SJSS SPANISH JOURNAL OF SOIL SCIENCE • YEAR 2020 • VOLUME 10 • ISSUE 2

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Letter from the Editor-In-Chief

I welcome you to the second issue of the tenth volume of the Spanish Journal of Soil Science. It contains four research articles; three of them on soil organic carbon in three geographically and pedo-diverse environments: fractionation and stability of soil organic matter in the Russian arctic zone; seasonal variability of CO_2-flows in anthropogenic soils of *Chinampas* in Mexico; and agronomic variables that control organic carbon stocks in Mollisols from the Argentine pampa. The fourth article is an interesting study on the influence of the age of forest plantations on the availability of aluminum and other soil chemical properties in Galicia. The issue is completed by a review article on soil rehabilitation methodologies in areas affected by mining activities in the Murcia region.

With this issue a series of changes has also been started. We have moved the SJSS portal to a new server of the Spanish Society of Soil Science and therefore the URL has changed, although the old one will continue to be active and redirected to the new one. The current address is: www.sjss.es. Also, from this year 2020, the logo of the Biodiversity Foundation will be incorporated into the Journal. The Biodiversity Foundation collaborates with SECS through an agreement in which both institutions seek synergies to increase the dissemination and visibility of the activities of both institutions.

As in the preceding issues, on behalf of the editorial team, we thank the authors for sending the papers and for their indulgence, despite the availability problems of the website at this transition stage, to the referees for the quality of the reviews, and finally to the team of Universia and of the Spanish Society of Soil Science for their professionalism in editing and managing the platform.

Sincerely,

EDITOR-IN-CHIEF
Dr. Rosa M. Poch

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COVER PHOTO: Atrapado entre la lava. Winner of the Photography Competition of the SECS 2020. Author: José Álvarez Rogel.

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Methods of soil recovery in quarries of arid and semiarid areas using different waste types

Métodos de recuperación del medio edáfico en canteras de zonas áridas y semiáridas mediante el uso de residuos
Métodos de recuperação do solo em pedreiras de zonas áridas e semi-áridas usando diferentes tipos de resíduos

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ABSTRACT

In the Region of Murcia, there are many abandoned quarries in which restoration processes have not been carried out, and there are others that have a restoration plan but soil rehabilitation has not been achieved. Open pit mining generates a great environmental impact in the area where the activity takes place since it alters the morphology of the earth's crust, pollutes the air, the surface and underground waters, eliminates the flora of the area and destroys the biotope, causing changes in the landscape and strong changes in ecosystems. There is an international concern to promote sustainable development and waste reuse. In the European Union and Spain there is a requirement to carry out a restoration plan for mining operations. Waste production is a big problem; ways of reusing waste without polluting the environment and reintroducing it into economic activity are sought. In this article, several techniques are compiled that have given satisfactory results in the restoration of mining spaces, mainly in the Region of Murcia (SE Spain), by using waste such as pig manure, marble debris, sewage sludge or compost of urban household waste. These wastes pose a problem due to their disposal if they are not reused, and their use to restore mining spaces is a good option against dumping, abandonment or incineration.

RESUMEN

En la Región de Murcia se encuentran abundantes explotaciones mineras abandonadas en las que no se ha llevado a cabo un proceso de restauración, y existen otras que disponen de un plan de restauración que no ha conseguido la adecuada rehabilitación del suelo. La minería a cielo abierto genera un gran impacto ambiental en la zona en que se desarrolla la actividad ya que altera la morfología de la corteza terrestre, contamina el aire y las aguas superficiales y subterráneas, elimina la flora de la zona y destruye el biotopo, causando modificaciones en el paisaje y fuertes cambios en los ecosistemas. Existe una preocupación internacional para promover el desarrollo sostenible, la reutilización de residuos y la exigencia en la Unión Europea y España para que se lleve a cabo un plan de restauración de las explotaciones mineras. La producción de residuos constituye un gran problema, se buscan maneras de reutilizarlos en las que no contaminen al medio ambiente y reintroducirlos en la actividad económica. En este artículo se recopila información sobre técnicas que han dado resultados satisfactorios en la restauración de espacios mineros, principalmente de la Región de Murcia (SE España), utilizando residuos como el purín de cerdo, restos de mármol, lodos de depuradoras o compost de residuos domésticos urbanos. Estos residuos suponen un problema por su eliminación si no se reutilizan y su uso para restaurar espacios mineros es una buena opción frente a los vertederos, abandonos o incineración de los mismos.

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RESUMO

Na Região de Múrcia, existem muitas explorações mineiras e pedreiras abandonadas, nas quais não foi realizado qualquer processo de restauração, e outras em que, embora possuindo um plano de restauração, a reabilitação do solo não foi alcançada. A exploração mineira a céu aberto gera um grande impacto ambiental na área onde se desenvolve a atividade, pois altera a morfologia da crosta terrestre, polui o ar e as águas superficiais e subterrâneas, elimina a flora da área e destrói o biótipo, causando alterações na paisagem e fortes mudanças nos ecossistemas. Existe uma preocupação internacional em promover o desenvolvimento sustentável e a reutilização de resíduos. Na União Europeia, incluindo em Espanha, há uma exigência para que se realizem planos de restauração das áreas onde ocorrem operações mineiras. A produção de resíduos é um grande problema; atualmente, procuram-se formas de reutilizar os resíduos sem poluir o ambiente e reintroduzi-los na atividade económica. Neste artigo, são compiladas técnicas que obtiveram resultados satisfatórios na restauração de espaços mineiros, principalmente na região de Múrcia (SE Espanha), utilizando resíduos como chorume de suínos, resíduos de mármore, lamas de ETAR ou composto de resíduos de sólidos urbanos. Estes resíduos representam um problema ambiental se não forem reutilizados. O seu uso para restaurar espaços de áreas mineiras é uma boa opção quando comparado com a sua inclusão em aterros, abandono ou incineração.

KEY WORDS
Circular economy, marble, mining, restoration, slurry.

PALABRAS CLAVE
Economía circular, mármol, minería, purines, restauración.

PALAVRAS-CHAVE
Atividade mineira, chorume, economia circular, mármore, restauração.

1. Introduction

Open-pit mining generates a great environmental impact because it alters the morphology of the earth's crust, pollutes the air, surface and underground water, eliminates the existing flora in the area and destroys the biotope. This activity can also affect the health conditions of the surrounding inhabitants. By causing these modifications, negative visual impacts and strong ecological changes in the affected ecosystems are generated in the landscape (Gunn and Bailey 1993; Sheoran et al. 2010; Sort and Alcañiz 1996).

This article presents the important mining heritage that the Region of Murcia (SE Spain) has. The importance that this activity has had, especially in the Northwest of Murcia in the nineteenth and twentieth centuries, has left some mining spaces that should be the subject of an environmental policy.

The regional government is clearly committed to the mining industry, an economic activity that it plans to protect and promote through regulations that would have a validity of at least 25 years and that favors the exploitation of quarries over any other use of the territory. The analysis of the cartography included in the mining guidelines reveals that 59 population centers and 241 housing groups would be less than two kilometers from quarries. The Autonomous Community hardly poses restrictions on mining activity for environmental reasons: only in the case that industrial activity affects a protected natural area. Important steps have been taken but there is still not enough action to protect and enhance the vestiges left by this activity.

At present, the mining regulations require a restoration plan to be carried out before starting exploitation in order to prevent or reduce possible adverse effects on the environment and health caused by mining activity, and to establish the necessary preventive measures so that these impacts either do not occur, or affect the environment to a lesser extent. In addition, the land must be rehabilitated once the operation has been completed. Directive 2006/21/EC, on the management of waste from extractive industries requires the rehabilitation of areas where mining waste facilities have been located, as does the pre-existing Spanish mining legislation. This Directive is carried out, on a basic basis, by Spanish Royal Decree

975/2009 of June 12 on the management of waste from extractive industries and the protection and rehabilitation of space affected by mining activities. Restoration and rehabilitation are among the most important measures, as it allows the exploited land to be integrated into its ecological and landscape environment, and to develop an alternative use to mining once its exploitation has ended (Escribano and Mataix 2007). With restoration, the aim is to return the original condition of the land, whereas with rehabilitation or recovery, a different use is achieved from that which the land had before being exploited.

In this article, the terms restoration and rehabilitation will be dealt with indistinctly, since the aim is to show that the soil must acquire such conditions that it is capable of serving as a biotope, allowing the development of living organisms, especially vegetation, regardless of the subsequent use to which the recovered space is destined.

Currently, there are abundant mining operations in which a restoration process has not been carried out and they have been abandoned, as in the case of the Region of Murcia. In addition, in other cases restoration plans have not achieved the correct rehabilitation of the soil. Thus, the objective of this review is to compile the most relevant information on the techniques used for the recovery of soils in open-pit mining spaces, which serve as a basis for future rehabilitation.
Restoration in arid or semi-arid areas, as is the case in the Region of Murcia, is much slower than in rainy areas due to the scarcity and torrential nature of the rainfall at certain times and the intense solar radiation in these areas contribute to desertification, which subjects the plants to severe water stress, contributing as a whole to increased soil erosion (Miralles et al. 2009).

2. State of the art 2000-2019

As a preliminary approximation, a bibliometric analysis was carried out based on the Scopus database, which allowed us to determine the interest of the scientific community in the restoration of open-pit mining spaces. In this sense, 446 documents were located for the period 2000-2019 using the “quarry restoration” search. Of these documents, only 338 corresponded to scientific articles.

Focusing our attention on these articles, the country with the highest number of publications on the subject was Spain (Figure 1). In the Spanish contributions, 35 of them were related to Murcia. In addition, in this group of countries with the greatest number of publications, the European countries stand out, indicative of the interest in solving an environmental problem such as restoration after mining activity.

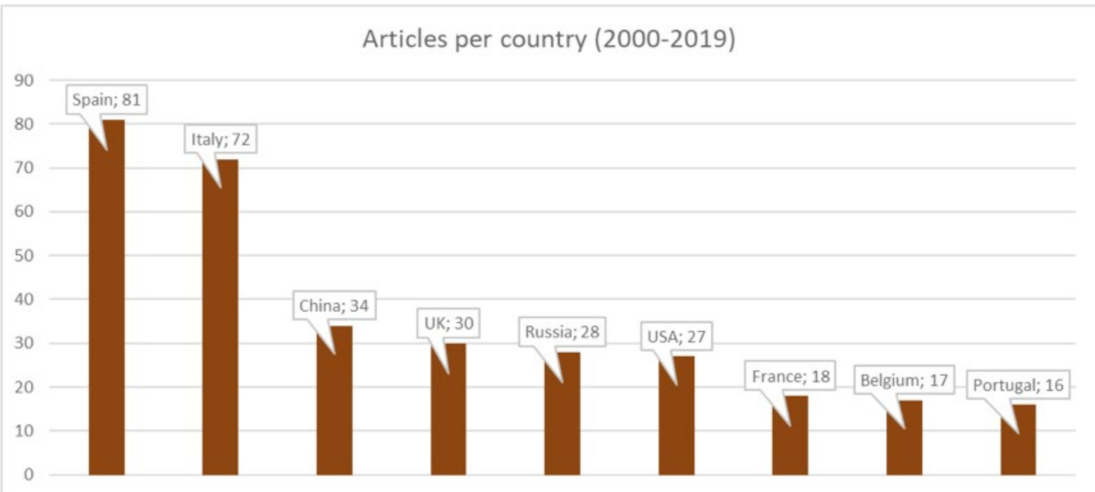

Figure 1. Number of published articles on quarry restoration published by country in the period 2000-2019 (own source).

In terms of the subdisciplines in which Scopus distributes the published articles, the one corresponding to "Environmental Science" stands out above all others, followed by the publications in "Earth and Planetary Sciences", as shown in **Figure 2**.

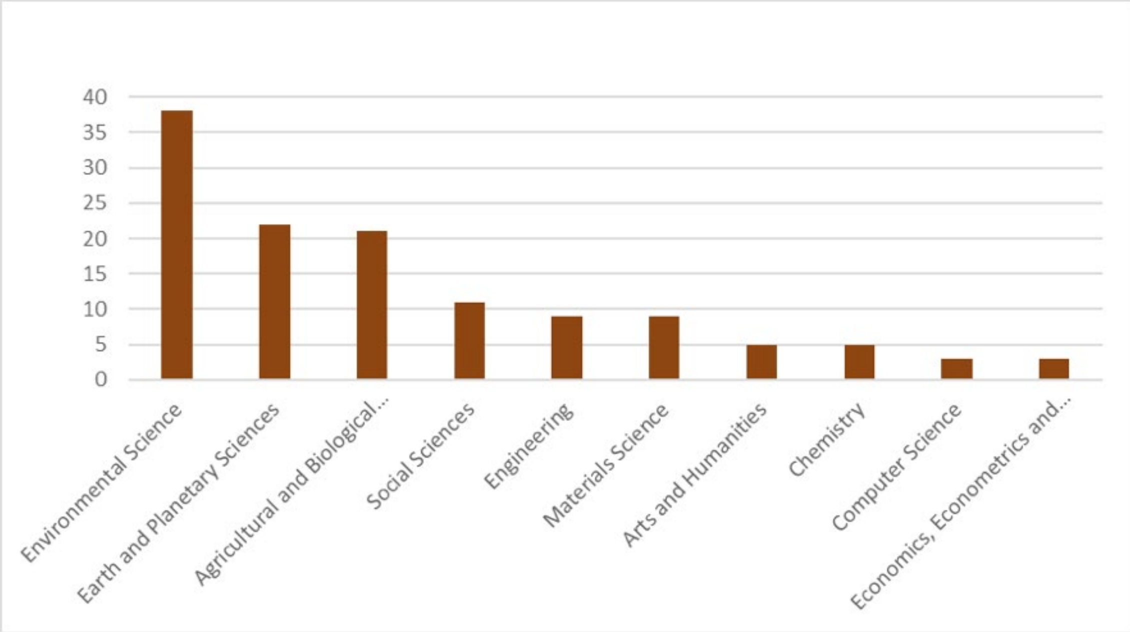

Figure 2. Subdisciplines where the articles published on quarry restoration in the Scopus database in the 2000-2019 period are grouped (own source).

In the studies with Spanish authors on waste used in mine restoration, those where sludge is used stand out, especially those that use sludge from wastewater treatment (**Figure 3**).

To conclude this preliminary analysis, the most frequently cited articles by Spanish authors in which the term "waste" is used are the following: (a) based on "pig slurry, municipal solid waste, marble waste", the publication of Castro-Gomes et al. (2012); (b) if we consider "sewage sludge, quarry solid waste", the most cited article is that of Luna et al. (2016a).

