LFOM and POXC fractions across a range of ecosystems.

Soils were analyzed from 75 different sites with varying soil types and climate conditions along a latitudinal transect in Chile. These sites included native forest, prairie, abandoned cropland, cultivated land, and sites under woody perennial cover. Labile soil C and bulk soil properties were measured, spectroscopic measurements of the LFOM and bulk soil were carried out, and correlations and multivariate methods were used to ascertain the relationships among the data.

4.2 Materials and methods

4.2.1 Study area

This study was conducted across a 2000 km transect, from 32°00' S to 51°00' S, spanning from the semiarid central region of Chile to the far rainy south (Fig. 4-1). In general, climates associated with the sites used in this study are characterized by concentrated rainfall during the southern hemisphere's winter season (June to September) and a drier summer season (Bonilla and Vidal, 2011; Bonilla and Johnson, 2012; Lobo et al., 2015). Sites were categorized according to aridity regime (UNESCO, 2010), calculated as the ratio of mean annual precipitation (MAP) to potential evapotranspiration (ET) (UNEP, 1997, 1992), and also by considering the length of the dry season. Climate information was obtained from two different meteorological stations, Dirección General de Aguas (DGA) and Instituto de Investigaciones Agropecuarias (INIA); all the stations were in the vicinity of soil sampling sites. The mean annual temperature (MAT), MAP, and ET of each site are shown in Table 4-1.

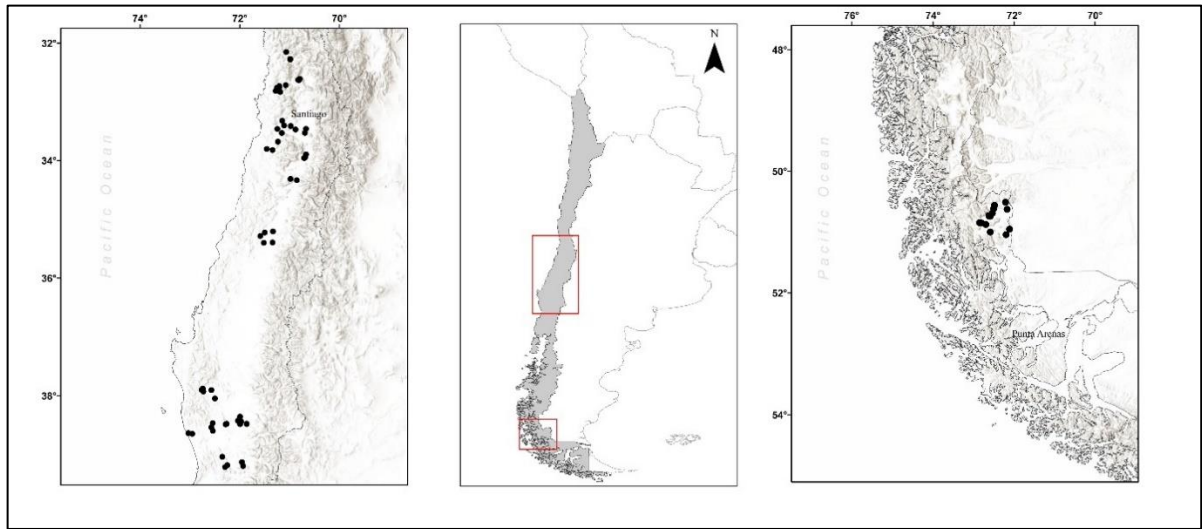


Figure 4-1. Map of sampling sites in central Chile (right) and southern Chile, Torres de Paine (left).

4.2.2 Land use

Sites were located in multiple types of land use areas including a biosphere reserve (Torres del Paine), natural sites, and cultivated sites. The land uses were sorted into five categories: 1) Native forest (NF) classified by the presence of Patagonian oak (*Lophozonia obliqua*) and Lenga (*Nothofagus pumilio*), 2) Natural prairie (NP) comprised of sites in the Patagonian Steppe, inhabited by desert shrubs and tuft grasses, 3) Woody perennial (WP) sites, including forest plantations, orchard or vineyards, 4) Cultivated (C) sites planted with cereals and/or vegetables and 5) Non-cultivated (NC) sites that were previously cultivated, but were no longer under agricultural management. Soil physico-chemical properties are listed in Table 4-1 according to land use.

Table 4-1. Mean values for soil properties categorized according to climatic regimes and land use. Values are means \pm standard deviation of the n indicated in the second column.

	n	MAP (mm)	MAT (°C)	ET (mm)	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	C:N	pH
Aridity Regime^(*)										
Arid	6	207	15	1405	18 \pm 3	42 \pm 16	34 \pm 9	24 \pm 8	18 \pm 3	7.7 \pm 0.2
Semiarid	22	400	14.8	1110	18 \pm 4	41 \pm 19	32 \pm 10	28 \pm 10	21 \pm 7	7.3 \pm 0.6
Subhumid	5	635	14.7	1007	19 \pm 3	41 \pm 17	35 \pm 12	24 \pm 7	16 \pm 3	6.2 \pm 0.4
Humid	9	1057	12.2	870	18 \pm 3	43 \pm 16	30 \pm 8	27 \pm 14	17 \pm 2	5.5 \pm 0.2
Hyperhumid	17	1615	12	780	16 \pm 3	44 \pm 11	37 \pm 10	19 \pm 9	16 \pm 4	5.7 \pm 0.3
Cold semiarid	16	401	7.5	870	20 \pm 0	45 \pm 11	40 \pm 9	16 \pm 6	11 \pm 4	6.0 \pm 0.6
Land use										
Native forest	5	-	-	-	19 \pm 2	43 \pm 15	37 \pm 8	20 \pm 9	15 \pm 3	5.6 \pm 0.2
Natural prairie	13	-	-	-	20 \pm 0	46 \pm 9	39 \pm 9	15 \pm 4	11 \pm 5	6.1 \pm 0.7
Abandoned crop	15	-	-	-	17 \pm 4	40 \pm 15	33 \pm 8	27 \pm 9	15 \pm 4	6.6 \pm 1.1
Cultivated	34	-	-	-	17 \pm 4	40 \pm 18	34 \pm 11	27 \pm 9	17 \pm 3	6.6 \pm 0.9
Woody perennial	7	-	-	-	19 \pm 1	39 \pm 22	32 \pm 11	30 \pm 17	17 \pm 4	6.4 \pm 0.9

*: According to UNEP (1992) and UNESCO (2010).

MAT: Mean annual temperature; MAP: Mean annual precipitation; C:N: carbon/nitrogen ratio.

Table 4-2. Two-way ANOVA of the effects of climate and land use on organic fractions (a), and variation partitioning analysis of organic fractions explained by climate, land use, and shared effects (b).

Organic Fractions	a) Two-way ANOVA		b) Variation Explained (%)		
	Climate	Land use	Climate	Land use	Share effects
SOC	***	ns	55.5	0.5	3.6
POXC	***	ns	26.5	0	11.7
POXC/SOC	***	ns	62.9	0	6.5
LFOM	ns	ns	0	0	34.8
WSA	***	ns	3.0	52.7	7.2

*** Statistically significant difference ($P < 0.001$).

ns: Non-significant relationship ($P < 0.05$).

LFOM: Light Fraction organic matter; POXC: Permanganate oxidizable carbon; SOC: Soil organic Carbon; WSA: Water stable aggregates.

4.2.3 Soil sampling and physico-chemical analysis

A total of 75 sites were sampled between January and February over four years (2013, 2014, 2016, and 2017). At each site, two points were randomly assigned at a distance of 100 m from one another for soil sampling. Before soils were collected from the topsoil horizon, plant residue and O horizon were removed. Depending on the site, first horizon depths ranged from 8 to 29 cm. The same methodology was used for this procedure as previously reported in Ramírez et al. (2019). For physico-chemical analysis, samples were air-dried and sieved (2 mm). The SOC was determined by potassium dichromate ($K_2Cr_2O_7$) oxidation (Walkley and Black, 1934). The pH was measured in a 1:2.5 soil/water suspension (Sadsawka et al., 2006). Particle size distribution (clay, silt, sand) was measured using the hydrometer method (Gee and Bauder, 1986). Total N was measured using the Kjeldahl digestion method. Finally, water stable aggregates (WSA) were measured using a wet-sieving apparatus (Eijkelpamp, Giesbeek, Netherlands) and were quantified as the proportional mass of stable aggregates in the 1–2 mm range relative to the mass of the whole soil, as presented by Kemper and Rosenau (1986).

4.2.4 Labile SOC fractions

Permanganate oxidizable carbon (POXC) was measured according to the methodology of Weil et al. (2003), with slight modifications as detailed by Culman et al. (2012). Duplicate air-dried soil samples (2.5 g) were placed in test tubes. Deionized water and 0.2 M KMnO_4 were added to each tube. The tubes were shaken for 2 min at 240 oscillations per minute, then allowed to settle for 10 min. Following dilution with deionized water the absorbance of the supernatants was measured at 550 nm. The POXC concentration was determined by generating a calibration curve with known standard solutions.

The soil samples were analyzed for light fraction organic matter (LFOM) using a modified version of the method described by Janzen et al. (1992). Specifically, 10 g of soil was weighed and added to 40 ml NaI with a density of 1.7 g cm^{-3} . The tubes were shaken, and then allowed to settle for 48 h before floating material was removed, and the remaining substance was filtered using a fiberglass filter in a Buchner funnel. The dried organic material was separated from the surface of the filter paper with a brush before being weighed. The LFOM was reported as g kg^{-1} soil because in degraded soils, mostly those collected from semiarid regions, the amount of LFOM was negligible and not sufficient for elemental C analysis.

4.2.5 FTIR methods

Whole soils and LFOM were dried and ground and scanned undiluted (neat) on the mid-infrared spectrometer from 4000 to 400 cm^{-1} . Specifically, a Digilab FTS 7000 (Agilent Technologies, Walnut Creek, CA) spectrometer with a KBr background, KBr beam splitter, and Peltier-cooled DTGS detector was used. The samples were analyzed in diffuse reflectance, at 4 cm^{-1} resolution, and each spectrum consisted of 64 co-added scans. Bands were assigned according to Parikh et al., (2014).

4.2.6 Statistical Analyses.

Correlations between soil properties and climate variables with SOC data were tested for significance using Pearson's correlations in Sigma Plot 13.0 (Systat Software, San Jose, California). Two-way analysis of variance (ANOVA) followed by a multiple range test was achieved using the least significant difference (LSD, $p < 0.05$) in Statgraphics plus 5.1 (Manugistics, Inc., Rockville, MD, USA) and was used to compare differences in the labile organic fractions and soil physico-chemical properties that were separately determined for each climate and land use type. The Unscrambler 10.3 software package (CAMO, Norway) was used to perform principal components analyses (PCA) on the mid-infrared spectral data.

A canonical redundancy analysis (RDA) followed by variation partitioning was conducted to identify relationships between environmental variables and spectral data. The analysis was performed using Canoco software v5.0 (Microcomputer Power, USA). Redundancy analysis is a constrained version of PCA which summarizes linear relationships between response variables that are explained by explanatory variables. In the RDA analysis, arrows pointing in the same direction indicate positive correlations, while those pointing in opposite directions suggest negative correlations, with arrow length indicating the magnitude of the correlation. Variables (Table 4-1) that were correlated according to the RDA were included. This can potentially introduce some overfitting but including all response variables and environmental factors was deemed beneficial in order to illustrate a more inclusive set of relationships. The average whole soil and LFOM spectra were baseline corrected using the baseline offset feature in Unscrambler 10.3 (Camo Software, Norway) before being used in the RDA analysis. Spectral bands were selected from the average whole soil and LFOM spectra using the peak picking feature in ThermoGRAMS software (Thermo Fischer Scientific Inc., Massachusetts, USA). The absorbance data was mean centered, and a PCA of whole soil and LFOM mid-infrared spectra was performed using Unscrambler 10.3 software (Camo Software, Norway). Component loadings were used to determine the contribution of spectral bands to the variation in soil total organic C and to the variation in POXC content.

4.3 Results and discussion

4.3.1 Climate and land use effects on labile SOC fractions

In this study, the variability of POXC and LFOM was examined across a set of climates and land use types in Chile. In most cases (Table 4-2), climate accounted for at least 26% of the total variance in SOC, WSA, POXC, and POXC/SOC. Variation in LFOM was associated with shared effects between climate and land use only. Total unexplained variance may be explained by others factor not included in this study such as topography, soil type, vegetation, and management history (Duval et al., 2018; Wiesmeier et al., 2019). The results showed a significant negative effect ($p < 0.05$) of MAT on POXC ($r = -0.47$) and LFOM ($r = -0.57$) (Table 4-3). A strong and positive correlation was observed between MAP and POXC ($r = 0.47$), however, no relationship was found between LFOM and precipitation. POXC content was significantly higher ($p < 0.05$) in cold semiarid and in hyper humid conditions than in drier climates (Table 4-4). The proportion of POXC in SOC (POXC/SOC) decreased when climate became more humid and cooler, ranging from 3.2% in soils from arid-semiarid climates to just 1.2% in hyper humid soils. Warmer areas are inherently low in SOC and also have lower overall net primary productivity. SOM decomposition is strongly accelerated by warm conditions, therefore rate of transformation of organic materials incorporated into the soil could be lower which might be reflected in a higher content of POXC relative to SOC (Álvaro-Fuentes et al., 2008; Lal, 2001).

Although there were no pure effects of land use type on LFOM, significant differences in LFOM ($p < 0.05$) were observed between agricultural land and undisturbed conditions (natural prairie and native forest), indicating that soil disturbance and agricultural practices can decrease LFOM content (Table 4-4). The absence of an explanatory effect of land use over LFOM and POXC demonstrates the importance of considering climate when soil management is compared under regional-scale conditions.

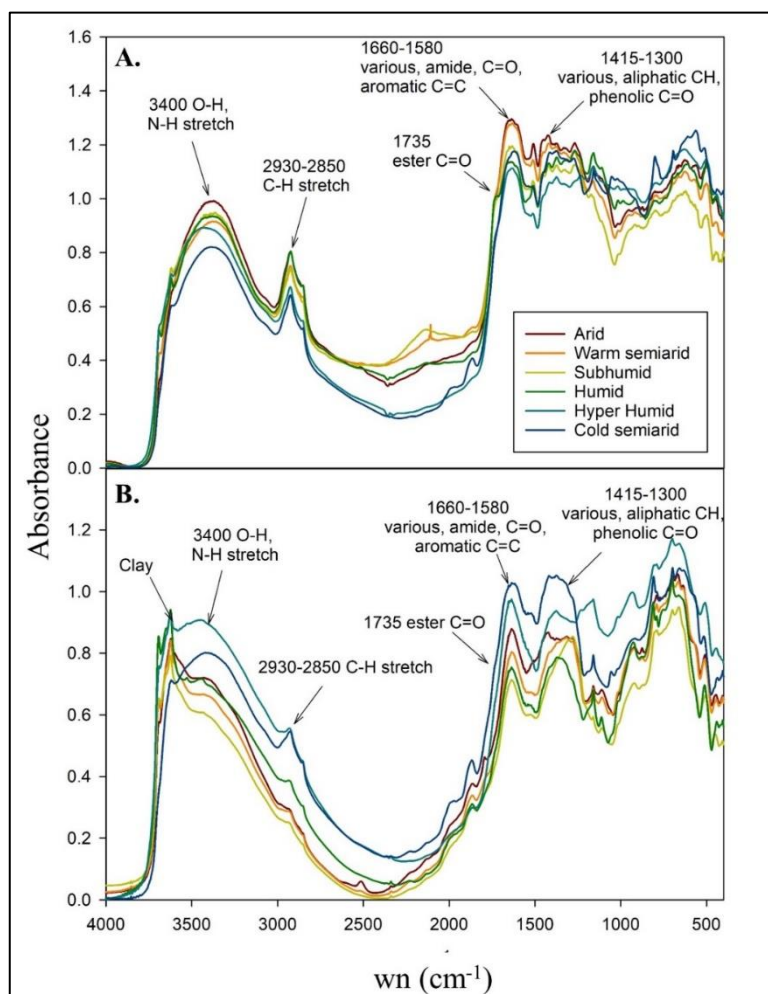


Figure 4-2. Average Fourier transform mid-infrared spectra for each aridity class. A) Light fraction organic matter, and B) whole soil spectra. Absorbances were baseline corrected using the baseline offset feature in Unscrambler 10.3 (Camo Software, Norway) in order to achieve a common low absorbance for all the averages.

Table 4-3. Pearson's correlation coefficient (r) among soil and climates attributes used in the study.

	LFOM	MAP	MAT	WSA	SOC	POXC/ SOC	Sand	Silt	Clay	C:N	pH
POXC	0.47*	0.47*	-0.47*	0.34*	0.82*	-0.44*	-0.13	0.45*	-0.25*	-0.26*	-0.25*
LFOM		0.00	-0.57*	0.29*	0.42*	-0.34*	-0.05	0.36*	-0.29*	-0.18	-0.29*
MAP			-0.19	0.49*	0.76*	-0.68*	0.04	0.12	-0.18	0.00	-0.60*
MAT				-0.63	-0.46	0.62	-0.09	-0.27	0.40	0.54	0.59
WSA					0.52*	-0.78*	0.08	0.08	-0.20	-0.51*	-0.74*
SOC						-0.73*	-0.01	0.35*	-0.33*	-0.17	-0.52*
POXC/SOC							0.04	-0.24*	0.18	0.36*	0.76*
Sand								-0.72*	-0.74*	-0.04	-0.07
Silt									0.07	-0.11	-0.14
Clay										0.18	0.24*
C:N											0.30*

(*) There is significant relationship between two variables ($p < 0.05$).

MAT: Mean annual temperature ($^{\circ}\text{C}$); MAP: Mean annual precipitation (mm/yr^{-1}); LFOM: Light fraction organic matter (g kg^{-1}); POXC: Permanganate oxidizable carbon (g kg^{-1}); SOC: Soil organic carbon (%); WSA: Water stable aggregates.

4.3.2 FTIR analysis in LFOM

The physical fractionation of the LFOM meant that infrared spectra could be directly obtained from the LFOM material as well as from the whole soil, and these differed dramatically (Fig. 4-2). Light fraction organic matter content is prone to rapid mineralization due to the labile nature of its constituents and the lack of protection by soil colloids (Turchenek and Oades, 1979), which is consistent with the absence of mineral spectral bands from clays at 3620 cm^{-1} and silicates at 1875 cm^{-1} observed in this study. Overall, several organic spectral bands were enhanced in the LFOM spectra. For example, the broad band at $3500\text{--}3200\text{ cm}^{-1}$ was much more pronounced compared to the whole soil spectra. This band is associated with OH or NH stretching, typical of fresh crop residues (Parikh et al., 2014). The peaks between 2930 and 2850 cm^{-1} , attributed to aliphatic CH stretching, were also more marked in LFOM spectra than in whole soil spectra. The peaks observed from 1700 cm^{-1} to 1250 cm^{-1} contain information about several functional groups such as esters (1730 to 1700 cm^{-1}) and carboxylic acids. Calderón et al. (2011) found similar results in a diverse soil collection from the North American Midwest, where LFOM was distinguishable from heavier fractions because of high absorbance at 3400 cm^{-1} (OH/NH), as well as between 1750 and 1350 cm^{-1} . As the soil undergoes net C mineralization, the mid-infrared absorbance of LFOM declines at 3400 cm^{-1} , and between 2920 and 2860 cm^{-1} , indicating that these could be regarded as labile moieties in LFOM (Calderón et al., 2011b). Previous research has indicated that the LFOM characteristics may be broadly similar even under different vegetation types and climatic conditions, suggesting similarity in the nature of the processes involved in the formation of LFOM (Bending et al., 2004; Gosling et al., 2013; Rumpel et al., 2012). Although, LFOM spectral responses showed little variation across climates, the gross chemical behavior generally featured common peaks, but with different band intensities (Fig. 4-2a). For instance, the peaks at 3400 cm^{-1} and 1660 cm^{-1} , attributed to stretching vibration O-H from water, alcohols, carboxylic acids, phenols, and N-H from amides, were observed most distinctly in the spectra of arid soils. As expected, these results show that the chemical composition of LFOM may differ across climate zones as a result of climatic influence on input quality

and decomposition processes, but a common sequence of molecular C transformation is followed during the early stages regardless of climate.

To test the influence of external factors on the composition of LFOM, an RDA analysis was carried out between FTIR functional groups (Fig. 4-2a) in response to edaphic and climatic factors. Along the first axis (Fig. 4-3a), the spectral bands were clearly clustered according to temperature regime. Light fraction organic matter spectra from arid, warm semiarid, and subhumid soils, with high POXC/SOC had high absorbance at multiple organic bands. These included 2110 (overtone of the --COH stretch), 3367 (O-H, N-H stretch), 2927 (aliphatic C-H stretch) and 1633 cm^{-1} (amide I). In addition, the results revealed the prevalence of relatively chemically complex LFOMs in low SOC soils. For instance, absorbances at 1512 cm^{-1} and 1633 cm^{-1} , possibly due to lignin derivatives, were more pronounced under arid/warmer conditions, and defined peaks at 2927 cm^{-1} at 3367 cm^{-1} were observed in soils from subhumid regions. These bands can be assigned to O-H and N-H stretch, aliphatic CH, amide I, and aromatic C=C, respectively. Data from the same study area have consistently indicated that semiarid soils have been more affected by SOC losses during recent decades (Ramírez et al., 2019).

LFOM composition is characterized by the presence of carbohydrates and aliphatic substances, which play important roles in the chemical composition of this fraction (Golchin et al., 1994). In this study, absorbance at 1016 cm^{-1} , attributed to polysaccharide C-O-C, C-OH stretch (Parikh et al., 2014), was higher in soils with high SOC and soil aggregation, mostly from colder climates (Fig. 4-3a). The polysaccharide-like characteristics of the LFOM in SOC-rich soils points to high C lability, and possibly to N-limited decomposition. The LFOM spectra from native forest soils were characterized by high WSA and LFOM content (Table 4-4) and also had high absorbance (Fig. 4-3a). The polysaccharide accumulation possibly resulted from temperature limitation of polysaccharide decomposition or, alternatively, from polysaccharide protection by soil physico-chemical mechanisms such as soil aggregation. In addition, low temperature is considered to be one of the dominant forces protecting soil C from decomposition because it limits the activity of microorganisms (Allison et al., 2010; Bradford et al., 2016). This

raises the possibility that a common decomposition sequence exists and is highly controlled by other ecosystem properties but is not strongly dependent on plant input characteristics. Thus, this common decomposition sequence can be altered by environmental factors that accelerate or delay the microbial transformation of raw material into more decomposition-resistant molecules (Grandy and Neff, 2008; Wickings et al., 2012).