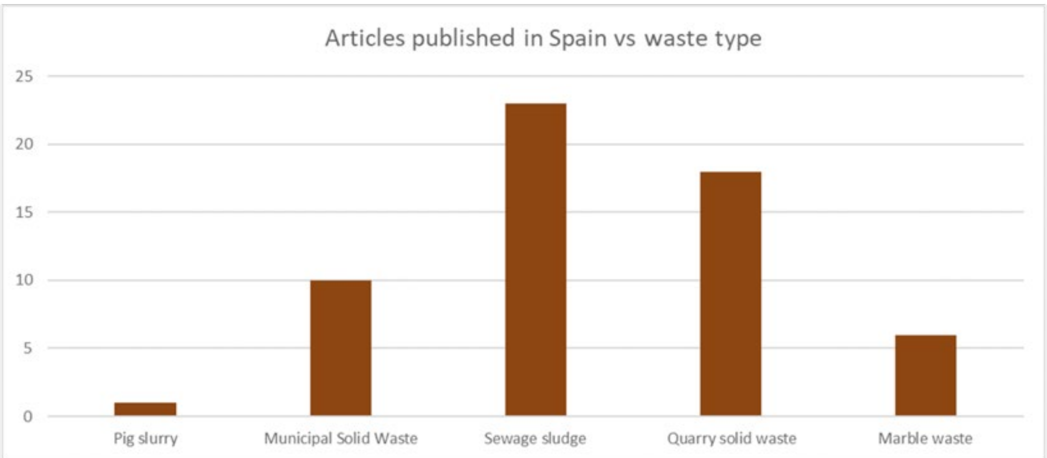

Figure 3. Scopus publications by Spanish authors in the period 2000-2019 according to the type of waste used in quarry restorations (own source).

3. Situation in the Region of Murcia

Nowadays, in the Region of Murcia, there are a large number of abandoned quarries in which no restoration plan has been carried out, as in the rest of Spain. In the case of Murcia, most of them are located in the northwest of the Region. According to the latest data prepared by the Regional Directorate General of Industry, Energy and Mines, in 2006 there were 88 aggregate quarries in the Region of Murcia, of which 54 were active, and 144 ornamental rock quarries of which 83 were still active, giving a total 273 existing exploitations in the Region of Murcia of which 157 were active. The Region of Murcia is one of the areas with the greatest geological potential on a national scale, as it represents about 12.6% of the extraction of marble and limestone. Many areas are affected by metalliferous mining activities. These areas are located in Cartagena and La Unión, so for more than 2,500 years until 1991, Phoenicians, Romans, Arabs, Carthaginians and Spaniards have been extracting zinc, lead, copper, silver, manganese, iron and tin (Conesa and Faz 2009). Thus, all these mining activities have caused a large accumulation of heavy metals and generated acid drainage in the mines (Faz et al. 2008).

A high percentage of the mountains of the Region of Murcia are formed by limestones; predominant in the mountain ranges of Moratalla, Villafuertes, Gavilán, Cerezo, Los Alamos, Mojantes, Quípar, Oro, Ricote, Manzanete, Pila, Corque Place, Quibas, Espuña, Carrascoy, among others. The public administration today is faced with a large number of abandoned areas, with consequent landscape degradation (some of which are used as uncontrolled landfills), and with active exploitations, which should be restored in the future.

The limestones are the rocks that are currently most exploited in the region, their main use being gravels and concretes, or masonry stone and ornamental rocks, which are marketed both inside and outside the region. Some of the best-known varieties of ornamental rocks from quarries located in the region are: Caravaca red, Cehegín red, Cehegín gray, Cehegín medium and Quipar red, which are Jurassic red nodular limestones; and the ivory cream of the Sierra de la Puerta, which is a Paleogene limestone. They are known as marbles, although from the geological point of view, they are sedimentary carbonate rocks.

The Region of Murcia has a Mediterranean climate with hot and dry summers and mild winters, although with frequent inland frosts, and rains in spring and autumn. The general characteristic of this climate is its scarcity of precipitation, concentrated over a few days of the year with a maximum in autumn. These rains, generally torrential, are produced when a mass of warm and humid air from the Mediterranean Sea rises over the coastal mountains, meets another mass of cold air and precipitates. These rains should be considered because of the high erosive power they can trigger when it comes to recovering mining spaces.

According to the Köppen climate classification, several climates are distinguished in the Region: semi-arid warm (BSh), semi-arid cold (BSk), and Mediterranean (Csa).

In the BSh climate the average annual temperature is above 18 °C and rainfall is scarce. Solar evaporation exceeds rainfall; it is a hot and dry climate. In the BSk climate, the average annual temperature is below 18 ºC. Rainfall is also scarce and evaporation like in the BSh also exceeds rainfall; this climate is cold and dry. The CSa has an average temperature of the coldest month less than 18 °C and higher than -3 °C, and that of the warmest month is greater than 10 °C and the temperature of the warmest month exceeds 22 °C. Precipitation exceeds evaporation and there are seasonal rains. Summer is dry, so the minimum rainfall coincides with the period of higher temperatures. The rainiest season is not winter.

4. Keys for the recovery of the environment

The soil is the fundamental pillar for recovery of the open mining areas, and next to it, the vegetation that is a key factor for landscape regeneration. If we add the need to reuse waste based on circular economy to these keys, we can conclude that the three basements in rehabilitation are soils, vegetation and the reuse of waste as the main source of organic matter. **Figure 4** shows the general restoration process of a quarry.

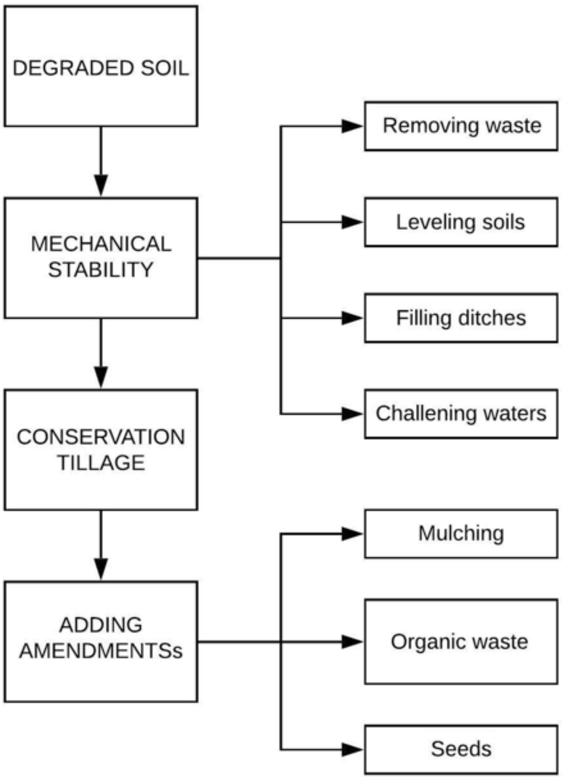

Figure 4. General restoration process of a quarry (own source).

The environmental properties affected by open-pit extractive activities are very diverse, altering both the morphology and the processes that affect the territory, changing them completely. If we focus on the example of the Region of Murcia, extrapolated to other arid and semi-arid Mediterranean areas, open pit limestone mining is particularly harmful due to the type of extraction (Luna et al. 2016b). In addition, these areas are susceptible to desertification and very sensitive to erosion due to the absence or scarcity of vegetation cover. Therefore, in order to restore an area without soils or with highly degraded and often unstructured soils, a surface layer with adequate physical, chemical and biological properties should be prepared. The usual strategy in the restoration is therefore to re-establish the edaphic cover, but in many cases, to build a new edaphic environment - a Technosol (IUSS Working Group WRB 2015).

After mechanical stabilization of the environment, efforts should be directed towards improving the soil by adding organic materials (e.g. through waste) and then revegetation, in such a way that allows the landscape lost after mining to be returned to its previous state.

Before creating this layer of "new soil" or artificial soil created *ad hoc* (Technosol), it is necessary to clean the surface, removing materials or debris (artifacts) from the soil that are not useful for its recovery and leveling it to prevent erosion. It is crucial to adapt the hydrography for proper rehabilitation and so the gullies and trails must be covered and the waters must be channeled to prevent the material supplied from flowing and dragging (Zornoza et al. 2017).

Once mechanical stability is achieved, the soil is prepared using tillage techniques to reduce compaction and bulk density and increase the porosity of the arable layer, which in turn facilitates seed germination and seedling development (Kabas et al. 2012). It is also necessary to recover the fertility of the soil, incorporating or covering the surface with organic waste that in turn remedies the soil, fertilizes and protects it.

The strategy based on the increase of organic matter in the soil is essential to guarantee a real rehabilitation of the soils, since soil organic matter is a property closely related to soil structure (Tisdall and Oades 1982), aeration, water retention and circulation, reduction of erosion rates, stimulation of biological activity and the increase of fertility by nutrient release (Smith et al. 1993; Shafi et al. 2007). Soil organic matter is universally recognized as one of the most important factors responsible for soil fertility and protection against pollution, degradation, erosion and desertification, especially in semi-arid areas (Senesi et al. 2007). This new substrate must be transformed into a fertile soil in the short and medium term, which improves biological activity, activates biogeochemical cycles, and accelerates the regeneration of plant communities (Caravaca et al. 2002; Heneghan et al. 2008; Domene et al. 2010; Soliveres et al. 2012).

To achieve this, the incorporation of organic matter must allow the structure to be neither too loose nor too compact (Jorba et al. 2002), and it must achieve good water infiltration, be resistant to erosion and contain nutrients and microorganisms that allow vegetation to grow (Banning et al. 2011; Muñoz-Rojas et al. 2016).

5. Organic matter and soil properties

The improvement of soil properties by application of organic matter is a strategy linked to the roots of the history of agriculture. It was traditionally based on the application of organic residues and waste from animal origin. However, a wide variety of amendments from many different sources are currently applied. These are also used in the restoration of soils in mining operations.

Waste from activities such as slaughterhouses, forest management, livestock, agriculture, households, wastewater treatment (biosolids) or food industries can be considered as organic products or waste (Navarro-Pedreño et al. 1995). Organic waste must have a biological origin and composition and contain the so-called bioelements, to a greater extent carbon, hydrogen and oxygen (C, H and O) and to a lesser extent nitrogen, phosphorus and sulfur (N, P and S).

5.1. Increase in soil organic matter

The application of organic waste to the soil is a useful tool to maintain and increase the amounts of organic matter (Mondini et al. 2008; Jorba and Vallejo 2008; Kabas et al. 2014). Organic waste can be used as a source of nutrients, improving soil fertility and stimulating the formation of aggregates and the development of microbial populations (Ortiz et al. 2012; Ye et al. 2002; Zanuzzi et al. 2009). Effective recycling of organic waste in soil requires optimization of waste management to minimize CO_2 emissions and optimize the efficiency of soil C retention. Since the main objective after the application of organic amendments is the increase of organic matter, it is crucial to carry out an exhaustive study on the stability and mineralization of organic C (Zornoza et al. 2012). Therefore, amendments can be implemented with nutrients that are not mineralized so quickly that the organic matter disappears before the vegetation cover is properly developed. In addition, the implementation of these amendments could be beneficial to the environment, as it prevents their incineration or uncontrolled dumping, thereby helping to promote the circular economy.

These amendments improve soil C sequestration by replacing labile organic carbon from aggregates with more stable compounds (Ojeda et al. 2015), contributing to plant growth (Hemmat et al. 2010). With the application of organic amendments, in addition to total organic carbon (TOC) the total nitrogen (TN) also increases. These increases are associated with increases in basal respiration and dehydrogenase activity, which are accepted as indicators of total soil microbial activity (García et al. 1997; Bastida et al. 2006). The effect of organic amendments on soil TOC depends on the chemical composition of amendments (Tejada et al. 2009), which determines the rate of their mineralization by soil microorganisms (Hahn and Quideau 2013).

5.2. Biological activation

Soil biological activity is related to soil health and will act as an indicator of the effectiveness of recovery procedures (Epelde et al. 2009). In this sense, biochemical properties are considered potentially sensitive, early and effective indicators of soil health in contaminated soils (Clemente et al. 2007). There is a direct relationship between the addition of organic matter with the stimulation of growth and the activity of the microbial community of soils degraded by mining, resulting in the mineralization of nutrients for plants (Alvarenga et al. 2014) and the increase of fertility and soil quality (Diacono and Montemurro 2010).

Soil organic matter is a source of energy and carbon for soil microorganisms, which promote the formation of micro and macro-aggregates (Lehmann and Rillig 2015). There is clear evidence that microorganisms are involved in the aggregation process because microbial activity controls the production of exudates that act as binding agents in aggregates (De Gryze et al. 2005). In any soil where clay is present, interactions between polysaccharide exudates, organic colloids and other decomposition products promote stability (Dontsova and Bigham 2005).

A group of these microorganisms, arbuscular mycorrhizal fungi (AMF), produce a proteinaceous material called glomalin present in roots, soil and hyphae, and this protein has the function of favouring the sequestration of C (Rillig 2004).

5.3. Structure and formation of aggregates

The application of organic waste, as indicated above, favors the formation of aggregates and thus the structure of the soil, so that it could lead to improved infiltration and water retention, making the soil more suitable for plant growth (Hueso-González et al. 2014). The stability of soil aggregates and the architecture of porous space affect water movement and storage, aeration, erosion, biological activity and plant growth (Bosch-Serra et al. 2017). At the same time, by achieving stable aggregates, the organic material is protected from microbial decomposition (Bronick and Lal 2005), erosion is reduced, root development is promoted (Tisdall and Oades 1982), soil structure degradation is prevented and water storage is favoured (Franzluebbers 2002). In short, organic matter amendments increase the stability of aggregates, contribute to the formation of new aggregates, increase porosity and water retention capacity, facilitate the development and establishment of vegetation and the formation of microbial communities and reduce erosion (Six et al. 2004).

In semi-arid environments, the presence of carbonates interferes with the relationship between clay minerals and soil aggregation, resulting in improved macro-aggregated stabilization in soils rich in carbonates, but with less porosity within macro-aggregates (Fernández-Ugalde et al. 2013). Moreover, the contribution of calcium carbonate in certain mining areas stabilizes the organic carbon added by organic amendments, minimizing losses of soil organic matter by mineralization (Zornoza et al. 2013).

5.4. Microbiota and enzyme activity

Soil microbiota helps the formation of soil structure, plant establishment and transformation of soil organic matter (Zink and Allen 1998). Many studies have demonstrated the importance of soil microbial communities for successful plant establishment and growth

(Kulmatiski et al. 2008; Epelde et al. 2010). However, extreme conditions caused by mining activities generally have a negative influence on soil biological activity (Asensio et al. 2013; Zornoza et al. 2013).

Not all organic waste produces the same effects on the soil microbiota. The microbial community responds faster to environmental changes than plants and is very sensitive (Harris 2009). However, many factors such as soil organic matter characteristics, soil moisture and soil temperature can affect biomass (Zhou et al. 2014) and soil microbial community structure (Hortal et al. 2015). The structure and activity of the soil microbial community may change in response to the quality of organic amendments. Therefore, soil processes mediated by microorganisms may also change depending on these changes in the microbial community (Lucas et al. 2014). In addition, since bacteria and fungi have different pH preferences (Rousk et al. 2009), the addition of alkaline amendments increases the pH of the soil, alters the balance between fungal/bacterial growth and the microbial structure of the community. This in turn can change the nature and magnitude of soil processes related to specific microbial groups (Zornoza et al. 2016). Changes in soil microbiota due to environmental factors should be reflected in the level of enzymatic activity of the soil (Kandeler et al. 1996). After the application of residues, there are changes in soil function that may indicate the evolution of microbial activity (Li et al. 2015). In general, organic amendments increase the enzymatic activities (b-glucosidase, alkaline phosphatase and urease) related to biogeochemical cycles of the elements in the soil (Pascual et al. 1997; Ros et al. 2003; Bastida et al. 2007; Tejada et al. 2009; Santos et al. 2014).

In semi-arid Mediterranean areas, indigenous microbial communities are well adapted to severe climatic conditions such as high temperatures and scarce rainfall, while new soil microbial communities from organic waste are more sensitive to water stress than native soil microbiota (Hueso et al. 2011). Organic amendments can also cause changes in the microbial populations of the native soil due to the diverse available substrates they provide (Luna et al. 2016b). Organic amendments have a strong effect on phospholipid contents, stimulating bacterial and fungal proliferation, as demonstrated by several authors (Marschner et al. 2003; Bastida et al. 2008; García-Orenes et al. 2013; Lazcano et al. 2013; Luna et al. 2016b). The profiles obtained from the phospholipid analysis were positively correlated with the total carbon content (TOC), the total N content and the total P content.

6. Revegetation

In order to restore degraded arid and semi-arid areas with significant rates of erosion such as the Region of Murcia, it is necessary to establish a vegetation cover in addition to restoring the soil (Zornoza et al. 2017).

In arid and semi-arid regions, due to low rainfall, severe water stress is produced in plants. Normally the species used are seedlings or greenhouse plants, which experience post-transplant shock after sowing, associated with moisture or nutritional stress (Jacobs et al. 2005; Bateman et al. 2018). Therefore, it is necessary to use plant species adapted to water stress, the nutrients existing in the restored area and the climatology of the site, since these have developed morphological and physiological adaptations that allow them to survive and grow in difficult conditions (Clemente et al. 2004).

The use of native species for restoration is of special interest to maintain the equilibrium of the ecosystem. In addition, native plants adapt to climatic and soil conditions and provide the basis for natural ecological succession (Méndez et al. 2007). Native plants have greater possibilities of survival, growth and reproduction under conditions of environmental stress than plants introduced from other environments (Adriano 2001; Antonsiewicz et al. 2008). It is a very important and basic strategy in all mining operations to have a seed bank of the existing species prior to exploitation, or failing that, prepare one with the plants of the closest environment with the same conditions as those that were initially in the quarry. In this way, it will be possible to use species characteristic of the area that can guarantee a better success in revegetation.

The beneficial effects of revegetation are very relevant from a soil and landscape point of view. Plants reduce water erosion by intercepting rain (Roundy et al. 2017; Tromble 1987), favor the increase of water infiltration (Huang et al. 2015; King et al. 2012; Piñol et al. 1991) and promote soil regeneration.

The application of organic matter inputs as a restoration technique increases the growth of plants due to the increase of available nutrients, mainly from organic amendments (Maisto et al. 2010; Moreno-Peñaranda et al. 2004). Thus, the appropriate combination of organic amendments and revegetation promotes the growth of plants (Caravaca et al. 2002) and, ultimately, the landscape recovery of the restored area.

7. Waste types applied as amendments

The European Commission (EC), during 2014 and 2015, made proposals focused on the circular economy and published an action plan that aims to "treat waste as a resource and convert Europe towards a circular economy". The action plan suggests as an objective for 2030, among others, to prepare 65% of municipal waste for reuse and recycling.

In addition to environmental degradation, one of the main environmental problems in the world is the production and accumulation of waste, so the objectives established by the European Union (EU) must be taken into account. Waste generation together with the depletion of many resources, lead the EU towards a zero-waste strategy through the circular economy, in which the value of products, materials and resources is maintained in the economy for as long as possible and waste generation is reduced (Almendro-Candel et al. 2018). An essential part of the circular economy is the use of urban solid waste (MSW) in soils as a source of nutrients, mainly through prior composting (Almendro-Candel et al. 2019).

Given the need to integrate European zero-waste strategies and the adoption of the circular economy, the recovery of mining operations is an opportunity for the convenient use of organic waste that allows the recovery of soil and landscape of areas affected by extractive activities. Therefore, this section focuses on contrasting the use of different amendments that have been used in the rehabilitation of mining spaces and their results, based on those applied in the Region of Murcia. The accumulation of waste is a problem and for this reason it is important to look for ways to reuse them without polluting the environment. In the studies and examples analyzed, mulching with peat and other materials, sewage sludge, compost, pruning waste, pig slurry and inorganic marble waste are considered, as well as combinations between them.

These amendments have been studied because of their excess in the Region of Murcia, as they pose a problem for their elimination. It is very common for farmers to abandon pruning remains in wadis and wastelands or carry out uncontrolled incineration. Municipal solid waste is a problem due to its abandonment in uncontrolled dumps and sewage sludge would soon overtake us if we did not do anything with it. In the Region of Murcia, the pig sector is one of the most important in Spain, with a census of 2,145,408 heads producing more than 6.9% of the country's output (MAPA 2019). This generates a large amount of waste, about 6.5 hm^3 per year, and generates management problems for producers. In addition, in the Region, the marble natural stone extraction industry processes 147,000 m^3 of product per year, generating 128,120 t of inert waste, of which only 10% is recovered (Zornoza et al. 2017).

7.1. Mulching

One of the techniques used for the restoration of degraded mining soils is the application of mulch by using different types of peat. This acts by limiting water loss through evaporation, improving filtration and root growth, as well as establishing vegetation and reducing soil erosion (Shao et al. 2014; Hueso-González et al. 2015). In addition, the plant remains and the mulch increases the stability of soil aggregates (Wright and Upadhyaya 1998).

Along with mulching, there are other strategies associated with it such as the use of plastic, stones or wood chips, which are effective measures to prevent water loss (Bakker et al. 2012; Devine et al. 2007). At the same time as plant mulch reduces the evaporation of soil water (Laliberté et al. 2008), soil protection is favoured and mulching is efficient for improving the establishment of vegetation (Valdecantos et al. 2009).

However, it has been observed in some experiments that the use of a vegetable mulch does not produce significant effects on the survival rate of plants in arid environments where annual precipitation is very low and only little rain can reach the soil due to interception by the mulch cover (Grantz et al. 1998; Luna et al. 2016b). In addition, plant mulches can have a negative effect on the activity of certain enzymes such as glucosidase, suggesting that mulch can prevent the entry of plant debris and native organic matter caused by the barrier created by mulch itself (Qiu et al. 2014).

By applying pruning residues as a mulch, we prevent uncontrolled incineration of them as they form a focus of disease. Moreover, the process of optimal dehydration expels large amounts of CO_2 into the air. In addition, the abandonment of pruning residues in ramblas and wastelands constitutes a serious risk during torrential rains and contributes to the spread of pests and diseases. Mulching may not always provide the expected beneficial effects on soil microbial activity as in some cases the effects on alkaline phosphatase and urease activities are positive and in other negative (Luna et al. 2016b). Several authors have found a significant negative effect on enzymatic activities related to C and P (b-glucosidase and alkaline phosphatase, respectively), while microbial biomass was not significantly affected by the type of coverage. Therefore, since these enzymes are frequently immobilized in clay and humic fractions (Nannipieri 2006; Bastida et al. 2012), it can be suggested that mulch has a stronger impact on the extracellular environment than on microbial growth (Luna et al. 2016b).

The positive effect of mulch on vegetation is related to improvements in soil water content, which would provide benefits for plant establishment, particularly in arid and semi-arid areas with severe water scarcity (Bautista et al. 2009).

Regarding the use of other surface materials, Luna et al. (2016b) did not observe clear positive effects with the use of gravel on the growth of plants and found a general negative effect using wood chips as mulch. Consistently, several authors found that wood chips could inhibit plant growth due to allelochemical compounds (Duryea et al. 1999; Rathinasabapathi et al. 2005). In addition, differences between plant species were found (Escós et al. 2000; Armas and Pugnaire 2005). Previous studies also reported negative effects (Kruse et al. 2004), no effects (Fernández et al. 2011; Santana et al. 2014) and positive effects (Badía and Marti 2000; Bautista et al. 2009). Contradictory effects have therefore been found in the case of mulch by using wood chips.

7.2. Compost from municipal solid waste

Composted material from municipal solid waste (MSW) mainly induces an increase in TOC and glomalin content in soil (Luna et al. 2016a). The increase in TOC content by compost is caused by the stable nature of the amendment (García et al. 1992; Ros et al. 2003; Dearden et al. 2006).

According to Luna et al. (2016b), composting increases electrical conductivity (EC) ($P < 0.05$) the first few days of being applied, but soil salinity decreases over time. This initial increase in EC may result from the incorporation of low molecular weight organic compounds or the release of salts during the decomposition of organic substances (González-Ubierna et al. 2012; Mingorance et al. 2014; Pérez Gimeno et al. 2016). The decrease over time of the EC may be due to the leaching of ions by rain, which contributes to reduce soil salinity (González-Ubierna et al. 2012).

Regarding soil carbon metabolism, five years after the application of MSW compost in the work carried out by Luna et al. (2016b), basal soil respiration is only sensitive to the application of further amendment and is similar to that of the natural soil of reference.

Compost increases microbial biomass and alters the composition of the community towards a community dominated by fungi. Fungi are capable of degrading more recalcitrant organic material (Boer et al. 2005); therefore, an increase in fungal biomass in soils modified with compost could be attributed to the presence of stabilized substrates that give a competitive advantage to fungi. Moreover, the development of vegetation cover on soils treated with compost could contribute to the entry of cellulose and lignin into the soil. Fungi are able to degrade these C polymers through their enzymatic systems (Boer et al. 2005; Baldrian et al. 2010). According to the study of Luna et al. (2016b), bacterial PLFA and Gram+/Gram- ratios increase in treated plots, reaching values similar to those observed in undisturbed reference soils. Gram+ bacteria communities are more resistant to drying and re-wetting than Gram- bacteria because of their physiological characteristics, i.e. the presence of a strong, thick and interconnected peptidoglycan cell wall (Schimel et al. 2007). In fact, the Gram+/Gram- ratio has been suggested as an indicator of resistance to disturbances in microbial communities (De Vries and Shade 2013). A shift to a more dominant Gram+ microbial community (high Gram+/Gram- values) can be seen as a mechanism to adapt to a semi-arid climate.

7.3. Sewage sludge

Sludge, in general, has stood out in its results for the creation of greater stability of soil aggregates (García-Orenes et al. 2005). Some results, however, indicate that it is not much larger than the reference soil and reaches a lower TOC than unaltered natural soils (Luna et al. 2016b). The organic carbon fraction of the sludge from wastewater treatment is more biodegradable than that of this composted material and can be rapidly hydrolyzed by enzymatic activity (Cook and Allan 1992), which would explain the difference in the results in the use of this waste. In general, composted sewage sludge (biosolids, as several types of treated sewage sludge that can be used as soil conditioner are termed) is a preferential organic waste used in agriculture and environmental rehabilitation.

Soils treated with sludge also have higher concentrations of actinobacteria, which can probably be attributed to the high content of these commonly found in wastewater treatment plants (Bitton 2005; Wang et al. 2014). However, the content and proportion of microbial biomass, both in restored and natural soils, responds over time to seasonal changes and vegetation development (Bastida et al. 2007; Baldrian et al. 2010).

7.4. Combination of MSW compost and sewage sludge

The combination of both residues apparently produces a sum of its positive properties in the soil. These affect enzyme activity, improve soil microbiological properties and improve soil respiration (Luna et al. 2016b).

The amendments of sewage and compost produce a positive correlation between enzymatic activities and the TOC and TN contents, favouring soil productivity and fertility (Luna et al. 2016b). This could be related to the higher organic matter content in plots modified with compost, as well as the stimulation of plant development that provides organic matter inputs to the soil (Bastida et al. 2008).

Compost and sludge contribute to increased plant growth, for example in the species *M. tenacissima, A. cytisoides and A. terniflora* (Valdecantos et al. 2011). However, opposite impacts on plant survival and growth have been observed. On the one hand, plant survival rates decrease slightly in the first months of planting, probably due to a high organic matter content, which increases soil salinity and the possible lack of nutrients associated with the increment of soil microbial activity. On the other hand, organic amendments are beneficial for plant growth in the following months, as they improve nutritional conditions (Luna et al. 2016a). In a semi-arid region under Mediterranean climate, the mean survival value of *M. tenacissima* of 92% was found four years after sowing. Values above 40-60% were noted by Valdecantos et al. (2011) and Oliet et al. (2012) for different species of plants in Mediterranean conditions. This was considerably above the value of 10% reported by Rokich (1999) in a Mediterranean mining restoration 2 years after sowing. The species that developed mechanisms to resist water stress during the summer period are of great interest. *M. tenacissima* is best suited for successful recovery under the semi-arid Mediterranean climate in the short term (Luna et al. 2018).

7.5. Sewage sludge, MSW compost and gravel

In addition to the properties commented on by the previous combination, the addition of inorganic, gravel-sized materials to these amendments introduces a noticeable physical change, favouring the creation of pores and spaces available for rapid infiltration and root development. This combination could be of great interest and application in certain environments where it is necessary to increase macroporosity and facilitate water infiltration and gas exchange.

From the point of view of the biological activity of soils, it should be pointed out that when this mixture of materials has been used, the detected dehydrogenase activity is much greater than if only organic amendments to MSW compost or sludge were applied. This may be correlated with an observed increase in vegetation cover with gravel (Masciandaro et al. 2004; Mukhopadhyay et al. 2016; Luna et al. 2016b), favored by changing physical conditions in the environment.

7.6. Pig slurry

The use of slurry as an amendment can solve two problems: recovery of a by-product and rehabilitation of degraded mining areas.

Slurry has a high organic matter content and can affect soil structure in several ways. The slurry provides sodium, magnesium and calcium (Na, Mg and Ca, respectively) in addition to the three main nutrients, nitrogen, phosphorus and potassium (N, P and K). Interchangeable cations (K^+, Na^+, Mg^{2+} and Ca^{2+}) affect soil aggregation. Sodium mainly causes dispersion of clay particles and destabilization of aggregates (Crescimanno et al. 1995). The dispersion of the aggregates generally leads to the formation of soil crusts, causing slow infiltration and high particle mobility by surface runoff. In calcareous soils, both Ca^{2+} and Mg^{2+} reduce clay dispersion (Amezketa and Araguees 1995). In addition, adsorbed K^+ limits clay dispersion due to its hydration energy, which is equivalent to 72% of that of adsorbed Na^+ (Levy and Torrento 1995). Carbonates probably prevent the decomposition of organic matter, which can be deduced from the evolution of the respiration rate during a growing season and from differences in the stability of aggregates when mineral and organic fertilizers are compared.

The introduction of pig slurry in fertilization strategies benefits porosity, mainly in the range area of 25-200 μm, but this is a transitory effect. These pores are associated with capillary water movement, soil aeration, rapid water drainage and root growth (Bosch-Serra et al. 2017). The dose for pig slurry should be established through the thresholds imposed by legislation on the addition of total nitrogen to soil to avoid contamination by salinity and highly soluble and water-movable nitrates (Directiva del Consejo 91/676/EEC). The dose of pig slurry applied increases the initial salinity values significantly and if unsuitable doses are used, they can be limiting initially in the establishment of the plants (Bosch-Serra et al. 2000). Furthermore, according to Salazar et al. (2009), high doses of slurry have significantly more nitrates than low doses and the highest values of nitrates occur at the surface. The application of pig slurry contributes to the improvement of soil fertility by facilitating greater colonization of natural vegetation (Kabas et al. 2012).