4.3.3 FTIR analysis in whole soils

The results showed that whole soil spectra contained absorbance bands attributable to mineral and organic matter. The arid whole soil spectrum featured peaks at 1870 cm^{-1} attributed to Si-O bonds, which is characteristic of quartz and sands, and 3620 cm^{-1} associated with hydroxyl stretching of clay minerals, as well as a small peak at 2510 cm^{-1} representing carbonates that was absent on the spectra of all other aridity regimes (Fig. 4-2b). This fits with other studies that have suggested that arid and semiarid areas in Chile frequently contain calcareous alkaline soils (Casanova et al., 2013). There was a tendency for specific regions of the spectrum to become more intense for whole soil samples from cold and humid areas, which may indicate less degradation of organic matter compared to warmer areas. More specifically, the broad peak around 3400 cm^{-1} was particularly pronounced in the whole soil spectra of soils from cold semiarid and hyper humid climates, which are also characterized by high SOC content (Fig. 4-3b).

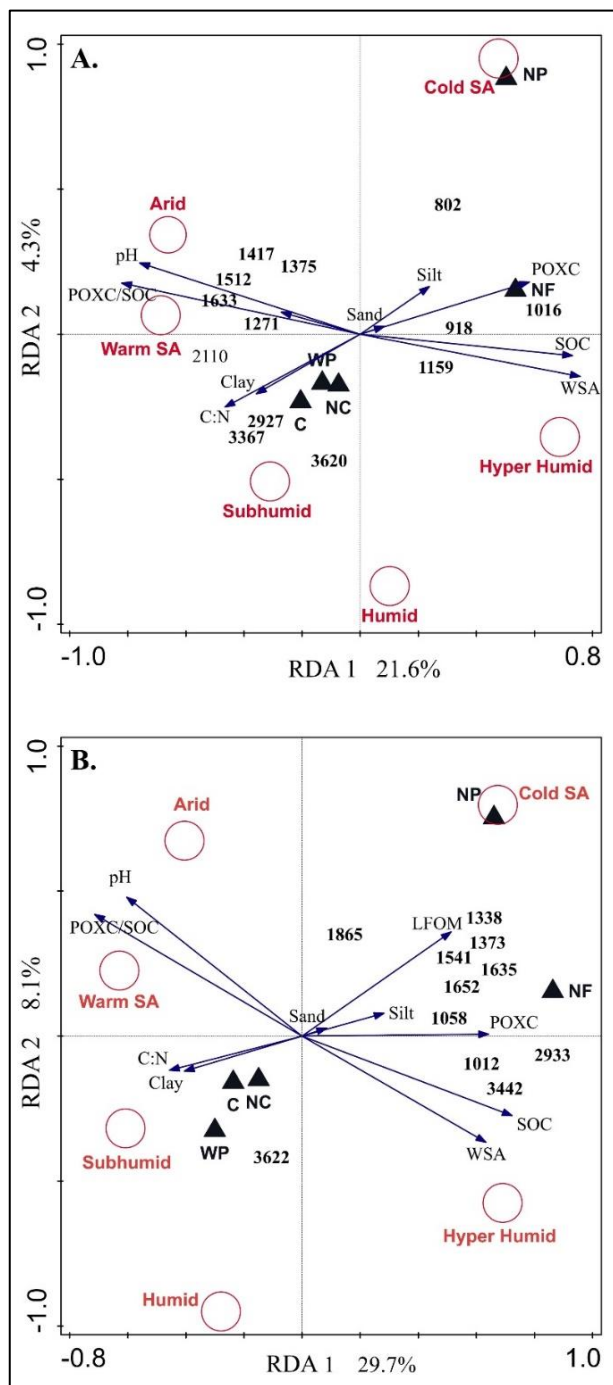


Figure 4-3. Redundancy analysis (RDA) showing the relationships between FTIR bands, SOC labile fractions (POXC and LFOM), and soil properties and environmental factors including aridity regime and land use. WP: Woody perennial; C: Cultivated; NC: Non-cultivated; N: Natural prairie, NF: Native forest. A) Light fraction organic matter of soils, and B) whole soils.

The RDA analysis indicated that the MidIR absorbance data for whole soils contained several spectral bands that were related to specific soil properties. For instance, as expected, sand content was positively correlated with absorbances at 1865 cm^{-1} , corresponding to silicate Si-O absorbance. Soils from cooler (cold semiarid, hyper humid, humid) and warmer (warm semiarid, arid, and subhumid) climates were separated into two groups according to pH, POXC/SOC, and spectral properties (Fig. 4-3b). Aromatic structures declined under warmer climates, and there was aliphatic enrichment in soils higher in SOC and POXC content, mostly soils from hyper humid sites. Higher absorbances for C-O stretching of polysaccharides around $1012\text{--}1058\text{ cm}^{-1}$ and OH/NH functional groups at 3442 cm^{-1} were observed for colder climates. Similarly, previous studies have shown that soils high in POXC contain increased proportions of aliphatic chemical species (Calderón et al., 2017; Margenot et al., 2015; Romero et al., 2018). The aliphatic component of SOM is affected by inputs of aliphatic-rich OM, and deposition of aliphatic compounds from increased microbial biomass during residue decomposition (Baddi et al., 2004; Hsu and Lo, 1999).

Stabilization of SOM often includes physical mechanisms, such as aggregation or sorption, chemical protection, and perturbations, such as N enrichment, that serve to accelerate or inhibit the activity of some enzymes or microbial groups (Grandy and Neff, 2008; Lützow et al., 2006). On a country-wide scale, the concentrations of POXC and LFOM significantly increased ($p < 0.05$) with SOM and soil aggregation. Thus, SOC was positively correlated with both POXC ($r = 0.82$) and LFOM ($r = 0.34$). In addition, WSA was also positively correlated with both POXC ($r = 0.42$) and LFOM ($r = 0.29$) (Table 4-3). These results indicate that MidIR data has certain chemical moieties that are more prevalent in samples with good soil quality attributes. Along with POXC and LFOM, soil aggregation also correlated positively with aliphatic components and the band at 1635 cm^{-1} corresponding to amide or ketone C=O (Fig. 4-2a and Fig. 4-2b), whereas whole soils with lower aggregation, mainly those from warmer climates, had aromatic absorbance bands in their LFOM spectra (Fig. 4-2a). Consistent with these findings, a recent study by Sarker et al. (2018) showed that structures such as O-alkyl and di-O-alkyl are positively associated with aggregate stability

and soil structure, whereas aromatic C fractions are negatively correlated with soil aggregation, possibly due to aggregates being protected by coatings associated with water repellency.

Negatively charged clay minerals promote sorption of organic groups contributing to SOM stabilization (Wiesmeier et al., 2019). An inverse relationship ($p < 0.05$) was found between clay and POXC ($r = -0.25$) as well as between clay and LFOM ($r = -0.29$) (Table 4-3). However, these relationships greatly improved when clay and the proportion of POXC to total SOC content (POXC/SOC) were analyzed separately for warmer areas ($r = -0.44$; $p = 0.01$). This may indicate the functional relevance of clay minerals in the formation of stable soil C through chemical binding of newer and less chemically complex compounds in warmer areas (Bach et al., 2018; Jones and Donnelly, 2004; Post and Kwon, 2000). In addition, negative relationships were also observed between C:N and POXC ($r = -0.26$) and between C:N and SOC ($r = -0.17$) (Table 4-3). These inverse trends are consistent with previous results observed for other labile or active fractions strongly associated with POXC, such as soil microbial biomass (Wardle, 1992). Previous studies have demonstrated that soil C:N ratio is an important factor in controlling microbial communities (Chu et al., 2010; Shen et al., 2013), indicating that in many soil ecosystems soil N rather than soil C may influence the immobilization of SOM by microbial biomass (Wiesmeier et al., 2019). In addition to the RDA analysis, PCA was performed in order to identify the spectral regions explaining the major spectral differences in LFOM spectra (Fig. 4-4) and in whole soil spectra (Fig. 4-5) according to SOC and POXC content. Overall, the LFOM soil spectra of cooler SOC-rich soils had different spectral features compared to soils with lower SOC content, mostly from warmer areas (Fig. 4-4a). However, these patterns were more clearly defined in whole soil spectra, as samples with increasing SOC tended towards the right of the PCA plane (Fig. 4-5a). The loadings of component 1 for LFOM indicate that the spectral differences were associated with higher absorbances in the 1042 cm^{-1} - 1120 cm^{-1} region, which can be attributed to C-O stretching of polysaccharides (Fig. 4-4c). In the case of whole soils, component 1 loadings show that the spectral differences were associated with higher absorbances at several organic bands: 2930 , 1680 , 1160 , and 1060 cm^{-1} (Fig.

4-5c), whereas component 2 loadings were negatively correlated with bands corresponding to silicate (Si-O) and clay minerals at 1875 cm^{-1} and 3620 cm^{-1} , respectively. This is consistent with the fact that clay content was lowest in soils with the highest levels of SOC content (Table 4-1, Table 4-4). As such variations in the spectral composition do occur, clay minerals do not seem to affect SOC stabilization in SOC-rich soils.

Table 4-4. Mean values and standard deviations for soil total organic carbon (SOC), permanganate oxidizable carbon (POXC), and LFOM (light fraction organic matter) categorized according to aridity regime and land use type of the 74 soils studied.

	SOC (g kg^{-1})	POXC (mg kg^{-1})	POXC/SOC (%)	LFOM (g kg^{-1})	WSA (%>0.25mm)
Aridity regime*					
Arid	14.9 ± 4.6^a	457 ± 95^a	3.2 ± 0.3^a	9.0 ± 2.4^a	64.2 ± 8.9^{ab}
Warm semiarid	15.7 ± 6.9^a	470 ± 161^a	3.2 ± 0.8^a	9.6 ± 8.5^a	64.1 ± 11.8^a
Subhumid	17.4 ± 7.5^a	439 ± 143^a	2.6 ± 0.3^a	3.9 ± 1.1^a	75.1 ± 8.1^b
Humid	29.2 ± 7.0^{ab}	424 ± 79^a	1.5 ± 0.2^{bc}	10.0 ± 4.3^a	89.5 ± 7.8^c
Hyperhumid	77.7 ± 36.5^c	796 ± 256^b	1.2 ± 0.3^c	38.2 ± 40.8^a	89.2 ± 4.1^c
Cold semiarid	44.7 ± 14.9^b	727 ± 188^b	1.8 ± 0.5^b	108.0 ± 101.5^b	86.9 ± 9.0^c
Land use					
Native forest	66.8 ± 33.5^b	806 ± 186^a	1.4 ± 0.5^a	70.1 ± 51.8^{ab}	82.3 ± 12.6^{ab}
Natural prairie	44.9 ± 16.2^{ab}	700 ± 192^a	1.7 ± 0.5^a	113.2 ± 110.8^a	89.4 ± 5.5^b
Non-cultivated	38.0 ± 36.4^{ab}	611 ± 241^{ab}	2.4 ± 1.1^{ab}	20.0 ± 28.3^{bc}	75.4 ± 17.8^a
Cultivated	30.9 ± 29.5^a	518 ± 213^b	2.4 ± 1.1^b	12.1 ± 12.8^c	74.5 ± 14.3^a
Woody perennial	33.2 ± 32.7^{ab}	499 ± 270^b	2.1 ± 0.8^{ab}	14.1 ± 13.3^{bc}	79.1 ± 9.7^{ab}

*: According to UNEP (1992) and UNESCO (2010).

Different letters within each column indicate significant differences between treatments at $P > 0.05$.

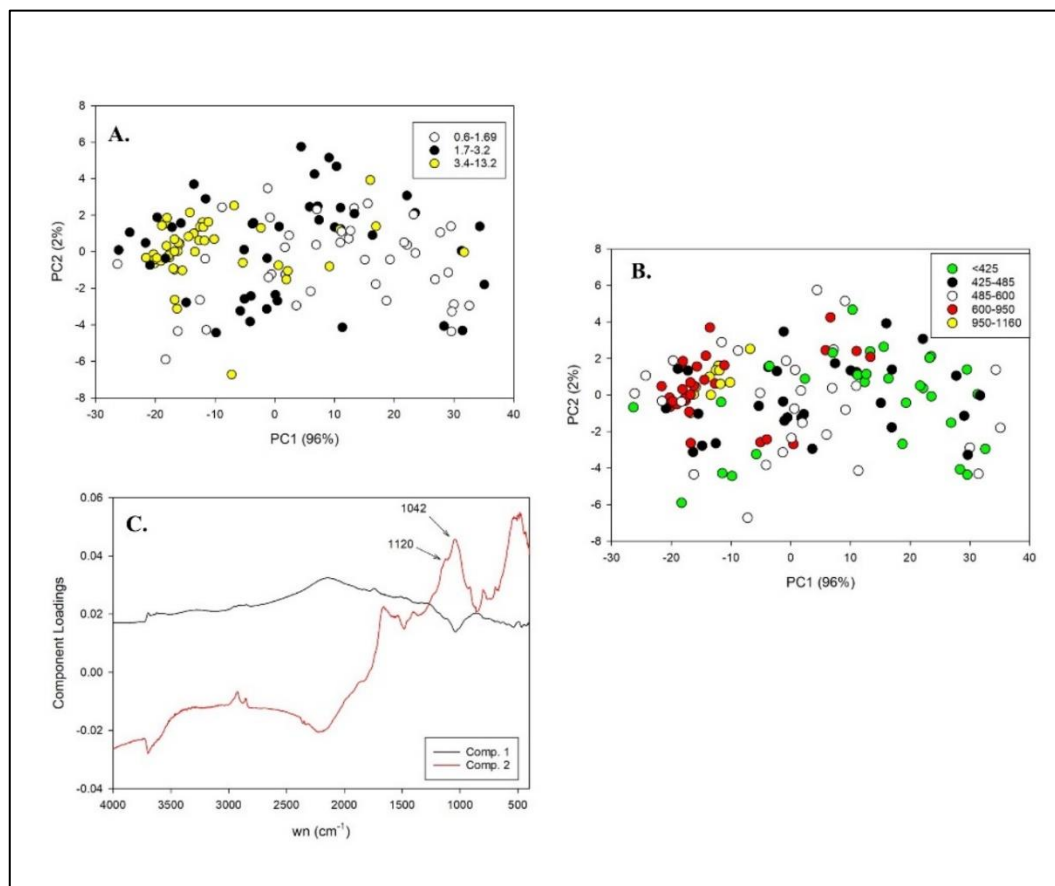


Figure 4-4. Principal components analysis (PCA) of the mid-infrared spectral data for light fraction organic matter (LFOM). Color coded according to (A) soil total organic C (%), and (B) POXC content (mg kg⁻¹). The component loadings for the PCA are shown in (C).

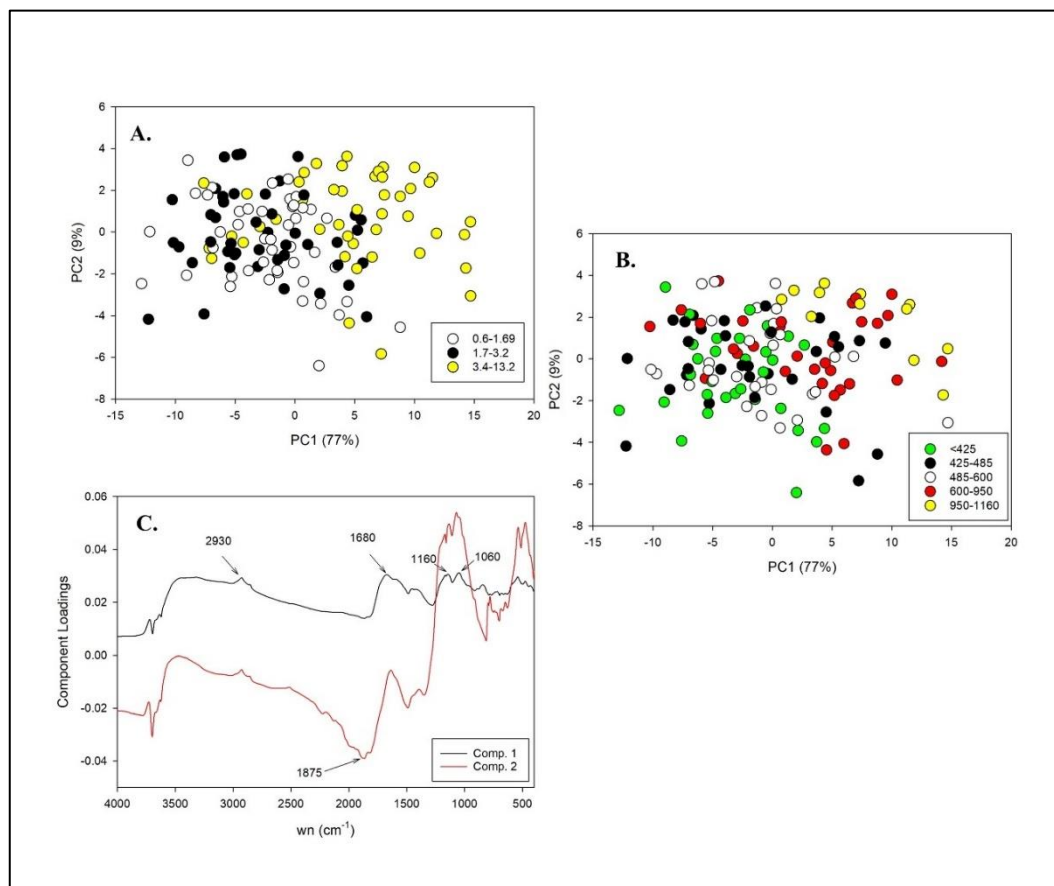


Figure 4-5. Principal components analysis (PCA) of the mid-infrared spectral data for whole soils. Color coded according to (A) soil total organic C (%), and (B) POXC content (mg kg⁻¹). The component loadings for the PCA are shown in (C).

4.3.4 Relationship between POXC and SOC content

Permanganate oxidizable C was strongly correlated with SOC in all the 75 sites studied. Moreover, as mentioned above, POXC fractions followed a similar spectral correlation with SOC (Fig. 4-5b). The line of best fit between SOC and POXC was hyperbolic rather than linear ($R^2 = 0.69$; Fig. 4-6), as SOC values higher than 60 g kg⁻¹ were not associated with further increases in POXC values. To determine whether the chemical composition of POXC varied under a wide range of edaphic and climate conditions, two correlation analyses between POXC and MidIR absorbances were carried out: one for whole soils from

cooler climates regimes with high level of SOC, and one for whole soils under warmer climates (Fig. 4-7).

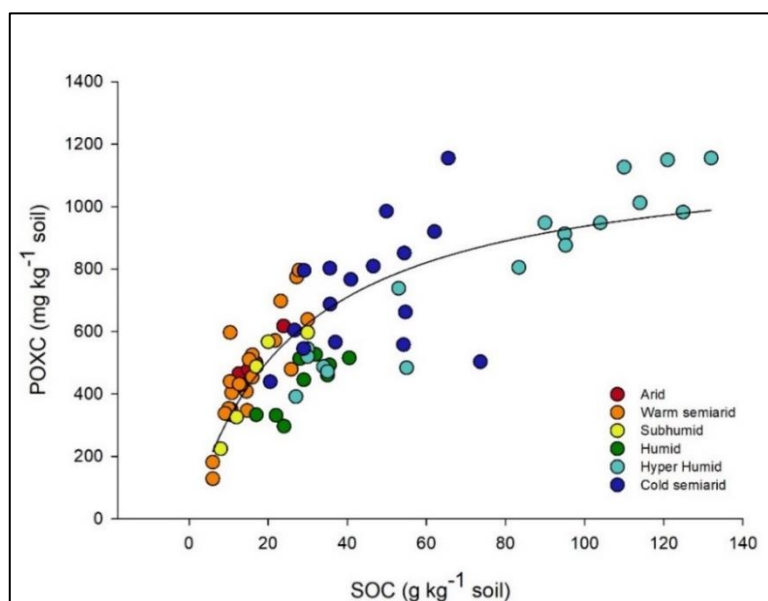


Figure 4-6. Hyperbolic fit lines for soil organic carbon (SOC) vs. permanganate oxidizable carbon (POXC). Red symbols are arid sites, orange symbols are warm semiarid sites, blue symbols are cold semiarid sites, green symbols are humid sites, light blue symbols are humid sites, and blue symbols are cold semiarid sites.

These results suggest that increases in POXC are associated with different compositional parameters in soils from different climates. Permanganate oxidizable C was negatively correlated with clay absorbance bands at 3630-3700 cm^{-1} in soils from warm climates, likely indicating that the accumulation of alkyl C in soils is due to its intimate association with clay mineral surfaces. For SOC-rich cold soils, absorbances at 3450-2820 and 1730-1000 cm^{-1} were positively correlated with POXC. These spectral regions contain many of the bands associated with a variety of organic moieties (Parikh et al., 2014). For warmer soils, a different set of spectral bands were positively correlated with POXC. These were the aliphatic CH band at 2930-2850 cm^{-1} , and a broad peak at 1190 cm^{-1} corresponding to polysaccharide.