Contrary to what is often observed for solid animal manure, the application of liquid pig manure does not always increase the C content in the soil (Plaza et al. 2005; Carter and Campbell 2006; Angers et al. 2010), partly because of its low C content and rapid mineralization (Rochette et al. 2000; Chantigny et al. 2001; Pardo et al. 2011). The organic matter fraction of pig manure is composed largely of fast-decomposing organic C that may not contribute significantly to stabilizing organic matter. In addition, its high labile C and available N and P contents could accelerate the decomposition of native soil C (Peu et al. 2007; Rochette et al. 2000). Therefore, the application of carbonates seems to be useful to stabilize fresh and very labile organic matter, as is the case with pig slurry.

On the other hand, due to the release of root exudates in the rhizosphere, the application of pig slurry increases TOC and TN, improves soil structure and provides nutrients for microbial populations, which are the basis for ensuring ecosystem recovery. In addition, pig slurry is a good fertilizer due to the large amount of nutrients provided, which are necessary to promote the development of vegetation. Therefore, the use of this waste to recover degraded soils is of great interest, although some procedures, such as liming, must be carried out to minimize the rapid mineralization of organic matter from this type of waste (Zornoza et al. 2012). An increase in soil organic matter content also favours an increase in soil cation exchange capacity (CEC) (Kabas et al. 2012). Zornoza et al. (2017) demonstrated that there was a significant increase in CEC after the application of pig slurry.

Nitrogen is an essential element for vegetation and microbial communities. The increase in N improves the nutritional conditions of the soil and helps the growth and development of the introduced plants. With the application of pig slurry, shortly after a year, production and the different extractions and losses of nitrogen tend to balance out their concentration (Salazar et al. 2002). However, this TN decreases with time, which could be due to plant absorption, and immobilization by microbial biomass. To prevent the lack of TN that could limit the development of vegetation, it would be interesting to include legumes among the species introduced in the revegetation, in order to increase the availability of this nutrient through biological fixation of atmospheric N (Zornoza et al. 2017).

The microbial population increases after the application of pig slurry due to the increase of soluble C. Availability of C should allow the possibility of growth of microorganisms (Pérez de Mora et al. 2005). In addition, pig slurry also contains microbial biomass that can be incorporated into the soil. However, with the depletion of the labile fraction of organic matter, microorganisms do not maintain their growth and return to initial values in about 25 days (Plaza et al. 2007; Pardo et al. 2011; Zornoza et al. 2013). The same trend was observed for b-galactosidase and b-glucosidase activities associated with soluble C dynamics (Zornoza et al. 2012). However, fungal growth is more stable over time, indicating a progressive and continuous growth that depends not only on the most labile organic compounds but also on the total organic carbon content of the soil. Fungi are able to degrade more complex organic compounds (Griffith and Bardgett 2008), which may allow for more stable growth over time.

The addition of marble waste in combination with pig slurry produces an accumulated bacterial growth (Zornoza et al. 2016). Bacterial and fungal growth rates are dependent on the pH and soluble and labile fractions of organic matter. From pH 4 to pH 8, the growth of fungi to bacteria changes about 30 times. The fungi are favored by a lower pH. This trend towards the different effect of pH on bacteria and fungi has previously been observed (Rousk et al. 2009, 2010; Fernández-Calviño et al. 2011). Pig slurry promotes faster microbial growth probably due to the higher content of soluble labile organic compounds (Zornoza et al. 2016).

The use of pig slurry has positive effects on the rehabilitation of mining soils, improving soil properties and increasing germination (Pardo et al. 2011; Zornoza et al. 2017).

7.7. Pig manure

Pig manure is an environmentally attractive amendment to prevent the formation of acid drainage from metal mines due to its high pH and presence of lime. It can be incorporated as part of long-term remediation process in abandoned mines and areas affected by mining activity.

The basic characteristics of pig manure are 13.5% humidity, 57% of $CaCO_3$ and 28.1 g/kg of TN. The study consulted shows that pig manure increases pH and improves conditions for plant growth (Faz et al. 2008).

With the application of pig manure, studies have shown a preliminary increase in pH, TN, organic carbon and equivalent calcium carbonate content, and a reduction in Zn, Bp and Cd, as well as a decrease in EC. It has also been observed that the pH does not have the capacity to neutralise acid in the leachate in the short term when this waste is applied (Faz et al. 2008). Aggregate stability increases significantly with the application of pig manure and in addition, it produces a large fungal growth in soils and increases the concentrations of the saturated PLFAs (Zornoza et al. 2016). Several studies show the ability of pig manure to efficiently adsorb metal, reducing their availability in soil (Beesley et al. 2011; Park et al. 2011; Kelly et al. 2014; Zornoza et al. 2016).

7.8. Marble waste

Waste from these calcareous quarries usually has a high apparent density and a massive structure, which gives them low filtration rates, triggers soil erosion processes and increases runoff (Moreno de las Heras et al. 2008). Its combined use with organic materials can be positive since carbonates and clays stabilize organic carbon and make it more inaccessible for microbial attack (Bernal et al. 1991). Therefore, the application of marble reduces the degradability of organic compounds. This is of particular interest for improving the accumulation of C in regenerated soils from the point of view of C sequestration (Shrestha and Lal 2006). Studies have shown that the application of marble sludge leads to an increase in soil pH and favours the accumulation of soil organic matter (Zornoza et al. 2017). The presence of calcium from marble sludge can favour the bonds between clay minerals, and promote intermolecular interactions between organic and inorganic compounds, forming aggregates (Baldock and Skjemstad 2000; Clough and Skjemstad 2000).

According to Kabas et al. (2012), marble waste inhibits plant development, probably due to the presence of higher contents of salts and clays, whereas according to Risser and Baker (1990), these residues can be used to neutralize the acidity of many types of mining waste. This favours the establishment of vegetation and increases the availability of nutrients such as K, C, Mg, Mo or P, which are more mobile at pH about 6. The application of marble waste stabilizes organic matter, microorganisms and enzymes involved in the degradation of more complex molecules to degrade organic matter, such as arylsterase, which could be used as an indicator of organic matter stability. If only marble waste is used, the biochemical properties of the soil do not increase, indicating that changes in these properties are mainly due to the organic matter provided, verified with the use of pig slurry (Zornoza et al. 2013). The application of marble sludge together with pig slurry increases the growth of native vegetation, increases vegetation cover and biodiversity (Kabas et al. 2012).

7.9. Pig manure with marble waste

This waste combination, as mentioned previously, offers aspects that should be considered. It was observed that this combination can reduce the available water but also improves the establishment of vegetation. It appears that the application of lime or alkaline materials together with the use of double doses of pig manure is a reasonable alternative for remediation of soils in acidic mining areas. However, it has been indicated that salinity should be considered with medium- and long-term monitoring (Faz et al. 2008).

8. Conclusions

In the XXI century, the mining industry stands out as one of the most important sectors when providing essential materials for economic development. However, great effort needs to be made to improve the economic, social and environmental aspects of this industry. This includes the need to rehabilitate the affected areas taking into account all the social agents involved in its development (administration, neighborhoods, town halls, etc.).

This review works on the importance of organic matter in the restoration of soils in areas affected by mining, in particular by open-pit extractive activities. It aims to highlight the great possibilities presented through the research and work generated in the last 20 years. The use of organic waste of many different kinds is feasible; however, not only the positive aspects have to be considered, but also the negative ones such as possible pollutants that are emitted as a result of the quarrying activity. It should be noted that most of these are expected to be gases (CO_2) from the combustion of the engines of the machines and the trucks used to transport the quarry materials and the waste. As a preventive measure in this regard, it would be advisable to have the ISO 14,000 standard and to have an environmental management system that establishes periodic maintenance of vehicles and/or equipment in general. This maintenance contributes to improving combustion and making it as efficient as possible, generating the least amount of carbon dioxide. In short, pollution is a limiting factor for the use of waste.

The major conclusions are that the increase of soil organic matter, the improvement in revegetation and the increase in water retention capacity are the most noteworthy aspects in a semi-arid environment such as the Murcia region.

As mentioned above, waste production is a crucial problem. Therefore, different ways of reusing waste that do not pollute the environment and can be reintroduced into economic activity in the frame of circular economy are sought.

9. Future actions

Future lines of research, complementing soil and vegetation aspects, are proposed. The first could be the improvement of habitat conditions for wildlife and the use of wildlife as an indicator, although the wildlife colonization of a restored quarry does not occur immediately because there is no habitat to support it. The main improvement for the fauna, in the beginning, is the restoration of vegetation and recovery the original habitat. Except for some species of birds and mammals quite elusive to human presence, wildlife may be present in the early stages of the exploitation if restoration is initiated from the early stages (favouring the landscape evolution and habitat). The diversification of the flora species used in plant restoration results in a significant improvement in the landscape. Even more importantly, it supports a greater number of wildlife species and increases the stability of the system. For this reason, a non-homogenous restoration is important. Other actions that can specifically help wildlife are sowings on debris or other altered spaces in which cereals and other cultivated plants are planted as a first-rate food source for many birds and mammals, as evidenced by numerous small plots used in hunting.

In line with the above, a high biodiversity footprint remains a key area of work. This includes both efforts to minimize the potential negative impact on biodiversity and to seek opportunities to increase it. Achieving a net positive impact (NPI) is a challenge for the future.

To achieve the target, it is proposed to work with major building materials companies to help actively reverse the trend of declining global biodiversity. As a proposal for future lines of work, it could be agreed to work within the following objectives:

- Reduce the ecological footprint on climate change through improved energy efficiency of installations, the use of renewable energy and special low carbon footprint cements.
- Improving knowledge and control of persistent organic pollutants, through systematic measurements, development and implementation of best practices, reporting on progress made.
- Reduce water consumption in operations and aim to improve knowledge of water flows by developing practices for their control.
- Analyze the potential of biodiversity in our quarries to study and develop biodiversity management programs; and aim to reduce the impact of quarries on biodiversity.

In conclusion, the general objective, is to achieve a positive net balance in nature after mining activities.

The second future line is the creation of artificial lagoons in the quarry and gravel exploitation sites that collect rainwater, thus creating lake spaces of great ecological value. These would serve as a refuge for many aquatic species and allow the development of some amphibians and invertebrates. Instead of generating a large single lagoon, it is preferable to build several small lagoons with different ecological slope conditions, etc., because the possibilities for different types of fauna are greatly increased. Once space and spatial distribution are known, it will be necessary to generate the gaps in the lagoon in which rainwater will be deposited. In this type of design, there should be at least two depths, one more shallow to allow amphibian spawning and larval growth, and a deeper one for temporary maintenance of adults and other wildlife species that need more time in its development like some dragonflies, beetles, etc.

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Influence of *Pinus pinaster* age on aluminium fractions in acidic soils

Influencia de la edad de Pinus pinaster *en las fracciones de aluminio de suelos ácidos*
Influência da idade de Pinus pinaster *nas frações de alumínio em solos ácidos*

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ABSTRACT

The influence of plantation age on the chemical properties of acidic soils was studied in 16 plots in adult *Pinus pinaster* stands established in Galicia (NW Spain). The Al fractions in the soil solid phase and the total Al in soil solution were determined in the upper soil layer (0-20 cm) and the lower soil layer (20-40 cm) in each plot. The pH, total C and N, exchangeable Ca, Mg, Na, K, and Al and Al saturation (% Al) were determined in the solid fraction. Aluminium was extracted from the solid phase with the following solutions: ammonium oxalate (Al_o), sodium pyrophosphate (Al_p), copper chloride (Al_{cu}) and ammonium chloride (Al_{NH_4}). The total Al in the liquid phase was also determined. All soil chemical parameters, except total N, C/N ratio and % Al, were significantly influenced by soil depth. The mean pH was lower in the upper than in the lower layer (4.57 vs. 4.97), but the opposite was observed for the organic C (77.2 vs. 50.4 g kg^{-1}), the effective cation exchange capacity (eCEC) (9.43 vs. 6.25 $cmol_{(+)}$ kg^{-1}), P (8.95 vs. 4.65 mg kg^{-1}) and the exchangeable cations. Organic matter, total N and eCEC were significantly and positively correlated with plantation age ($r = 0.69$ in the upper layer and $r = 0.82$ in the lower layer, $p < 0.01$; $r = 0.62$, $p < 0.05$ in the upper layer and $r = 0.78$, $p < 0.01$ in the lower layer; $r = 0.77$, $p < 0.01$ in the upper layer and $r = 0.85$, $p < 0.0001$ in the lower layer, respectively), and pH_{KCl} was negatively correlated with plantation age ($r = -0.55$ in the upper soil layer and $r = -0.61$ in the lower soil layer, $p < 0.05$). The concentrations of the different Al forms in all soils decreased in the order $Al_p > Al_o > Al_{cu} > Al_{NH_4}$. Highly stable organo-aluminium complexes (Al_{p-cu}) predominated over moderate and low stability complexes (Al_{cu}) in all soil plots. The highly stable organo-Al complexes were significantly more abundant in the lower layer, whereas the opposite was observed for the exchangeable Al and the total Al in soil solution. The concentrations of all Al forms (except Al_{p-cu}) were significantly and positively correlated with plantation age (Al_o $r = 0.50$, $p < 0.05$ for the upper layer and $r = 0.67$, $p < 0.01$ for the lower layer; Al_p $r = 0.64$, $p < 0.01$ for the lower layer; Al_{cu} $r = 0.84$ for the upper layer and $r = 0.83$ for the lower layer, $p < 0.0001$; Al_{cu-NH_4} $r = 0.65$ for the upper layer and $r = 0.78$ for the lower layer, $p < 0.01$; Al_{NH_4} $r = 0.76$, $p < 0.01$ for the upper layer and $r = 0.84$, $p < 0.0001$ for the lower layer; total Al in soil solution $r = 0.61$ for the upper layer and $r = 0.60$ for the lower layer, $p < 0.05$). Stepwise linear regression analysis showed that plantation age, pH and total C explained between 67% and 93% of the variance in the Al forms. In all regression models, plantation age was a significant predictor variable for the different Al fractions, except total soluble Al, which is an important variable to consider in the study of chemical properties in forest soils.

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RESUMEN

La influencia de la edad de las plantaciones sobre las propiedades químicas de suelos ácidos se estudió en 16 parcelas de Pinus pinaster adulto establecidas en Galicia (Noroeste de España). Se determinaron las fracciones de Al en la fase sólida del suelo y el Al total de la disolución del suelo en la capa superior del suelo (0–20 cm) y en la capa inferior del suelo (20–40 cm) en cada parcela. El pH, el C y N total, Ca, Mg, Na, K y Al cambiables y la saturación de Al (% Al) se determinaron en la fracción sólida. El aluminio se extrajo de la fase sólida con las siguientes soluciones: oxalato amónico (Al_o), pirofosfato sódico (Al_p), cloruro de cobre (Al_{cu}) y cloruro amónico (Al_{NH_4}). También se determinó el Al total en la fase líquida. La profundidad del suelo tuvo un efecto significativo en todos los parámetros químicos del suelo, excepto la relación N total, C/N y % Al. Los valores medios de pH fueron más bajos en la capa superior (4,57 vs. 4,97), lo contrario se observó con el C orgánico (77,2 vs. 50,4 g kg^{-1}), con la capacidad de intercambio catiónico efectiva (CICe) (9,43 vs. 6,25 $cmol_{(+)}$ kg^{-1}), con el P (8,95 vs. 4,65 mg kg^{-1}) y los cationes cambiables. La edad de la plantación se correlacionó significativamente y positivamente con la materia orgánica, el N total y la CICe ($r = 0,69$ en la capa superior y $r = 0,82$ en la capa inferior, $p < 0,01$; $r = 0,62$, $p < 0,05$ en la capa superior y $r = 0,78$, $p < 0,01$ en la capa inferior; $r = 0,77$, $p < 0,01$ en la capa superior y $r = 0,85$, $p < 0,0001$ en la capa inferior, respectivamente), y negativamente con el pH_{KCl} ($r = -0,55$ en la capa superior y $r = -0,61$ en la capa inferior, $p < 0,05$). La concentración de las diferentes formas de Al en todos los suelos disminuyeron en el orden $Al_p > Al_o > Al_{cu} > Al_{NH_4}$. Los complejos organo-alumínicos de alta estabilidad (Al_{p-cu}) predominaron sobre los complejos de estabilidad media y baja (Al_{cu}) en todas las parcelas. Los complejos órgano-Al de alta estabilidad fueron significativamente más abundantes en la capa inferior, mientras se observó lo contrario con el Al cambiable y el Al total en disolución del suelo. La edad de las plantaciones se correlacionó significativa y positivamente con todas las formas de Al (excepto Al_{p-cu}) (Al_o $r = 0,50$, $p < 0.05$ en la capa superior y $r = 0,67$, $p < 0,01$ en la capa inferior; Al_p $r = 0,64$, $p < 0,01$ en la capa inferior; Al_{cu} $r = 0,84$ en la capa superior y $r = 0,83$ en la capa inferior, $p < 0,0001$; Al_{cu-NH_4} $r = 0,65$ en la capa superior y $r = 0,78$ en la capa inferior, $p < 0,01$; Al_{NH_4} $r = 0,76$, $p < 0,01$ en la capa superior y $r = 0,84$, $p < 0,0001$ en la capa inferior; Al total en la disolución del suelo $r = 0,61$ en la capa superior y $r = 0,60$ en la capa inferior, $p < 0,05$). El análisis de regresión lineal stepwise mostró que la edad de la plantación, el pH y el C total explicaron entre 67% y 93% de la varianza en las formas de Al. En todos los modelos de regresión, la edad de las plantaciones fue una variable predictiva para las diferentes fracciones de Al, excepto el Al total de la disolución, que es una variable importante a considerar en el estudio de las propiedades químicas en suelos forestales.

RESUMO

A influência da idade das plantações nas propriedades químicas dos solos ácidos foi estudada em 16 parcelas de pinheiro-bravo (Pinus pinaster) adulto estabelecidas na Galiza (Noroeste de Espanha). A frações de Al na fase sólida do solo e o Al total na solução do solo foram determinadas, em cada parcela, na camada superficial do solo (0–20 cm) e na camada sub-superficial (20–40 cm). O pH, C e N totais, Ca, Mg, Na, K e Al de troca e a saturação de Al (% Al) foram determinados na fração sólida. O Al foi extraído da fração sólida com as seguintes soluções: oxalato de amónio (Al_o), pirofosfato de sódio (Al_p), cloreto de cobre (Al_{cu}) e cloreto de amónio (Al_{NH_4}). O Al total na fase líquida também foi determinado. A profundidade do solo teve um efeito significativo em todos os parâmetros químicos do solo, exceto no N total, relação C/N e % Al. Os valores médios de pH foram menores na camada superior (4,57 vs. 4,97), o contrário foi observado com o C orgânico (77,2 vs. 50,4 g kg^{-1}), a capacidade de troca catiónica efetiva (eCEC) (9,43 vs. 6,25 $cmol_{(+)}$ kg^{-1}), o P (8,95 vs. 4,65 mg kg^{-1}) e os catiões de troca. A idade da plantação correlacionou-se significativa e positivamente com a matéria orgânica, N total e eCEC ($r = 0,69$ na camada superior e $r = 0,82$ na camada inferior, $p < 0,01$; $r = 0,62$, $p < 0,05$ na camada superior e $r = 0,78$, $p < 0,01$ na camada inferior; $r = 0,77$, $p < 0,01$ na camada superior e $r = 0,85$, $p < 0,0001$ na camada inferior, respetivamente) e negativamente com pH_{KCl} ($r = -0,55$ na camada superior e $r = -0,61$ na camada inferior, $p < 0,05$). A concentração das diferentes formas de Al em todos os solos seguiram a ordem $Al_p > Al_o > Al_{cu} > Al_{NH_4}$. Os complexos organo-alumínicos de alta estabilidade (Al_{p-cu}) predominaram sobre os complexos de média e baixa estabilidade (Al_{cu}) em todas as parcelas. Os complexos de alta estabilidade foram significativamente mais abundantes na camada inferior, enquanto o oposto foi observado com o Al de troca e o Al total em solução. A idade das plantações correlacionou-se significativa e positivamente com todas as formas de Al (exceto Al_{p-cu}) (Al_o $r = 0,50$, $p < 0,05$ na camada superior e $r = 0,67$, $p < 0,01$ na camada inferior; Al_p $r = 0,64$, $p < 0,01$ na camada inferior; Al_{cu} $r = 0,84$ na camada superior e $r = 0,83$ na camada inferior, $p < 0,0001$; Al_{cu-NH_4} $r = 0,65$ na camada superior e $r = 0,78$ na camada inferior, $p < 0,01$; Al_{NH_4} $r = 0,76$, $p < 0,01$ na camada superior e $r = 0,84$, $p < 0,0001$ na camada inferior; Al total solúvel $r = 0,61$ na camada superior e $r = 0,60$ na camada inferior, $p < 0,05$). A analise de regressão linear stepwise mostrou que a idade das plantações, o pH e o C total explicavam entre 67% e 93% da variância nas formas de Al. Em todos os modelos de regressão, a idade da plantação foi uma variável preditiva significativa para as diferentes frações de Al, exceto o Al total solúvel, sendo uma variável importante a ser considerada no estudo das propriedades químicas em solos florestais.

KEYWORDS
Maritime pine, adult plantations, organo-aluminium complexes, forest soils.

PALABRAS CLAVES
Pino marítimo, plantaciones adultas, complejos organoalumínicos, suelos forestales.

PALAVRAS-CHAVE
Pinheiro-bravo, plantações adultas, complexos organo-alumínicos, solos florestais.

1. Introduction

The soils in Galicia (NW Spain) are characterised by being mostly acidic. This is due to the predominance of acidic rocks and the existence of open and subtractive systems (in which precipitation exceeds evapotranspiration) in the region. At the initial stages of soil formation, the most unstable primary minerals are already partly weathered and neoformation of secondary minerals takes place while there is still an important pool of non-weathered primary minerals (Macías et al. 1982). At this stage, nutrient elements are available to plants and the soil productivity is close to optimum. The pool of weatherable minerals is gradually reduced, 1:1 minerals are formed and Fe/Al oxy-hydroxides and the base cations are retained in the system. This reduces the degree of base saturation of the exchange complex, with Al ions becoming the most important cations (Macías et al. 1982; Macías and Camps 2020). The acid conditions influence the concentration of nutrients in soil solution and their availability to plants. Thus, while the availability of N, P, K, S, Ca, Mg and Mo is greatly decreased, the solubility of Al and Mn increases becoming toxic to plants (Macías and Calvo 1992; Brennan et al. 2004). These conditions have important consequences for plant production, especially for calcophiles and eutrophiles, with reductions in root elongation, symbiotic N_2 fixation and nitrification and increases in phosphate fixation and aluminium mobilization (Macías et al. 1982; Macías and Camps 2020). Active aluminium species prevail in most Galician soils (García-Rodeja and Macías 1984), with high Al contents in both solid and liquid phases (Álvarez et al. 1992, 2002, 2005) that can cause toxicity and nutritional imbalances in forest stands.

Vegetation plays an important role in determining Al forms in the solid and liquid phases of soil (Álvarez et al. 2002, 2005). Thus, in forest soils, the type of organic matter is closely related to the forest species and strongly influences the formation of Al-humus complexes. The formation of these complexes in the soil solid phase plays a major role in regulating Al activity in soil solution (Mulder and Stein 1994; Takahashi et al. 1995). In addition, aluminium bound to organic matter in the soil solution is less toxic than inorganic Al species (Adams et al. 2000; Matús et al. 2006), which may explain the lack of negative effects of Al on the growth of tree species in acid soils.

Pinus pinaster is one of the most important tree species in Spain, both in terms of extent and wood production. This species is considered non-site-demanding and it is mainly planted for timber production and for restoration and landscaping purposes in northern Spain. In their natural range, pines appear to be particularly well adapted to marginal habitats, in which combinations of various related factors enable them to compete successfully with other tree species. The widespread use of *Pinus pinaster* in Galicia, where it covers 0.53 Mha of land in pure and mixed stands, is due to its extraordinary adaptation to poor, acidic, shallow sandy soils (Eimil-Fraga et al. 2014). Many processes associated with growth and nutrient cycling in pine strongly affect the underlying soils. These processes generally lead to nutrient depletion and consequently to soil acidification, which in turn provide pines with a competitive advantage against other plant species (Richardson 1998).

As a complement of a previous study that assessed Al-forms in the solid phase of soils developed on different parent materials under young plantations of *Pinus pinaster* (Eimil-Fraga et al. 2015), the aims of the present study are to investigate if the plantation age influences the chemical properties of acidic soils under adult *Pinus pinaster*. This study focuses on some Al fractions in the soil solid phase and on the total concentration of Al in soil solution, comparing the results obtained in two different soil layers.

2. Material and Methods

The study was carried out in a network of 16 plots established as a chronosequence in adult reforested plantations of *Pinus pinaster* located in Galicia (NW Spain). The plot size was 25 × 40 m and the age of the trees ranged between 27 and 58 years. This species is usually managed on rotations of 25-40 years.

The plot soils are characterised by different types of parent material: granite, acid metamorphic rock and sedimentary rock. In each plot, three soil samples were obtained at random from the upper layer (0-20 cm) and from the lower layer (20-40 cm). The three samples from each layer were combined to form a composite sample for each depth. Composite soil samples were dried at 40 °C and sieved through a 2 mm mesh. The following parameters were determined in the soil solid fraction: pH in water and in 0.1 M KCl (Guitián and Carballas 1976); total C and N, by combustion in a CHNS LECO analyser (CHNS Truspec model); the exchangeable cations (Ca, Mg, Na, K and Al) displaced by 1 M NH_4Cl using the method proposed by Peech et al. (1947) and the effective cation exchange capacity (eCEC) as the sum of them (Kamprath 1970). The exchangeable cations were measured by atomic absorption (Ca, Mg, and Al) and atomic emission (Na and K) spectroscopy (Perkin Elmer, Optima 4300 DV model). Al saturation (% Al) was calculated as the ratio between exchangeable Al and the eCEC and was expressed as a percentage. The P concentration was measured by the Olsen method (Olsen and Sommers 1982).

Different forms of Al were extracted from the soil solid fraction by extraction with different reagents: the Al extracted with acid ammonium oxalate (Al_o) (ratio soil:extractant 1:100, shaking for 4 h in darkness) provided an estimate of total non-crystalline Al compounds (Blakemore 1978); the Al extracted with sodium pyrophosphate (Al_p) (ratio soil:extractant 1:100, with 16 h shaking) yielded an estimate of the total organically bound Al (Bascomb 1968); and the Al extracted with 0.5 M $CuCl_2$ (ratio soil:extractant 1:10, 30 min shaking) provided an estimate of organo-Al complexes of low and moderate stability (Al_{cu}) (Juo and Kamprath 1979). The Al extracted with unbuffered NH_4Cl was considered exchangeable Al (Al_{NH_4}) (Peech et al. 1947) (ratio soil:extractant 1:100 contact time, 12 h). Aluminium in the extracts was determined by atomic absorption spectroscopy (Perkin Elmer, Optima 4300 DV model). Subtraction of Al_{cu} from Al_p provided an estimate of the Al that forms highly stable complexes with organic matter (Al_{p-cu}), and subtraction of Al_{NH_4} from Al_{cu} provided an estimate of complexes of moderate and low stability (Al_{cu-NH_4}) (Urrutia et al. 1995).

The soil solution was prepared as aqueous extracts mixing soil with distilled water (soil: solution ratio, 1:10), with a contact time of 3 days. The extracts were filtered (0.45 µm) and total soluble Al was determined by visible spectrophotometry, with pyrocatechol violet (Dougan and Wilson 1974).

Forest plot characteristics included the tree diameter at breast height and total height of all trees in each plot, determined in 2012 when trees were between 27 and 58 years old. Site index (SI) was calculated as the dominant height of the stand (in metres) at a reference age of 20 years (Álvarez-González et al. 2005). Dominant height (H0) was calculated as the average total height of the 100 thickest trees per hectare. The stand basal area (G, $m^2\ ha^{-1}$) and number of trees per hectare (N, trees ha^{-1}) were also calculated for each plot. The forest plot characteristics are summarised in **Table 1**.

Table 1. Values of individual tree and stand parameters in the plots under study

Variable	Mean	Minimum	Maximum	Standard deviation
Age of plantation (years)	38	27	58	9.345
Diameter at breast height (cm)	31.35	7.60	67.20	9.142
Total height (m)	20.08	7.90	34.20	3.635
G (basal area, $m^2\ ha^{-1}$)	44.99	21.63	74.28	163.9
N (number of trees, trees ha^{-1})	596	240	930	8.133
H0 (dominant height, m)	22.60	17.22	27.37	3.044
SI (site index, m)	15.08	12.00	18.20	2.025

2.1. Statistical Analysis

The data were analysed to determine mean values and ranges of variation. Duncan's test was used to classify the mean values in order to examine all possible differences in relation to soil depth. Pearson's correlation coefficients were calculated to assess the linear relationships between variables. Stepwise regression analysis was applied to the candidate variables for inclusion in predictive models of the different forms of Al. The variance inflation factors (VIF), which represent a measure of the inflation in the variances of the parameter estimates due to collinearity between (independent) variables, were calculated using the VIF option in the MODEL procedure. The data were analysed using the MEANS, CORR, REG and GLM procedures in the SAS statistical package (SAS Institute 2004).

3. Results

3.1. General soil parameters

There were no significant differences in any of the assessed soil parameters between the parent materials. The mean values and standard deviations of the main chemical properties of soils in relation to soil depth are shown in **Table 2**. All soil chemical parameters, except total N, C/N ratio and Al saturation in the cation exchange complex (% Al), were significantly influenced by soil depth (**Table 2**).