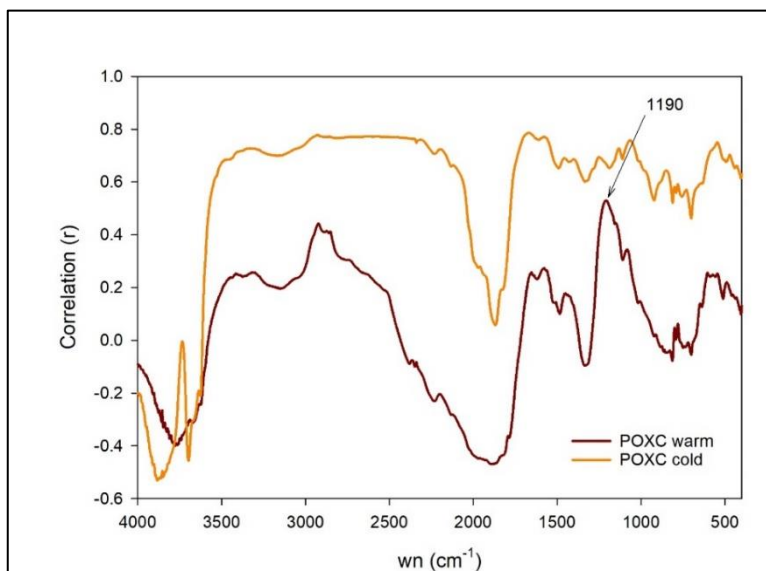


Figure 4-7. Correlation (r) between whole soil absorbance and POXC. The warm correlation refers to soils from the semiarid, arid, and subhumid sites ($n=68$). The cold correlation refers to soils from the cold semiarid, humid, and hyper humid sites ($n=69$).

4.4 Conclusions

The results of this study provide evidence that climate and ecosystem properties play important roles in organic matter lability. Under a wide range of climates and land use types, LFOM chemical composition appears to maintain a relatively stable spectral composition, but with different band intensities across climatic regimes. Thus, in cooler areas, which are typically characterized by SOC-rich soils with high levels of POXC and aggregation, LFOM composition was closely associated with aliphatic structures. However, in soils with low SOC from warmer regions, LFOM contained more chemically complex structures. The results of this work suggest that, in all sites, a common decomposition sequence for LFOM exists, but when LFOM becomes part of the soil, it is highly controlled by other ecosystem properties such as climate, soil aggregation, and clay minerals, which accelerate or delay the microbial transformation of raw materials into more decomposition-resistant compounds. Permanganate oxidizable C was negatively correlated with clay absorbance in warmer areas, likely indicating that the accumulation of alkyl C in soils from these areas might be due to an intimate association with surface minerals.

Furthermore, POXC was strongly correlated with and exhibited similar spectral features to SOC content. It was also significantly influenced by climate. This study, therefore, advances our understanding of the labile SOC fraction dynamic in soils and how they can respond to different environments as early indicators of changes in soil carbon, which may contribute to identifying areas that are more vulnerable to SOC losses.

4.5 Acknowledgments

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5 EFFECTS OF ANNUAL CROPS ON SOIL ORGANIC CARBON STOCKS UNDER SEMIARID AND HUMID CLIMATES IN CHILE, 1980–2016

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5.1 Introduction

Soil organic carbon (SOC) is a critical driver in natural and agricultural systems due to its role in a range of soil functions associated with water dynamics, erosion control, nutrient provision, soil structure, and biodiversity (Lal, 2016; Schmidt et al., 2011). SOC is fundamental for supporting long-term agricultural productivity, as well as for mitigating the effects of greenhouse gases, and adapting agricultural systems to climate change (Lal, 2015, 2014; Powlson et al., 2011). Factors that control long-term dynamics in SOC include land use and management, soil type, and climate (Paustian et al., 2016).

The production of annual crops of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) is of great importance in Chile. They are major crops in terms of planted area covering a wide range of climatic regions (Espinoza et al., 2012; ODEPA, 2017). Areas cultivated by annual crops have been characterized by intensive agriculture resulting in the erosion and depletion of soil fertility, and degradation of natural vegetation (Muñoz et al., 2007). Currently, the decline of soil C in agroecosystems is mainly due to cultivation (Luo et al., 2010). Cultivation practices change the quality and quantity of C inputs to the soil, and impact soil physical properties affecting carbon decomposition.

Carbon inputs to soil are mainly controlled by biomass productivity of the cropping systems, which is a function of the variable climate, soil conditions, fertilizer inputs and agronomic management (Luo et al., 2016). The soils located in more arid conditions are more vulnerable to SOC loss and degradation (Lal, 2001; Ramírez et al., 2019). These soils facing significant periods of water shortages are inherently low in SOC (Álvaro-Fuentes et al., 2008). Water shortages make it difficult to reach high levels of SOC and thus increase

crop production (Albaladejo et al., 2013). As a consequence, to achieve a maximum yield, agricultural systems have been managed intensively under irrigation resulting in an accelerated C and N mineralization.

Chilean SOC stocks are very heterogeneous, both spatially and temporally (Bonilla and Johnson, 2012; Ramírez et al., 2019), mainly due to different soil types and climate conditions (Doetterl et al., 2015; Ramírez et al., 2019; Reyes Rojas et al., 2018). Therefore, SOC storage is likely to respond differently to agricultural practices in the country (Chenu et al., 2019; Guo and Gifford, 2002). It has already been demonstrated that climatic regimes under cooler and rainier conditions can store as much SOC as the original native vegetation and may sometimes even surpass that amount. In contrast, semiarid regions are more vulnerable to SOC loss (Ramírez et al., 2019). This paper seeks to answer the following research questions: Can soil carbon stock losses be partly avoided by identifying the threshold amount of C or plant input that is put into the soil under annual cropping systems in semiarid areas?

The availability of a reliable model to predict changes in SOC stocks will help us to understand how a system works and provide an inexpensive and expedient alternative to long-term field studies, although clearly only providing a simulation of reality. One of the most widely used models for predicting SOC stock is the Rothamsted Carbon model (RothC, Coleman and Jenkinson, 1999), it requires a few easily obtained inputs and produces reliable simulations (Farina et al., 2013). RothC has been proven effective in a broad range of climates and in diverse grassland, arable and forest ecosystems (Cerri et al., 2007; Coleman and Jenkinson, 1999; Francaviglia et al., 2012; Kaonga and Coleman, 2008; Nieto et al., 2010; Skjemstad et al., 2006). Thus, the purpose of this study was to evaluate the effects on soil organic carbon storage during the cultivation of annual crops in a semiarid and humid environment using the RothC model with historical data collected in 1980 as well as in 2014-2016.

5.2 Material and methods

5.2.1 Study area

The study was carried out in the central zone (Metropolitana, O'Higgins and Maule regions) and in a southern zone (Araucanía region) of Chile. The central zone is characterized by a semiarid climate with an average annual evapotranspiration (ET) of 865 mm, a mean annual temperature (MAT) of 15°C and a mean annual precipitation (MAP) between 300-500 mm. In the southern zone, the climate is typically rainy, cool and temperate, with an ET of 650 mm, a MAT of 12 °C y MAP of 1200 mm (Bonilla and Johnson, 2012; Lobo et al., 2015).

In Chile, conventional tillage has been the common practice, comprising the burning of stubble and additional disk-harrowing operations for seedbed preparation and weed control (Alvear et al., 2005; Varnero Moreno et al., 2015). This is followed by an intensive and irrigated agriculture in semiarid and subhumid areas (Ovalle et al., 1999). These areas are dominated by monocultures of maize, since many producers, especially those that have larger surfaces, have achieved a great specialization in maize cultivation. Maize in rotation with wheat is very common because it is a winter crop that allows for the immediate use of the soil following the harvest (Concha et al., 2004; Rouanet et al., 2005). The dominant soil orders are Mollisols, Inceptisols followed by Alfisols and Entisols. Although in southern Chile most of the area is under traditional management, their climatic features allow for the use of management practices like zero tillage. The soils are commonly used for small-grain crops, permanent pasture, or forest plantations (Stolpe, 2005). Typical rotations are wheat-lupin (*Lupinus albus* L.), and wheat-potato (secano interior). The Andean foothills are dominated by volcanic ash soils (Andisols) characterized by high SOC content, and the presence of evergreen forest ecosystems (Panichini et al., 2017). The western part of the central valley (secano interior sur) is dominated by Inceptisols and Ultisols.



Figure 5-1. Pictures of the sites from which data were used for RothC simulations. The letters correspond to soil Series CIREN names. LGS: Los Lingues; LIU: Liucura; TAL: Talca, SSF: Santa Sofía; SPT: San Patricio; MAL: Malihue; SDG: San Diego; LAP: Las Perdices; DAO: Duao; ARC: Araucano; QTP: Quintrilpe; LAU: Lautaro.

5.2.2 Soil sampling and analytical methods

Five sites for maize and seven sites for wheat were selected (Figure 5-1). These soils were initially sampled in 1968 and later in 1994 for various field surveys conducted by Chilean Natural Resources Information Center (CIREN) (CIREN, 2002, 1997b, 1996b, 1996a), which is the official repository institution of soil surveys and serves as Chile's official source of soil information (Casanova et al., 2013). At that time, the sites were georeferenced and local landmarks surrounding the sites were noted. In the second sampling, a total of 51 sites were relocated with GPS and sampled in the summers of 2014, 2016 and 2017. In both surveys, soils were collected from the first horizon described by CIREN. These values were later harmonized to depths of 20 cm using 'mpspline' from R package GSIF (Bishop et al., 1999; Hengl et al., 2016). The Carbon organic stock was determined by Walkley and Black (1934) and soil bulk density (BD) was measured by the core method (Blake and Hartge, 1986). Pedotransfer functions were used to predict BD when the data was not available (Heuscher et al., 2005). Finally, the water-stable aggregates (WSA) were measured using a wet-sieving apparatus (Eijkelkamp, Giesbeek, Netherlands) and calculated as the proportional mass of stable aggregates in the 1–2 mm range relative to the whole soil, as presented by Kemper and Rosenau (1986). The SOC content (g C kg^{-1}) in the topsoil horizon were converted into SOC stock (t ha^{-1}) as follows Eq. (5-1),

$$SOC_{stock} = BD \times SOC \times D \times 0.1 \quad (5-1)$$

where, BD represents the bulk density (g cm^{-3}), SOC is the SOC content (g C kg^{-1} soil) and D indicates the thickness of the top horizon (cm).

5.2.3 The Rothamsted Carbon model

RothC includes five pools of SOC: decomposable plant material (DPM), resistant plant material (RPM), microbial biomass (BIO), humified OM (HUM) and inert OM (IOM) (Coleman and Jenkinson, 1996). These pools decompose at different rates; the most rapid decomposition occurs in DPM, followed by BIO, RPM, and finally HUM decomposes the slowest; material in the IOM pool is assumed to be inert and does not decompose. The DPM/RPM ratio of incoming plant material depends upon the vegetation type (1.44 for crops). Key drivers of SOC turnover are plant input and the SOC decomposition rate which, in RothC, is modulated by clay content and climate. The decomposition of each compartment of the model, except IOM, follows a first-order exponential decay based on Eq. (5-2),

$$y = y_0 - (1 - e^{-abckt}) \quad (5-2)$$

where y is the amount of C that decomposes in one month, y_0 is the initial amount of C in a particular pool (t C ha^{-1}), a , b and c are the rate modifying factors for temperature, soil moisture, plant retainment factor (if the soil is vegetated or not), k is the rate constant for the given pool (yr^{-1}) and t is 1/12 to convert k to a monthly time step (Coleman and Jenkinson, 1996).

5.2.4 Input data

The main input data for running the RothC model include monthly climate data (ET, T and P), clay content (%), carbon stock (t C ha^{-1}) and plant residues (t C ha^{-1}). The sites were categorized according to the aridity index (AI) (UNESCO, 2010), calculated by the ratio of average annual precipitation (MAP) to potential evapotranspiration (ET) (UNEP, 1997, 1992). Lower AI values in extremely arid regions imply that all precipitation is essentially converted into evapotranspiration. In order to facilitate the interpretation of our results we use 1-minus Aridity index as surrogate of aridity. Climate information was obtained from different meteorological stations managed by two Chilean institutions: Dirección General de Aguas (DGA) and Instituto de Investigaciones Agropecuarias (INIA). ET data, based on values of Penman–Monteith equation, was only available for the last decade. For estimating missing ET information, Hargreaves and Samani (1985) equation was used, which requires average minimum and maximum air temperature and extraterrestrial radiation. The coefficient of this equation was calibrated by using measured ET by Penman–Monteith.

5.2.5 Annual C inputs estimates

The plant residue input is the amount of carbon that is put into soil (t C ha^{-1}), including carbon released from roots during crop growth. The annual C input was estimated using a series of plant C allocation coefficients for each crop for some common agricultural crops in Canada (Bolinder et al., 2007). The method is specific for particular crop and management practices and is driven by agronomic yields (Figure 5-2). The yields were collected base on annual data reported from Oficina de Estudio y Políticas Agrarias (ODEPA). The C input from plant derived materials corresponds to the sum of C_R , C_S , C_W , C_E and C_P , which were estimated using Eq. (5-3), Eq. (5-4), Eq (5-5) and Eq. (5-6) as follows,

$$C_P = Y_p \times 0.45 \quad (5-3)$$

$$C_S = Y_p (1 - HI)/HI \times 0.45 \quad (5-4)$$

$$C_R = Y_p (S:R \times HI) \times 0.45 \quad (5-5)$$

$$C_E = C_R \times Y_E \quad (5-6)$$

where, C_R is the sum of C inputs from roots, C_S is the stubble and chaff, C_w is the weeds, C_E is the exudates, C_P is the plant portion with a primary economic value, harvested and exported from the ecosystem. Y_p is the dry matter yield of above-ground product ($\text{g m}^{-2} \text{yr}^{-1}$), HI is the harvest index expressed as dry matter yield of grain/total above-ground dry matter yield, S:R is the shoot:root ratio, and Y_E is the extra-root C (rhizodeposit C) expressed as factor relative to recoverable roots.

The allocation C within different crop plant parts can also be expressed using relative C allocation coefficients (R_P , R_S , R_P , R_E), which can be readily used to calculate the corresponding value of C_P , C_S , C_R , and C_E , if C_P is known (Table 5-1). Thus, the estimated were made substituting C_P from Table 5-1 and then using Eq. (5-7), Eq. (5-8) and Eq. (5-9) as follows,

$$C_S = (R_S/R_P) \times C_P \quad (5-7)$$

$$C_R = (R_R/R_P) \times C_P \quad (5-8)$$

$$C_E = (R_E/R_P) \times C_P \quad (5-9)$$

Table 5-1 . Relative annual plant C allocation coefficients for small-grain cereals, grain–corn and soybeans used to estimate NPP and C input to soil for Canadian agroecosystems.

Crop	R_P	R_S	R_R	R_E
Wheat	0.335	0.482	0.110	0.073
Barley	0.322	0.482	0.118	0.078
Oats	0.319	0.283	0.241	0.157
Grain-maize	0.386	0.387	0.138	0.089

The effects of tillage practices on annual C input were determined using default parameters (Bolinder et al., 2007), where $S_p = 0$, $S_S = 1$, $S_R = 1$, and $S_E = 1$, where S_p , S_S , S_R , and S_E are the proportions of C in product, above-ground residue, roots, and extra-root C, respectively, that are returned to soil. If a portion of a fraction is removed (e.g. wheat straw removed for feed or bedding), $S_S < 1$. Thus, for small-grain cereals $S_S = 0.15$, and for grain corn and soybeans $S_S = 0.1$. Then, the annual C input C_i to soil is determined using the following Eq. (5-10),

$$C_i = (C_P + S_P) + (C_S + S_S) + (C_R + S_R) + (C_E + S_E) \quad (5-10)$$

5.2.6 Model simulations and scenarios

Each model was first run in inverse mode to equilibrium to generate the C input required to match the initial stock of SOC. In order to incorporate the effects of different land use from 1980 to 2016, a total of 36 specific management files were created per site with the annual C input for each year of study. Additionally, a total of 36 climates files with monthly climate data were created per site for the same period of time. For simulations, sites cropped with maize and wheat were selected. Six different cropping rotations were compared in the study area (Table 5-2). The target rotation for each site was identified by farmer interviews and farming reports (Concha et al., 2004; Novoa et al., 1991; Rouanet et

al., 2005). Additionally, field management information was complemented with high-resolution satellite images typically available since the year 2004.

5.2.7 Roth C output modification

Two independent equations were developed for improving predictions on semiarid and subhumid areas (eq. 9) and for humid and hyper humid (eq.10). Microsoft Excel-Solver tool was used to estimate the coefficients of each equation by minimizing the sum of the square of the difference between measures values of SOC and SOC output of RothC. The WSA values used for deriving the eq. 9 and eq.10 were estimated by using the Artificial Neural Network (ANN) technique. The ANN analysis was develop using multilayer perceptrons with a backpropagation algorithm, which was implemented using logistic function in neuralnet R Package (Günther and Fritsch, 2010; Team, 2014). In the developed ANN model, the mean annual temperature and annual precipitation were used as predictors and the number of neurons in the hidden layer was two. The modified SOC outputs are calculated as follows Eq. (5-11) and Eq (5-12),

$$SOC = -0.0263 \times WSA + 0.985 * SOC_{RothC} \quad (5-11)$$

$$SOC = -0.002 \times WSA + 1.141 * SOC_{RothC} \quad (5-12)$$

where, SOC represents the modified output ($t\ C\ ha^{-1}$), WSA is the water stability aggregate (% WAS in 1–2 mm), and SOC_{RothC} is the authentic model output ($t\ C\ ha^{-1}$).

Table 5-2. Input data used for Roth C simulations (1980-2016). Maize-Bean, (MB); Wheat-Maize (WM); Native Vegetation-Maize (NVM); Continuous Maize (CM); Wheat Lupine (WL); Wheat Lupine-Pasture (WLP). Conventional Tillage (CT) and No-tillage (NT).

Aridity regime	Series	MAT (°C)	MAP (mm)	ET (mm)	SOC (Mg C ha⁻¹)	Clay (%)	WSA (%>0.25 mm)	Soil order	Crop^{a,b} System	Management practices
Semiarid	SDG	14.8	384	1037	73.1	35	0.79	Molisol	MB	CT (1980-2016)
	LGS	14.9	498	1096	62.9	24	0.84	Alfisol	WM	CT (1980-2016)
	LAP	14.8	384	1037	21.4	8	0.43	Inceptisol	NVM	Abandoned / CT (2013-2016)
Dry Subhumid	TAL	14.4	627	982	38.5	44	0.66	Alfisol	WM	CT (1980-2016)
	LIU	14.4	627	982	61.1	26	0.75	Inceptisol	CM	CT (1980-2016)
	DAO	14.4	627	982	70.0	28	0.78	Inceptisol	CM	CT (1980-2016)
Humid	SSF	12.3	1002	849	59.1	41	0.92	Alfisol	WL	CT (1980-2016)
	ARC	12.1	1141	783	59.4	49	0.92	Ultisol	WL	CT/NT (1995-2016)
	LTA	12.1	1141	652	65.3	27	0.88	Inceptisol	WLP	CT (1980-1995) / NT (1995-2009) / Pasture (2009-2016)
Hyper humid	SPT	11.2	1148	652	149.1	34	0.89	Andisol	WL	CT / NT (1995-2016)
	QTP	11.2	1642	652	73.1	39	0.81	Andisol	WL	CT / NT (1995-2016)
	MAL	12.2	2094	700	137.4	32	0.86	Andisol	WL	CT / NT (1995-2016)

^a Plant inputs calculated by Bolinder et al. (2007) approach for particular crops driven by annual yields provided by ODEPA (Ministry of Agriculture, Government of Chile).

^b Average plant inputs (Mg C ha⁻¹) of a) MB: 1.4 (1980-1990), 1.7 (1991-2000), 2.3 (2001-onwards); b) WM: 1.2 (1980-1990), 1.6 (1991-2000), 2.3 (2001-onwards) c) NVM: 0.2 (1980-2012); 3.9 (2013-onwards); d) CM: 2.1 (1980-1990); 2.6 (1991-2000); 3.7 (2001-onward); e) WL: 0.9 (1980-1995), 2.9 (1996-onward); f) WLP: 0.9 (1980-1995); 2.9 (1996-2013); 1.5 (only pasture 2013-onward).

5.3 Results and discussion

5.3.1 RothC model validation

The analysis of data revealed that within the last 36 years, the intensification of crop production has increased constantly between 1979 and 2015 (Figure 5-2). Overall, our field data observations showed that SOC stock have decreased in semiarid zones and may have increased in more humid zones (Figure 5-3).

The RothC model is a suitable tool to analyze and predict the effects of annual croplands of Chile on SOC stocks from 1980 to 2016, even when using indirect determination of the plant input using crop yields, harvest index and shoot to root ratio data. The model is able to simulate the declining trends observed for C stocks from semiarid to humid areas (Figure 5-3). The simulated SOC content and storage had a close relationship with the measured data for semiarid, subhumid and humid areas. The results of the validation by the comparison of the model outputs with a set of measured data to all sites in the four climatic areas are presented in (Table 5-3). The simulations by RothC during the period 1980 and 2014, 2016 shows a deviation between simulated and measured from -6.2% to 26.1% for semiarid and subhumid areas. The greater overestimates were in semiarid LGS Series by about 4 t C ha⁻¹ yr⁻¹, and for subhumid LIU and DAO Series with 11 t C ha⁻¹ yr⁻¹, and 10 t C ha⁻¹ yr⁻¹, respectively. Limitations of RothC in semiarid regions have previously been reported (Farina et al., 2013; Lobe et al., 2005). For instance, an unrealistically small input of C to predict how much C must enter the soil each year to maintain the measured or estimated stock of SOC. However, our data approximated the annual C input estimated from crop yields. The C inputs to soils were 0.46 and 0.41 t ha⁻¹ year⁻¹ for paddy and upland soils, respectively (Tables 2-3). On average, depending on the crop system and the management practices, the annual C input were from 1.4 to 3.7 (Table 5-2).