The pH_{water} and pH_{KCl} were significantly higher in the lower soil layer (20-40 cm) than in the upper soil layer (0-20 cm). The concentration of exchangeable Ca reached values of between 0.22 and 0.56 $cmol_{(+)}$ kg^{-1} in both layers. The concentration of exchangeable Mg ranged between 0.10 and 0.21 $cmol_{(+)}$ kg^{-1} and the concentration of exchangeable K varied between 0.10 and 0.14 $cmol_{(+)}$ kg^{-1}. The concentrations of Ca, Mg and K were significantly higher in the upper soil layer than in the lower one. The concentration of P (8.95 vs. 4.65 mg kg^{-1}), the amount of organic matter (77.2 vs. 50.4 g kg^{-1}) and the eCEC (9.43 vs. 6.25 $cmol_{(+)}$ kg^{-1}) were also significantly higher in the upper than in the lower soil layer. The Al saturation was lower in the upper soil layer (82%) than in the lower layer (83%), although the difference was not significant (**Table 2**).

In both soil layers, C and pHwater were significantly and negatively correlated ($r = -0.87$, $p < 0.0001$ in the upper layer and $r = -0.54$, $p = 0.0307$ in the lower layer), as were C and pHKCl ($r = -0.87$, $p < 0.0001$ in the upper layer and $r = -0.70$, $p = 0.0024$ in the lower layer). The Al saturation was also negatively correlated with

Table 2. Mean values (and standard deviations) of general soil parameters in relation to soil depth, with the respective p values for the one factor ANOVA. Different letters indicate significant differences ($p < 0.05$) between layers (Duncan's test).

Variable	p value	0-20 cm layer	20-40 cm layer
pH_{water}	0.0001	4.57 (0.30) b	4.97 (0.22) a
pH_{KCl}	0.0017	3.88 (0.33) b	4.22 (0.21) a
Ca ($cmol_{(+)}$ kg^{-1})	0.0001	0.56 (0.31) a	0.22 (0.06) b
Mg ($cmol_{(+)}$ kg^{-1})	< 0.0001	0.21 (0.06) a	0.10 (0.03) b
K ($cmol_{(+)}$ kg^{-1})	0.0134	0.14 (0.05) a	0.10 (0.03) b
C (g kg^{-1})	0.0167	77.2 (3.41) a	50.4 (2.49) b
N (g kg^{-1})	-	0.33 (0.13)	0.25 (0.12)
C/N	-	23.3 (4.54)	20.4 (4.28)
eCEC ($cmol_{(+)}$ kg^{-1})	0.0129	9.43 (3.80) a	6.25 (2.95) b
% Al	-	81.7 (5.90)	83.2 (4.38)
P (mg kg^{-1})	0.0056	8.95 (4.89) a	4.65 (3.04) b

pH_{water} ($r = -0.59$, $p = 0.0160$ in the upper layer and $r = -0.70$, $p = 0.0026$ in the lower layer) and with pH_{KCl} ($r = -0.69$, $p = 0.0033$ in the upper layer and $r = -0.80$, $p = 0.0002$ in the lower layer).

Regarding the relationships between the general soil parameters and forest plot characteristics, the pH_{KCl} was negatively correlated with plantation age in the upper soil layer ($r = -0.55$, $p = 0.0286$) and in the lower cm soil layer ($r = -0.61$, $p = 0.0118$) (**Figure 1**). The amount of organic matter in both soil layers was positively correlated with the age of plot ($r = 0.69$, $p = 0.0034$ in the upper layer and $r = 0.82$, $p = 0.0001$ in the lower layer) (**Figure 2**). The plantation age was also significantly and positively correlated with total N ($r = 0.62$, $p = 0.0101$ in the upper layer and $r = 0.78$, $p = 0.0004$ in the lower layer) and eCEC ($r = 0.77$, $p = 0.0005$ in the upper layer and $r = 0.85$, $p < 0.0001$ in the lower layer).

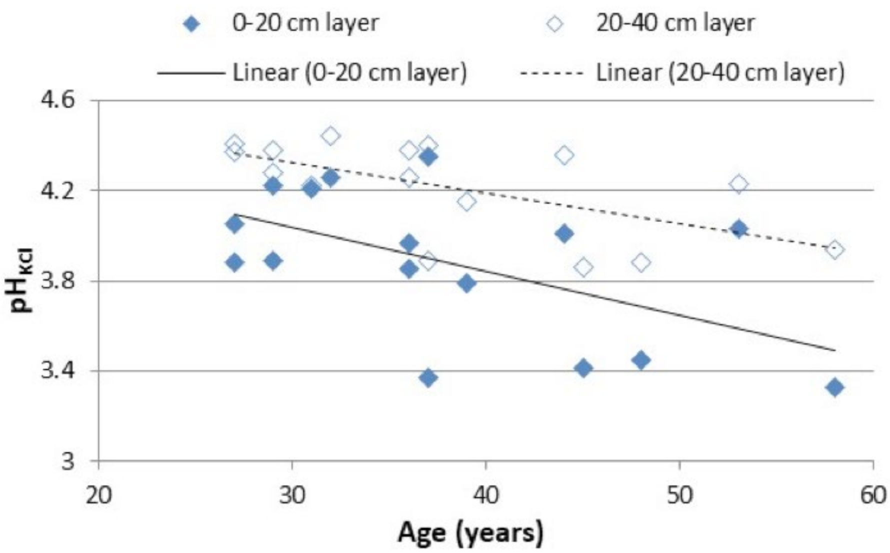

Figure 1. Relationship between plantation age and pH_{KCl} in the upper soil layer (0-20 cm) and the lower soil layer (20-40 cm).

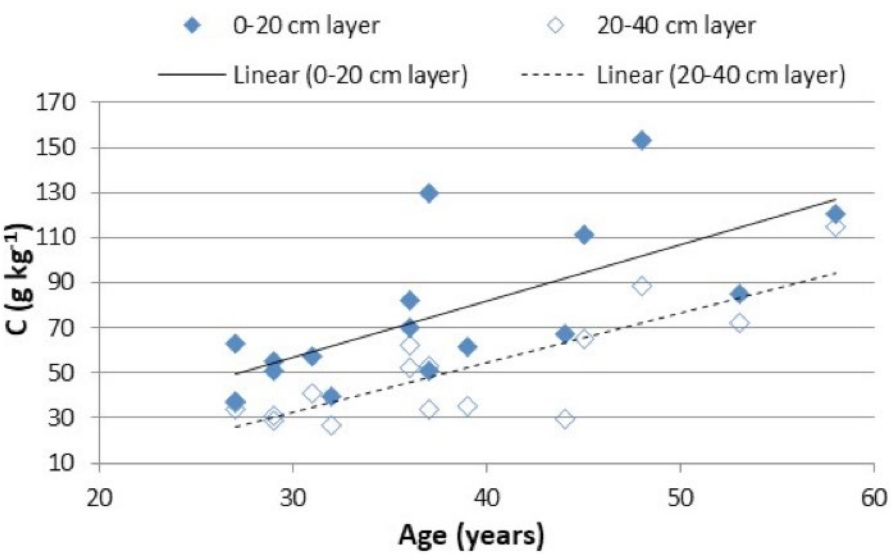

Figure 2. Relationship between plantation age and total C in the upper soil layer (0-20 cm) and the lower soil layer (20-40 cm).

The site index, a parameter related to forest production, was negatively correlated with the percentage of Al in the cation exchange complex in the upper layer (r = -0.62, p = 0.0101) and positively correlated with the pH_{KCl} in the lower soil layer (r = 0.60, p = 0.0133).

3.2. Al fractions in the solid phase and total Al in the soil solution

The concentrations of non-crystalline aluminium (Al_o), which varied between 4.67 and 5.73 g kg^{-1}, were higher in the lower than in the upper soil layer, but the difference was not significant. The concentration of organically bound aluminium (Al_p) reached values of between 5.97 and 7.44 g kg^{-1} and was also higher in the lower layer, although again the difference was not significant (**Table 3**). The concentration of Al_o in both soil layers was significantly and positively correlated with plantation age (r = 0.50, p = 0.0468 for the upper layer and r = 0.67, p = 0.0041 for the lower layer), whereas for Al_p, correlation with plantation age (r = 0.64, p = 0.0080) only occurred in the lower soil layer.

The concentration of organo-aluminium complexes of low and moderate stability (Al_{cu}) varied between 1.66 and 1.89 g kg^{-1} and were higher in the upper than in the lower layer. The Al extracted by $CuCl_2$ did not differ significantly in the two layers (**Table 3**). The concentration of Al_{cu} in both layers was positively and significantly correlated with stand age (r = 0.84, p < 0.0001 for the upper layer and r = 0.83, p < 0.0001 for the lower layer) (**Figure 3**).

Table 3. Mean values (and standard deviations) for Al extracted by acid ammonium oxalate (Alo), sodium pyrophosphate (Al_p), $CuCl_2$ (Al_{cu}), NH_4Cl (Al_{NH_4}), $Al_{p-cu} = Al_p - Al_{cu}$, $Al_{cu-NH_4} = Al_{cu} - Al_{NH_4}$ and total Al in soil solution in relation to soil depth. The values of p account for the one factor ANOVA. Different letters indicate significant differences (p < 0.05) between layers according to Duncan's test.

Variable	p value	0-20 cm soil layer	20-40 cm soil layer
Al_o (g kg^{-1})	-	4.67 (1.21)	5.73 (2.18)
Al_p (g kg^{-1})	-	5.97 (1.11)	7.44 (2.19)
Al_{cu} (g kg^{-1})	-	1.89 (0.52)	1.66 (0.97)
Al_{NH_4} (g kg^{-1})	0.0353	0.70 (0.32) a	0.48 (0.26) b
Al_{p-cu} (g kg^{-1})	0.0110	4.08 (0.99) b	5.78 (2.30) a
Al_{cu-NH_4} (g kg^{-1})	-	1.18 (0.29)	1.18 (0.75)
Total Al (mg L^{-1})	<0.0001	14.7 (5.93) a	5.20 (3.05) b

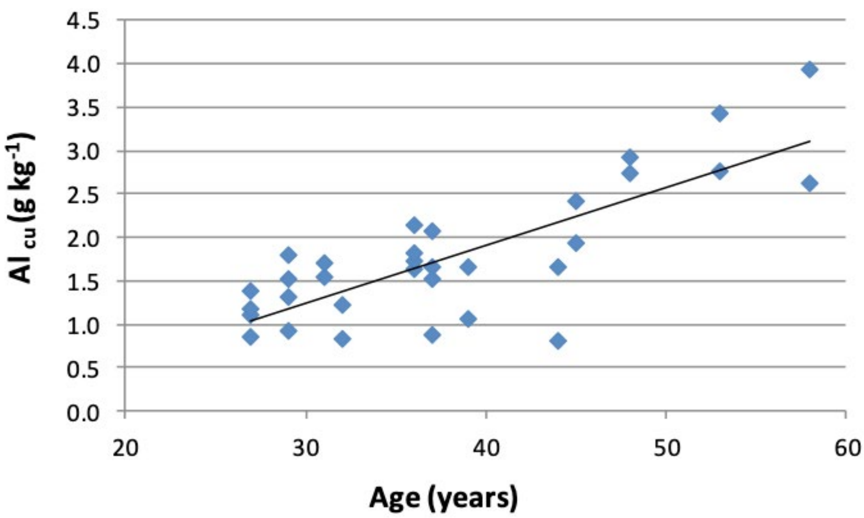

Figure 3. Relationship between plantation age and organo-aluminium complexes of low and moderate stability.

The highly stable complexes formed with organic matter (Al_{p-cu}) varied between 4.08 and 5.78 g kg^{-1} and were significantly higher in the 20-40 cm layer than in the 0-20 cm layer.

The concentration of complexes of moderate and low stability (Al_{cu-NH_4}) was 1.18 g kg^{-1} in both soil layers (**Table 3**). The Al_{cu-NH_4} was significantly and positively correlated with plantation age in both layers ($r = 0.65$, $p = 0.0068$ for the upper layer, and $r = 0.78$, $p = 0.0004$ for the lower layer) (**Figure 4**).

Aluminium extracted by NH_4Cl ranged between 0.48 and 0.70 g kg^{-1}. The concentration of Al_{NH4} was significantly higher in the upper soil layer ($p = 0.0353$) (**Table 3**). The concentration of AlNH4 in both soil layers was significantly and positively correlated with plantation age ($r = 0.76$, $p = 0.0006$ for the upper layer and $r = 0.84$, $p < 0.0001$ for the lower layer) (**Figure 5**)

The concentration of total Al in soil solution varied from 5.2 to 14.7 mg L^{-1} and was significantly

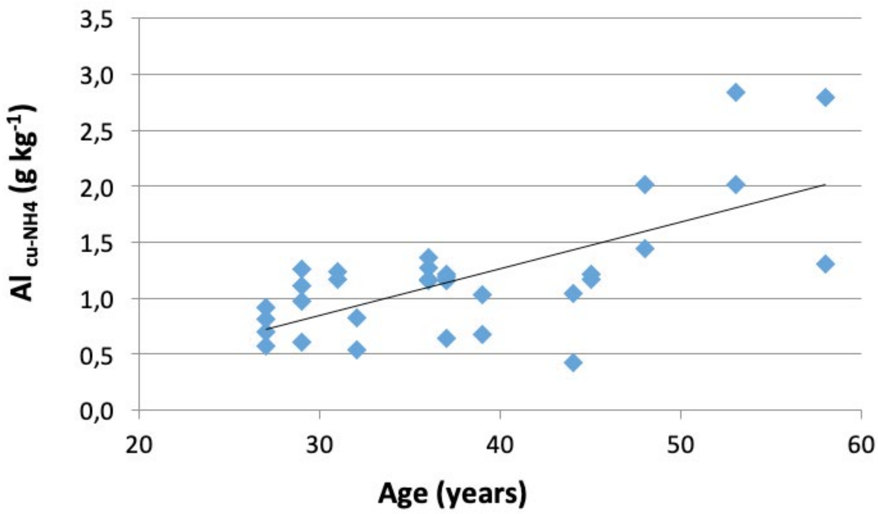

Figure 4. Relationship between plantation age and concentration of medium and low stability complexes.

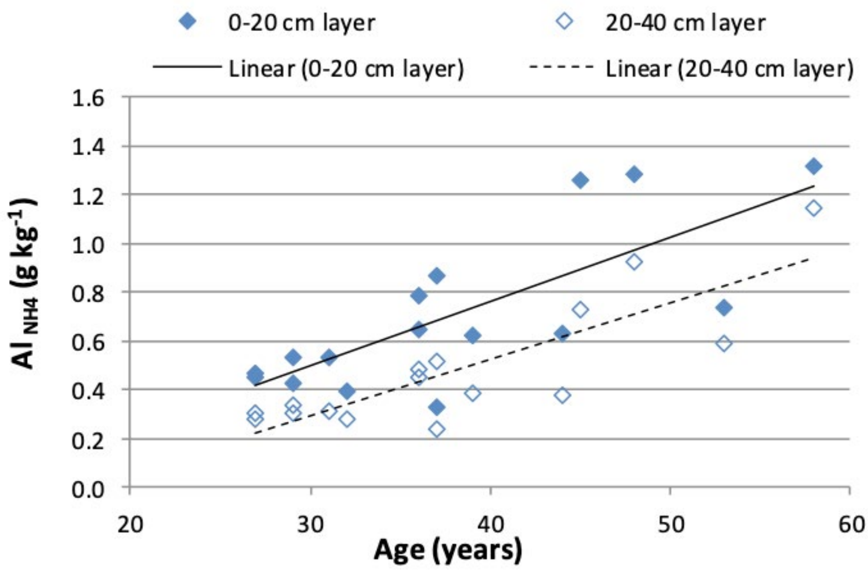

Figure 5. Relationship between plantation age and concentration of exchangeable Al in the upper soil layer (0-20 cm) and in the lower soil layer (20-40 cm).

higher in the 0-20 cm layer, coinciding with the low pH of this layer (Tables 2 and 3). The total soluble Al in both soil layers was positively and significantly correlated with stand age ($r = 0.61$, $p = 0.0113$ for the upper layer and $r = 0.60$, $p = 0.0142$ for the lower layer) (Figure 6).