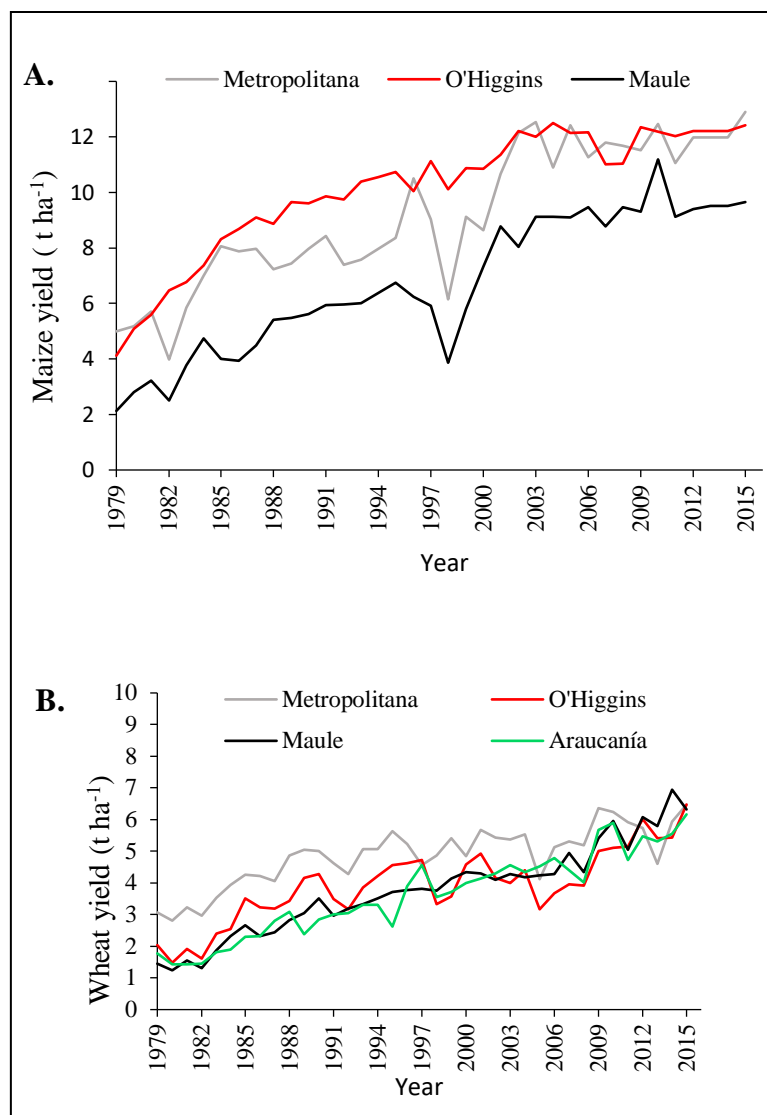


Figure 5-2. Annual grain yield from 1979 to 2015 in different regions of Chile: Metropolitana (arid/semiarid), O'Higgins (semiarid), Maule (subhumid), Araucanía (humid/hyper humid). A) Maize yield, and B) Wheat yield.

As conditions become cooler and wetter, the model markedly underestimated carbon stocks (Figure 5-3). In hyper humid climates, with highest initial C stock (over 100 t ha⁻¹), the deviation data between simulated and measured data ranged from -13.8% to -31.9% (Table 5-3). Most of these soils have been under no-tillage practices at least for the last two decades. Reduced tillage practices and the use of organic waste to cover the ground is considered to be an efficient way of increasing carbon sequestration in agricultural soils

(Lal, 2004b). It has already been shown that climatic regimes under cooler and rainy conditions can store as much SOC as the original native vegetation, and sometimes may even surpass it (Guo and Gifford, 2002; Ramírez et al., 2019). The poor performance of RothC under hyper humid regions, in this study represented by Andisols, might be explained by the first-order exponential decay function used by this model, which did not represent the real dynamics that are occurring. As mention in previous studies (Shirato et al., 2005, 2004; Takata et al., 2011), the original model could not explain the resistant character of humus decomposition in Andisols. Other factors such as amorphous inorganic materials, and pyrophosphate-soluble metals may highly control the size and turnover rate of soil C and contribute significantly to aggregation and organic matter stabilization (Asano and Wagai, 2014; Matus et al., 2014). While in semiarid areas, phyllosilicate clay content seem to be more relevant in SOC storage (Ramírez et al., 2019), which may explain the better adjustment obtained between simulated and measured data.

Table 5-3. Percentage deviation across all sites and aridity regimes.

Aridity regime	Series	RothC deviation^a (%)	RothC WSA fit deviation^b (%)
Semiarid	SDG	7	3.2
	LGS	14.1	9.6
	LAP	-6.2	-13.1
Dry Subhumid	TAL	6.1	-0.3
	LIU	31.7	25.6
	DAO	26.1	19.8
Humid	SSF	0.5	8.8
	ARC	-13.8	-1.3
	LTA	-26.3	-15.7
Hyper humid	SPT	-31.9	-22.2
	QTP	-20.1	-8.5
	MAL	-24.6	-13.8

^{a b} Deviation calculated as $[100 * (\text{modeled} - \text{measured} / \text{measured})]$.

Current analytical approaches suggest that SOC turnover is governed by physical accessibility rather than chemical recalcitrance (SOM, 2017). The WSA content for the different soil types ranged from 0.43% to 0.92%. Although we found that the output modification with WSA parameters for all sites improved the SOC predictions, the hyper humid zones always declined their carbon contents over the time (Figure 5-3). RothC in all hyper humid sites underestimated the SOC stocks by about four times. The total amount of residue produced by wheat-lupine rotation was estimated to be on the order of 3 t C ha^{-1} , these amounts were clearly much lower than the required C inputs $> 6 \text{ t ha}^{-1}$ to maintain the SOC level of Andisols soils even if all residues are incorporated into the soils.

5.3.2 SOC changes in response to plant input and climate

The simulation shows that wheat cropping has differentially impacted soil carbon stocks from drier to more humid conditions (Figure 5-4). The results evidence that SOC accumulation is constrained in more arid environments. The carbon losses increased when the aridity conditions were higher, reaching around 20%. In contrast, when the aridity conditions started to decrease, SOC storage increased up to 10%. Under the same amount of plant residues input, the responses of soils located in the semiarid and humid areas were different. The semiarid croplands were not able to keep their SOC stock, decreasing their levels drastically (Figure 5-4). Over the last 35 years, crop yields in Chile have increased because of the adoption of modern cultivation techniques (Figure 5-2). Although it may have contributed to the increase of residue and root input into the soil, it seems that similar plant input in semiarid areas has no positive effect on SOC storage when compared to more humid areas. The marked trends of SOC depletion exhibited by drier areas might be explained by distinct mechanisms associated with C storage, such as tillage, climate, and higher amounts of plant residue inputs might either accelerate decomposition, resulting in a lower carbon storage through decreased metabolic efficiency, and priming effects (Lange et al., 2015; Manzoni et al., 2012).

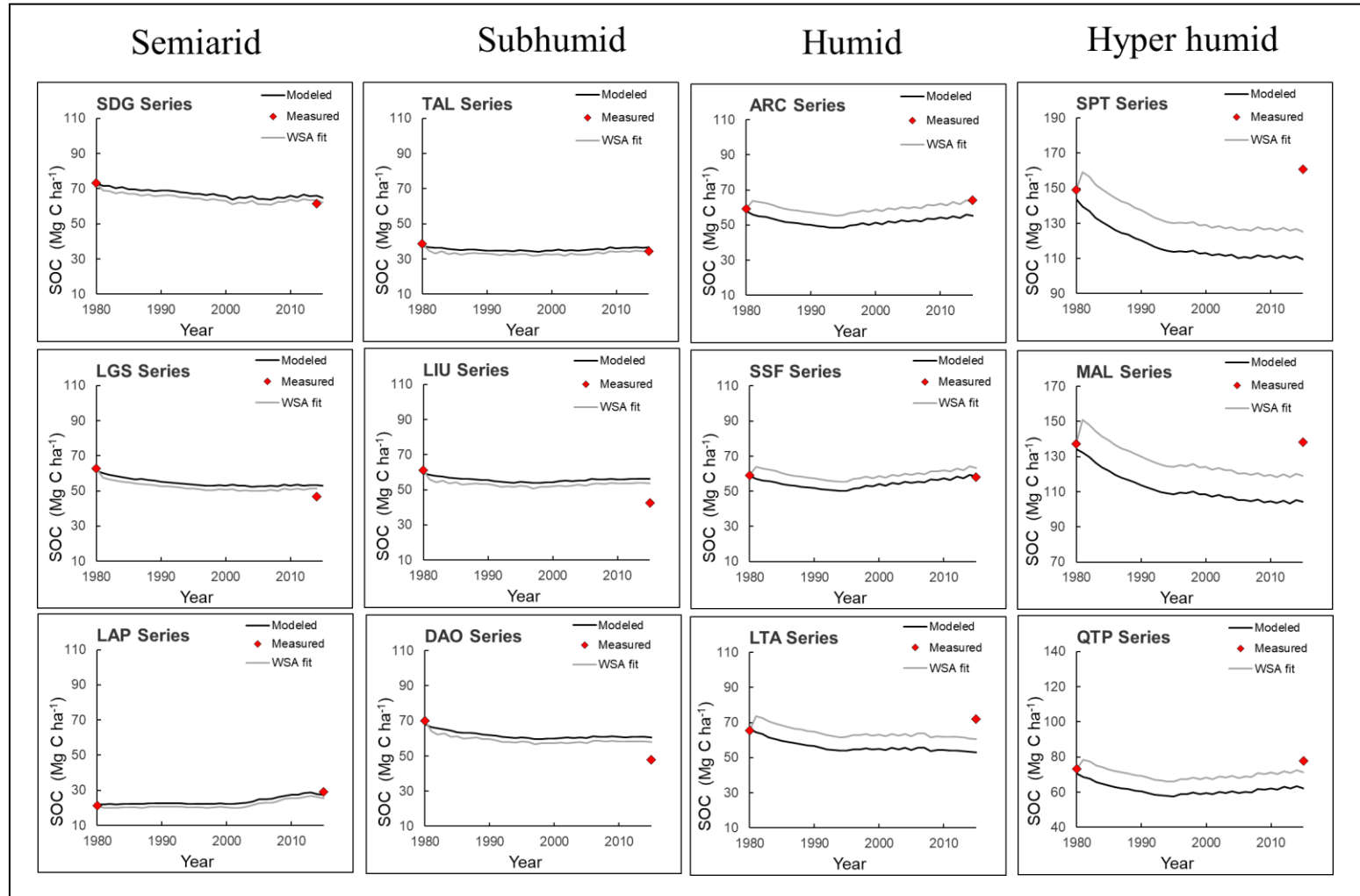


Figure 5-3. Measured and simulated organic carbon values in semiarid, subhumid, humid and hyper humid areas

Previous studies (Dimassi et al., 2014; Hermle et al., 2008) have reported that the response of SOC to tillage is dependent on climate and in particular precipitation, with a greater response in drier conditions. Consistently, the small variation observed in the 3D plot across more humid areas (Figure 5-4) indicate that tillage practices have no effects on SOC accumulation, and residue input do not appear to be an important factor on SOC accrual rates under this climate condition. Semiarid soils had the highest relative C loss and these areas are commonly under intensive crop production. In addition, in Chilean semiarid areas, the stubble cannot always be incorporated into the soil since moisture is required for this to be done. Therefore, the use of fire in stubble removal is an ancestral practice deeply rooted in Chilean agriculture (Varnero Moreno et al., 2015). Such observations suggest that an increase in plant input could not be a feasible alternative for C accumulation in arid and semiarid areas. However, the use of a reduced tillage system and the increases of belowground C inputs through the conversion to perennial vegetation could have potential restoration benefits on degraded land.