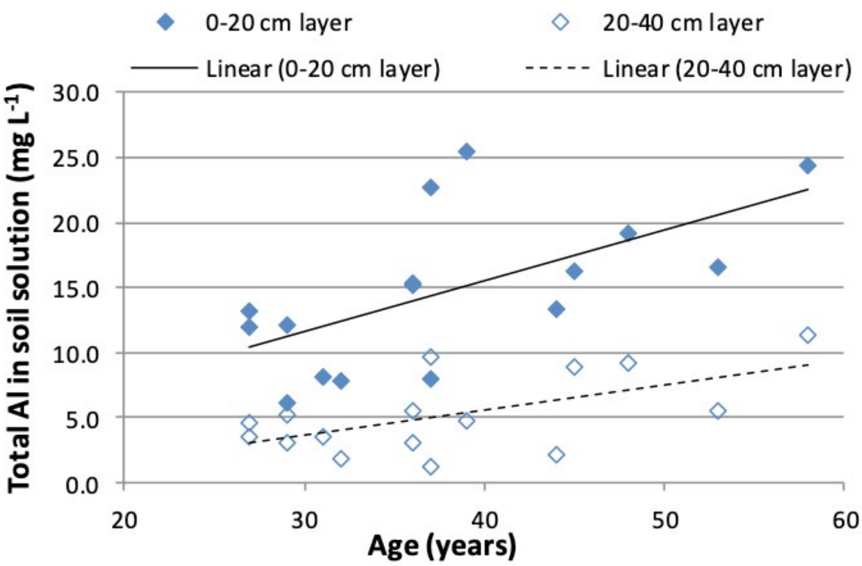

Figure 6. Relationship between plantation age and total Al concentration in soil solution for the upper layer (0-20 cm) and the lower layer (20-40 cm).

3.3. Regression analysis: Al forms

The stepwise linear regression showed that the plantation age, pH_{KCl} and total C explained 70% of variance in total non-crystalline Al (Al_o), all with positive coefficient in the equation (Table 4). For the organically bound Al (Al_p), plantation age, pHwater and total C explained 67% of variance, all with a positive influence (Table 4). Plantation age explained 62% of the variance in organo-Al complexes of low and moderate stability (Al_{cu}), reaching 74% when total C was included in the stepwise linear regression and 81% when age, C and pH_{water} were included. All variables had a positive effect on the regression (Table 4). Total C was the main parameter explaining the variance in exchangeable Al (88%), whereas age contributed to an additional 2% and the pH_{KCl} a 3% more with the last one entering negatively in the equation (Table 4). Most of the variation in total Al in soil solution was explained by pH_{water} (64%), with a negative influence, increasing to 67% when total C was included. Plantation age was included in all equations as an explanatory variable for the different Al forms, except total soluble Al in soil solution (Table 4).

4. Discussion

The soils under study were acidic and rich in organic matter. The concentrations of Ca, Mg and K (Ca < 0.3 $cmol_{(+)}$ kg^{-1}, Mg < 0.15 $cmol_{(+)}$ kg^{-1}, K < 0.12 $cmol_{(+)}$ kg^{-1}) in the lower layer can be considered deficient for forest plantations, and the concentrations in the upper layer intermediate (Ca 0.3-1.0 $cmol_{(+)}$ kg^{-1}, Mg 0.15-0.25 $cmol_{(+)}$ kg^{-1}, K 0.12-0.20 $cmol_{(+)}$ kg^{-1}) according to Bonneau (1995). The soils in all plots were "alic" (%Al > 60%) and the Al saturation was always higher than 80% in both layers (Buol et al. 1975), indicating that these soils would suffer stress due to excess aluminium as base saturation is less than 15% (Cronan and Grigal 1995).

The organic matter content increased with plantation age due to continuous deposition of plant debris. The positive correlation between eCEC and plantation age may be related to the increase in organic matter in the soil, thus explaining the strong correlation observed between C and eCEC ($r = 0.88$, $p < 0.0001$ in

Table 4. Regressions between different Al forms, plantation age and soil parameters

Dependent variable*		Independent variable				
		Variable	Estimate	p level	R2	VIF
Al_o (mg kg^{-1})	-29339 ($p < 0.0001$)	Age	94.8	0.0021	0.3162	1.89
		pH_{KCl}	6780.8	< 0.0001	0.5279	3.85
Al_p (mg kg^{-1})	-39312 ($p < 0.0001$)	C	548.5	0.0006	0.7057	5.35
		Age	73.3	0.0778	0.2590	2.10
		pH_{water}	8136.6	< 0.0001	0.4984	3.27
Al_{cu} (mg kg^{-1})	-6144.6 ($p = 0.0015$)	C	699.7	0.0005	0.6759	5.09
		Age	27.5	0.0099	0.6203	2.10
		C	228.2	< 0.0001	0.7427	5.09
Al_{NH_4} (mg kg^{-1})	1265.3 ($p = 0.0056$)	pH_{water}	1136.1	0.0027	0.8146	3.27
		C	46.4	0.0002	0.8800	5.35
		Age	8.6	0.0009	0.9020	1.89
Al_t (mg L^{-1})	61.48 ($p = 0.0032$)	pH_{KCl}	-320.1	0.0020	0.9307	3.85
		pH_{water}	-11.59	0.0032	0.6417	2.78
		C	0.60	0.1161	0.6714	2.78

* Aluminium forms extracted by acid ammonium oxalate (Al_o), sodium pyrophosphate (Al_p), $CuCl_2$ (Al_{cu}) and NH_4Cl (Al_{NH_4}) and total Al in soil solution (Al_t). Age: plantation age. C: Carbon. VIF: variance inflation factor.

the upper layer and r = 0.96, $p < 0.0001$ in the lower layer). Although the decrease in pH with age would lead to a diminution in the eCEC due to a decrease in the negative charge of organic matter, the increase in organic C supply appears to have a greater effect on the eCEC value. Although the pH of the older plots is lower, the value reached is still high enough to allow the deprotonation of the carboxylic groups of organic matter as it increases when pH raise above 4, according to Marschner et al. (2005).

The concentrations of the different Al forms in all soils followed the order $Al_p > Al_o > Al_{cu} > Al_{NH_4}$. The same order of abundance has also been observed in other Galician soils with *Pinus pinaster* (Eimil-Fraga et al. 2015) and in acidic soils with below *Pinus massoniana* and *Cunninghamia lanceolate* (Larssen et al. 1999). The greater extraction efficiency of Al by sodium pyrophosphate than by ammonium oxalate has frequently been observed in organic matter rich soils (Camps Arbestain et al. 2003; García-Rodeja et al. 2007; Ferro-Vázquez et al. 2014; Eimil et al. 2015), possibly related to the predominance of organo-aluminium complexes over inorganic compounds of low crystallinity or/and to the extraction of inorganic forms of low crystallinity by pyrophosphate (Kononova and Belchikova 1970; Kaiser and Zech 1996). Alo and Alp were significantly correlated with organic matter content in the lower soil layer (r = 0.77, p = 0.0004 and r = 0.66 p = 0.0051 respectively), indicating that both fractions (Al_o and Al_p) are associated with the soil organic matter, and highlights the important role of the organic matter in preventing the evolution of non-crystalline Al towards more crystalline forms (García-Rodeja et al. 1987; Álvarez et al. 2002). The concentrations of Alo and Al_p were much lower than those obtained by Eimil-Fraga et al (2015) in soils from young plantations of the same species (*Pinius pinaster*), as well as in other soils also developed on acidic rocks (granites and slates).

Highly stable organo-aluminium complexes (Al_{p-cu}) predominated over moderate and low stability complexes (Al_{cu}) in all plots (73.5% Al_{p-cu} and 26.5% Al_{cu}). Moderate and low stability complexes were more abundant in the plots with older trees, reflected by the strong correlations between Alcu and plantation age (r = 0.84) and Al_{cu-NH_4} and plantation age (r = 0.65).

The Al_{NH_4} was also higher in the plots with older trees ($r = 0.76$, $p = 0.0006$ for the upper layer and $r = 0.84$, $p < 0.0001$ for the lower layer) (**Figure 5**). The results obtained for exchangeable Al in terms of amount are consistent with those reported by Boruvka et al. (2005) for forest species. However, in the present study, most of the Al forms in the solid phase were more abundant than those observed in other studies in forest soils from other parts of Europe (Sweden, Poland) possibly related to different soil characteristics, derived from very different geology and climatic conditions (Berggren and Mulder 1995; Walna et al. 2005; Frankowski et al. 2013). The total Al in the soil solution was also more abundant in the plots with older trees (**Figure 6**) and the concentration was higher than the values reported in other forest soils in Galicia with pine presence (Fernández-Sanjurjo et al. 1998; Camps-Arbestain et al. 2004; Álvarez et al. 2005; Eimil-Fraga et al. 2015). The values obtained in the present study were also higher than those previously observed for different types of soils and forest species (Boudot et al. 2000; Dlouhá et al. 2009; Tejnecky et al. 2010; Collignon et al. 2012). These comparisons show that *Pinus pinaster* has a great capacity to resist high Al concentrations in the soil solid phase and in soil solution.

We previously studied the Al forms in soils under young *Pinus pinaster* plantations and developed on the same type of rock (Eimil-Fraga et al. 2015). The difference in the content of the Al forms and in the stability of the organo-aluminium complexes observed in both studies may be explained by differences in the plantation age. In the previous study (op.cit.), the plantations were all between 11 and 13 years old, while in the present study they are between 27 and 58 years old. In both young and old plots, there were clear differences in the C/N ratio (15 and 23, respectively) and in the pH value, which was slightly higher in the younger plantations (4.85 vs 4.57). The effect of the C/N ratio on Al forms is associated with the degree of humification of the organic matter, which is lower when the C/N ratio is high. The higher pH and the higher degree of humification of organic matter (lower C/N) favour the formation of highly stable organo-aluminium complexes. Therefore, it seems that the greater reactivity of organic matter in young plantations favours the formation of organo-aluminium complexes in the solid phase of the soil and that these complexes are more stable.

Focusing on plantations between 27 and 58 years in the present study showed that Al_o, Al_p and Al_{cu} increased with plantation age. These results can be explained by taking into account that the influence of the plantation age is not the same in all Al forms. Thus, while plantation age explains 31% and 26% of the variance in Al_o and Al_p respectively, it explains up to 62% of the variance in complexes of moderate and low stability ($Al_{cu\text{-}NH_4}$). The pH and the organic matter content are the other parameters contributing to explaining the concentrations of these Al forms. We observed a significant increase in the organic matter content and a decrease in soil pH in the soils as plantations become older, while the C/N ratio did not vary significantly. The greater amount of organic matter may explain the increase in non-crystalline Al (Al_o and Al_p) in the soils in older plantations in this age range, but the higher acidity of the plots in these plantations would cause a decrease in the reactivity of the organic matter. The higher acidity and the lower reactivity favour the formation of more labile Al-organic matter complexes, at the expense of more stable complexes (Eimil-Fraga et al. 2015), and they also favour an increase in exchangeable Al and Al in solution that can be observed in older plantations. Thus, at lower soil pH, as in the older plantations, the solubility of Al increases and some of the dissolved Al can interact with the organic matter in the soil to form labile complexes, some will be exchangeable Al and some may remain in soil solution (Mulder et al. 1989).

On the other hand, the more acidic pH and the higher C/N ratio of the upper soil layer (**Table 2**) would explain the lower presence of highly stable organo-aluminium complexes and the higher concentration of exchangeable Al and Al in soil solution in relation to the lower soil layer.

The regression models improved our previous findings in four young *Pinus pinaster* plots (Eimil-Fraga et al. 2015). In the present study, the equations were fitted with a larger set of stands, yielding more robust findings regarding prediction of Al forms. The R^2 values were higher in all linear regressions, and the input variables were very similar to the equations for young plots, and the difference in plantation age explained an important part of the variability in Al forms in the present study.

The study findings showed a clear increase in all Al fractions with plantation age. Most previous studies involving Al fractionation in forest soils have not considered the age of the tree species. Thus, although Álvarez et al. (2002), Walna et al. (2005) and Collignon et al. (2012) reported the stand age, they did not evaluate the relationships between this parameter and the different Al forms. The present findings highlight the need to adapt pinewood management by avoiding excessive lengthening of rotations or by promoting the natural regeneration of broadleaf species as the pine stands reach a mature stage of development.

The results of the present study cannot be fully compared with those of other studies because of the lack of previous studies relating plantation age and soil properties, in particular, Al fractions in the solid phase and the total Al in soil solution.

5. Conclusions

The study findings clearly show a relationship between soil aluminium chemistry and plantation age and thus contribute to a better understanding of the growth of *Pinus pinaster* in acidic soils. Older plots of *Pinus pinaster* may have a higher risk of Al toxicity, because they are more acidic and because of an increase in the low stability of the organo-aluminium complexes and the exchangeable Al, which could cause an increase in Al in soil solution. The high yields of young and adult plantations of *Pinus pinaster* in these poor and acidic soils confirms the resistance of this species to high Al concentrations, even in adult plantations, in which Al concentrations are particularly high.

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Changes in soil organic carbon fractions in a sequence with cover crops

Cambios en las fracciones de carbono orgánico del suelo en una secuencia con cultivos de cobertura
Alterações nas frações de carbono orgânico do solo numa sequência com culturas de cobertura

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ABSTRACT

Advances in cover crops practice, in the context of potential benefits for annual crop production and sustained soil quality were studied. A soybean-maize sequence with five winter cover crops (CC) species were studied at the Marcos Juárez INTA Experimental Station, Córdoba, Argentina. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T) and hairy vetch (VV) + triticale (T) mixture were tested as well as a control treatment (Ct) without a CC. The CC effect on the dynamics and balance of the soil organic C (SOC) and its fractions were examined. Maize and soybean yields did not show significant differences between the control and the CC treatments. The SOC stratification (0-0.10 and 0.10-0.20 m) with accumulation of residue on surface was due to the concentration of SOC and fractions that decreased with depth. The gramineous crops were more efficient in biomass production with more C input into the soil. Triticale showed positive C balance in OC and in the easily degradable fraction (labile) and an increase in the residue decomposition rate. CC had a positive impact on the more stable C stock (recalcitrant OC) in the sub-superficial layer than in the superficial one. The gramineae input was evident in the superficial layer and the most stable OC fraction, was concentered in the sub-superficial layer. Organic soil fractioning by particle size have been shown to be useful indicators for detecting changes produced by management practices in most experiments. This study demonstrated that the effect of cover crops on SOC and the labile fraction in the upper soil layer was strongly related with the high residue production.