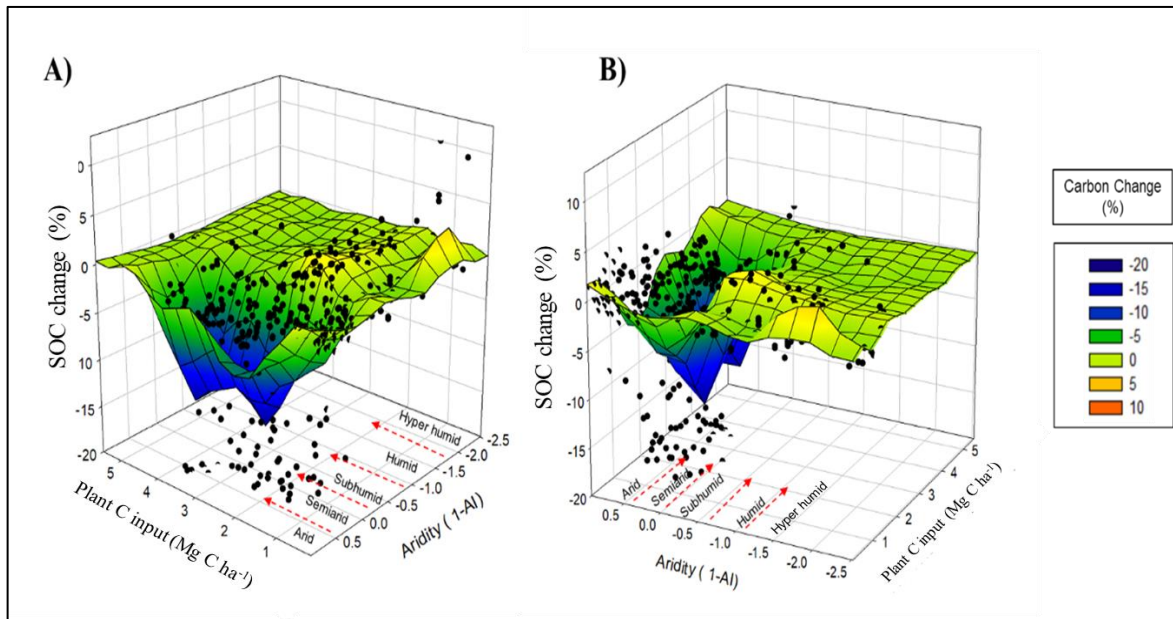


Figure 5-4. 3D plot displaying annual SOC changes (%) to climate and plant residue from annual cropping systems. A) Frontal view from semiarid areas, and B) frontal view from humid areas.

5.4 Conclusions

The RothC model was a suitable tool to analyze and predict the effects of climate and agricultural management changes on soil organic stock under certain climatic conditions, such as semiarid, subhumid and humid climates. However, in hyper humid areas represented by Andisols in this study, the model did not perform well. The comparison of observed and simulated data confirmed that trends can be effectively explained by the RothC model, where the carbon losses increased when the aridity conditions were higher in the study area. Our findings show that the amount of plant input seems to not be limiting the SOC storage under semiarid areas. According to the simulation results, the annual return of C required is too large and unrealistic compared to the crop yield in these regions to reach SOC gains. Thus, other agricultural management options such as less intensive tillage and the conversion to perennial vegetation might be potential practices for increasing C stock. In our simulations, there are sources of uncertainty such as indirect determination of the plant input. Despite this limitation, our approach to improve RothC output with soil aggregation data across different climates can be used for predicting future or explaining historical C stock trends as well as comparing scenarios of agriculture development in Chile.

6 CONCLUSIONS

Understanding and predicting SOC changes is critical in order to maintain the health of soils, to enhance agricultural productivity and to mitigate greenhouse gases in a context of ongoing climate change. This thesis examines the potential effect of climate, soil types and land use on SOC storage, which have been previously reported in several independent studies. Our findings indicate that SOC content have changed over the last three or four decades in agricultural lands in Chile. Mollisols in semiarid regions have been more impacted by SOC losses than those in the other regions surveyed. Furthermore, the results suggest distinct mechanisms of SOC protection in both warm and cold regions. This study shows that the labile fraction POXC/SOC ratio is relatively high in semiarid sites, showing that it can be a useful metric to identify ecosystems that have been historically vulnerable to C losses. Additionally, we provide empirical evidence, demonstrating that labile SOC fraction plays an important role as a driver of soil bacteria-archaea community. This leads us to consider the role of soil microorganisms as important for understanding and predicting land degradation processes associated with SOM decomposition. Overall, the results emphasize the importance of the intrinsic properties of each ecosystem for controlling the magnitude of these temporal SOC changes. Thus, a clear understanding of these factors is essential in order to achieve an accurate simulation model for SOC prediction. This was tested with the simulations obtained by the RothC model, where this model clearly performed better in some climates and types of soils. Chile has a large gradient of climate and highly organic soils and thus further efforts aimed at studying SOC should research each area separately paying close attention to hotspot areas, such as mollisols, permafrost and drylands.

7 FUTURE PERSPECTIVES

Given the current knowledge of SOC dynamics in soil, the following research areas could be pursued. In a first instance, efforts should focus on developing a map of SOC vulnerability that allows restoration practices to be addressed. Secondly, the implementation of inexpensive and measurement protocols target on soil GHG emissions and soil degradation are necessary. Our study has shown that spectroscopic methods may be successfully applied to the study of labile fractions, therefore, further research should examine how SOC fractions are distributed regionally in Chile. In addition, considering that semiarid areas are more vulnerable to SOC losses, an emerging issue is the study of mineralization of soil C in response to amendments or high C input species, often referred to as the priming effect, which could have negative impacts on SOC over time. In this thesis, potential relationships were explored between the most important SOC drivers. Thus, another challenge is to find a mathematical expression using machine learning methods, which should include microbial attributes and the most important soil physicochemical drivers identified for predicting SOC stocks.

Although research has focused on studying SOC in Chile, little effort has focused on soil freeze and SOC-rich soils in the context of climate change. Therefore, considering that in Chile a great part of the surface is covered by these types of soils, performance studies and models should address these areas in the near future. In this work, specifically, microbial diversity indexes were good candidates to help explain C lability. However, carbon decomposition also seemed to be explained predominantly by microbial composition. Knowledge regarding microbial composition and its functional traits remains limited, and further studies are required to understand the relationship between microbial composition and SOC. Therefore, microbial microcosm experiments may be conducted to study the differential response of microbial communities, eliminating other environmental variables. Considering the social, environmental and economical issues, the feasibility of SOC storing management through increasing crop residues practices may be limited. Therefore, a process-oriented approach shows the need to explore a wide range of alternative agricultural strategies. We suggest testing the cultivation of plant varieties or species with

greater root mass to deposit C in semiarid areas as an alternative to SOC restoration. In addition, there has been a general concern about the importance of soil biological indicators and their relevance soil monitoring programs of organic matter degradation. Our findings call for further research in these areas since their specificity to changes in soil management practices and land cover. Besides microbial indicators, the study of dissolved organic matter (DOM) plays an important role in the biogeochemistry of carbon, nitrogen, and phosphorus, and in the transport of contaminants in soils, a key challenge in understanding the role of DOM on the nature and extent of C sequestration in soils as in agricultural farms, forest lands, and wetlands (i.e., implication on global climate change).

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9 SUPPLEMENTARY MATERIAL

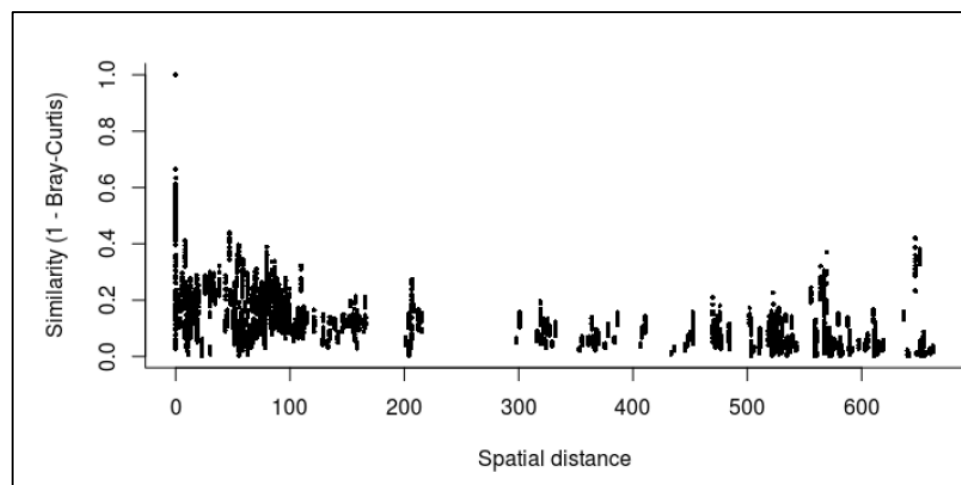


Figure 3-S1. Geographic distances and environment dissimilarity

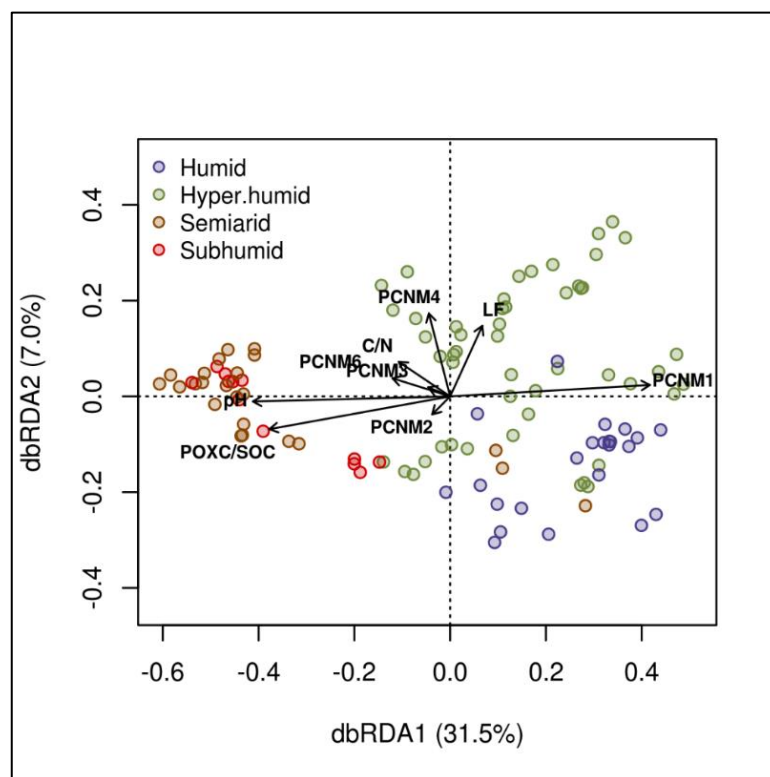


Figure 3-S2. Distance-based redundancy analysis (db-RDA) based on weighted UniFrac distances for bacterial community structure (ie, phylogenetic β -diversity), soil physicochemical properties and spatial variables. Variables and physicochemical vector correlation plots showing strengths and directions of relationships between physicochemical variables. Axis legends represent the percentages of variation explained by the axis.