RESUMEN

Se estudió el manejo de los cultivos de cobertura y sus potenciales beneficios para la producción de cultivos anuales y la calidad sostenible de los suelos. Se estudió la secuencia soja-maíz con cinco especies de cultivos de cobertura (CC) invernales, en la Estación Experimental INTA Marcos Juárez, provincia de Córdoba, Argentina. Los CC utilizados fueron: vicia sativa (VS), vicia villosa (VV), centeno(C), triticale (T) y una consociación de vicia villosa y triticale (VV+T), así como un tratamiento control (Ct), sin CC. Se estudió el efecto de los CC sobre la dinámica y el balance del carbono orgánico del suelo (COS) y sus fracciones. Los rendimientos de maíz y de soja no mostraron diferencias significativas entre el tratamiento Ct y los CC. La concentración del carbono orgánico del suelo (COS) y sus fracciones disminuyeron a medida que aumentó la profundidad produciendo la estratificación del COS (0-0,10 y 0,10-0,20 m) con acumulación de residuos superficiales. Las gramíneas como CC fueron más eficientes en la producción de biomasa con la consecuente incorporación de C al suelo. Dentro de éstas, el triticale mostró un balance positivo de C tanto en el total del COS como en las fracciones fácilmente degradables (fracciones lábiles) con una mayor velocidad de descomposición de residuos. Los CC tuvieron un impacto positivo en el aumento del stock

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del C más estable, carbono orgánico recalcitrante (COR), en el horizonte subsuperficial más que en superficial. El aporte de las gramíneas fue notorio en la capa superficial de suelo y la fracción de C recalcitrante se concentró en la capa subsuperficial. En la mayoría de los estudios se observó que el fraccionamiento del carbono orgánico del suelo es un indicador útil para detectar los cambios producidos por las prácticas de manejo. En este trabajo se demostró que en la capa superficial de suelo, el efecto significativo de los CC sobre el COS y la fracción lábil del mismo, estuvo fuertemente relacionado con la mayor producción de residuos.

RESUMO

Estudou-se a gestão de culturas de cobertura e os potenciais benefícios para a produção anual de culturas e a qualidade sustentável do solo. A sequência soja-milho foi estudada com cinco espécies de plantas de cobertura de inverno (CC), na Estação Experimental INTA Marcos Juárez, província de Córdoba, Argentina. As CC utilizadas foram: ervilhaca comum (VS), ervilhaca peluda (VV), centeio (C), triticale (T) e uma consociação de ervilhaca peluda (VV) + triticale (T), bem como um tratamento controlo (Ct) sem CC. Estudou-se o efeito dos CC sobre a dinâmica e o equilíbrio do carbono orgânico do solo (COS) e as suas frações. As produções de milho e soja não apresentaram diferenças significativas entre os tratamentos controle e CC. A estratificação do SOC (0-0,10 e 0,10-0,20 m) com a acumulação de resíduos na superfície foi devida à concentração de SOC e frações que diminuíram com a profundidade. As gramíneas foram mais eficientes na produção de biomassa com mais entrada de C no solo. O triticale apresentou um balanço positivo de C no CO e na fração facilmente degradável (lábil) e um aumento na taxa de decomposição do resíduo. O CC teve um maior impacto positivo na reserva do C mais estável (CO recalcitrante) na camada sub-superficial do que na camada superficial. O efeito das gramíneas foi evidente na camada superficial e a fração mais estável de OC concentrou-se na camada sub-superficial. O fracionamento do solo orgânico por tamanho das partículas demonstrou ser um indicador útil para detectar mudanças produzidas pelas práticas de gestão na maioria dos ensaios. Este estudo demonstrou que o efeito das culturas de cobertura no SOC e na fração lábil na camada superficial do solo estava fortemente relacionada com a elevada produção de resíduos.

KEY WORDS
Maize-soybean sequence, soil organic matter, soil quality, balance, recalcitrant index, Mollisols.

PALABRAS CLAVE
Secuencia soja-maíz, materia orgánica del suelo, calidad de suelo, balance, índice de recalcitrancia, Mollisoles.

PALAVRAS-CHAVE
Sequência milho-soja, matéria orgânica do solo, qualidade do solo, equilíbrio, índice de recalcitrância, Molissolos.

1. Introduction

Cover crops (CC) are crops that are seeded between cash crops and are not harvested, not incorporated into the soil as green manure, and not intended for grazing, like annual forage. The species most commonly used as CC are gramineae, and to a lesser extent leguminosae, which have the benefit of fixing nitrogen (N) from the atmosphere (Rimsky-Korsakov et al. 2015). Cover crops grown during periods when the soil might otherwise be fallow are a valuable management option for reducing soil erosion and nitrogen losses from agroecosystems. They improve soil quality but the impacts on crop yield depend on the type of cover crop, the commercial crop considered and the climate (Álvarez et al. 2017).

Using cover crops (CC) would be an efficient alternative during winter periods for producing an increase in crop residues in production systems with a high frequency of soybean (*Glycine max* L.) rotation. Various authors agree that winter species as CC increase the dry matter production and improve the carbon (C) input into the soil (Sainju et al. 2007; Ghiotti and Basanta 2008). Significant amounts of carbon rich residues in the soil positively modify the composition and quality of the soil organic matter (SOM), improving soil productivity (Restovich et al. 2011; Sainju et al. 2003). In this context, CC has been recommended for soybean monoculture in Argentina, where the production of crop residues may be insufficient for proper soil cover and protection (Novelli et al. 2011). Soybean, characterized by a high N demand for grain production, also causes a negative soil N balance (Landriscini et al.

2019). On the other hand, maize produces more residues with high values in the C:N ratio, which would favor slow decomposition that is beneficial for stabilizing SOM evolution (Morón 2004).

The content of soil organic carbon (SOC) is determined by the relationship between the C input into the soil and C losses, therefore, long-term fallow cropping systems (soybean or maize monocultures) reduce the carbon input whereas cover crops increase it significantly (Duval et al. 2016). Several authors have recommended the annual C input necessary for maintaining the SOC levels in different soils in the Pampas region, e.g. in the southeast of Córdoba province, Argentina. It was estimated that 3 Mg ha^{-1} $year^{-1}$ of C input from crop residues are needed for SOC levels of 36 Mg ha^{-1} (Cazorla 2013).

These agricultural systems typically include long periods of fall-winter fallow with a low annual C input into the soil (2-3 Mg C ha^{-1} $year^{-1}$) (Restovich et al. 2005).

In no-tillage systems, the C and N contents may differ between the surface and subsurface layers. The stratification ratio (SR) of SOC or total N (TN) is an index that relates these contents in two different soil layers and can be used as indicator of the dynamic soil quality (Franzluebbers 2002; Toledo et al. 2013). Furthermore, the SR of SOC is a good indicator of the SOC sequestration rate. Higher SR of SOC indicates that soil management enhances soil quality. This is because the topsoil layer is influenced by land management but the second and subsequent layers are less affected (Franzluebbers 2002). For SOC, stratification values higher than 2.0 are essential to maintain soil quality (Fernández-Romero et al. 2016). Stratification ratios allow a wide diversity of soils to be compared on the same assessment scale because of an internal normalization procedure that accounts for inherent soil differences (Franzluebbers 2002).

The SOC is unlikely to change in the short term (3-4 years), but not in the most labile fractions, associated with residues in the early stages of decomposition and linked to the coarser structural fractions of the soil (particulate organic carbon, POC) (Duval et al. 2016). The POC fraction is the most active and it is used as a soil quality indicator in the short term because it is sensitive to management-induced changes (Duval et al. 2013). In addition, these labile fractions were shown to be correct indicators of changes in crop sequences (Salvo et al. 2010) and they may also show early soil changes resulting from the inclusion of cover crops.

The SOC components can be compartmentalized into different fractions or pools. The labile fraction is composed of particulate organic matter, easily degradable by microorganisms with fast turnover rates, from months to a few years (Krull et al. 2003). The other stabilized or resistant fraction would be persistent in the soil over a scale of years (Bruun et al. 2007). This fraction can be defined as that which is slowly lost after cultivation and which increases proportionally as the SOC decreases.

There is little agreement about how to quantify soil organic matter (SOM) as a biochemical quality. Ideally, this "quality" must reflect biodegradability in the absence of physical protection and hence be based on chemical composition independent of physical position within the mineral matrix (Rovira and Vallejo 2007). A useful alternative is acid hydrolysis, proposed as a simple method to evaluate SOM quality. This procedure is easy to perform and can be applied to the large series of samples generally employed in ecological research. The non-hydrolyzable residue may include young SOM, but most radiocarbon studies have shown that the residue from acid hydrolysis is consistently older than the hydrolysable fraction, whether the hydrolysis is applied to the whole SOM (Pandey et al. 2014).

Even though there is no standard definition of "chemical recalcitrance" under natural conditions, operationally it refers to resistance to loss under selected chemical treatments (Pandey et al. 2014). Hydrolysis with acids like HCl (Plante et al. 2006) is a common procedure for obtaining the labile and recalcitrant fractions from the soil and for determining the SOM biochemical quality. Chemical recalcitrance removes proteins, nucleic acids and polysaccharides, whereas the non-hydrolysable residue contains resistant compounds such as lignin, aromatic, humified components and long-chain aliphatic compounds. The non-hydrolysable fraction represents the recalcitrant pool and its relative size with respect to the total C is termed the "Recalcitrance Index" (RI) (Rovira and Vallejo 2002).

We hypothesized that cover crops mainly modify the distribution of the labile organic fractions and the quality of the recalcitrant compounds within the surface layer.

The objectives of this study were to: 1. Evaluate the effect of different CC inclusions sown after a soybean-maize (*Zea mays L.*) sequence, on the dynamics of the SOC and its fractions in a soil in the southeast of Córdoba province, Argentina, during a crop cycle; 2. Determine the SOC and its fractions balance after 7 years of a soybean-maize sequence; 3. Assess the changes in the organic fractions with different degrees of recalcitrance and the Recalcitrance Index variations.

2. Materials and Methods

2.1. Experimental Site

The study site was located at the Marcos Juárez INTA Experimental Station, Córdoba, Argentina (32°42'44.65"S; 62°05'46.07"W) (**Figure 1**). The area is characterized by a temperate climate with an average annual temperature of 16.9 ºC and a mean annual precipitation of about 894 mm (Andreucci et al. 2016). The soil is a Mollisol, i.e. Typic Argiudoll (Soil Survey Staff 2010); developed in aeolian sediments (loess), with a wide variability in depth, texture, soil OM content and fertility (Álvarez and Lavado 1998). It is a dark, deep and well-drained soil in a flat relief position with a silt loam texture in the surface horizons (Horizons A). It is a typical representative of the good soils of the area with a wide aptitude for crops, forage and pastures, although they present a slight climatic limitation, especially in the west and northwest sector (Baigorria et al. 2019). The upper layer is slightly acidic (pH 6.4) with 32.6 g kg^{-1} OM, belonging to the Marcos Juárez series (INTA 1978). In semiarid and semihumid regions, the soils are characterized by a low OM content, and their dynamics are affected more strongly by water availability (Galantini et al. 2016).

Figure 1. Location of the experimental site at Cordoba province, Argentina.

2.2. Experimental design and treatments

A soybean-maize sequence with different winter cover crops used as predecessors was started in 2008, under no-tillage for the previous 9 years. The species used were common vetch (*Vicia sativa L.*) (VS), hairy vetch (*Vicia villosa* Roth) (VV), rye (*Secale cereale L.*) (R), triticale (*x Triticosecale Wittmack*) (T), hairy vetch (VV) + triticale (T) mixture, and a control treatment (Ct) without CC, that was kept weed-free with herbicide applications (**Figure 2** and **Table 1**). Rye and Triticale are gramineae (G) crop and the others leguminosae (L) species.

The long-term assay experiment was established on 150 m^2 (6 x 25 m) plots in a randomized split plot design with five treatments and three replications. The principal factor was the CC inclusion and the second factor was the CC fertilization (fertilized: f, and unfertilized: nf) applied to subplots. The fertilization factor was 100 kg N ha^{-1} for triticale and rye, 50 kg N ha^{-1} and 50 kg P ha^{-1} for the hairy vetch + triticale mixture and 50 kg P ha^{-1} for hairy and common vetch.

Table 1. Different cover crops species, scientific names and treatments code

Treatment	Scientific Name	Treatment Code
Cover Crop		CC
Common Vetch	*Vicia sativa L.*	VS
Hairy Vetch	*Vicia villosa Roth*	VV
Rye	*Secale cereal L.*	R
Triticale	*Triticosecale Wittmack*	T
Hairy Vetch + Triticale mixture	*Vicia villosa Roth x Triticosecale Wittmack*	VV + T
Control		Ct

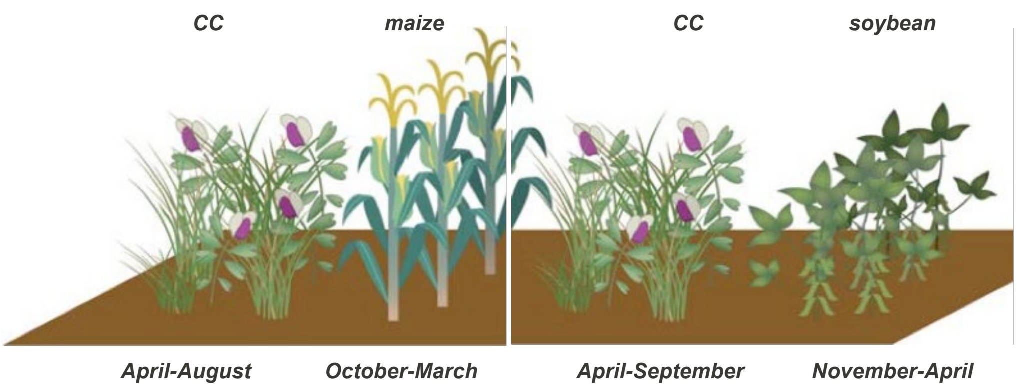

Figure 2. Scheme of the crop sequence in the long-term experiment with cover crops (CC): common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture. Harvest crop: maize and soybean. The months mentioned are the CC and crop sowing dates (González et al. 2017).

2.3. Cover crops and harvest crops

All CC were fertilized at seeding time and were killed in their reproductive stage by glyphosate application after harvesting the summer crop. The soybean and maize crops were sown between October and December each year. The seeding density was 270,000 and 65,000 plants ha^{-1} for soybean and maize, respectively.

At physiological maturity, the above-ground biomass of the cover crops was harvested manually from three 0.5 m^2 areas per sampling point at CC killing time. The total aerial dry matter yield was determined after drying in a forced-air oven at 65 ºC for at least 72 hours.

Soybean and maize yields were obtained by mechanical harvesting from three subsamples (1.0 m^2) per experimental unit. Grains were separated from all other vegetal material and dry matter weights were recorded separately.

2.4. Soil physical fractionation of SOM and soil analysis

After the soybean harvest, in 2017, soil samples (three replicates) were taken randomly at 0-0.10 and 0.10-0.20 m depths from each plot. The samples were air-dried, crushed and passed through a 2-mm aperture sieve.

The SOM was physically separated by wet sieving (Cambardella and Elliot 1992; Duval et al. 2013). Briefly, 50 g of previously air-dried and sieved soil was dispersed in 120-mL glass containers and mixed with 105 mL of distilled water. Ten glass beads (5 mm diameter) were added to increase aggregate destruction and reduce any potential problems created by sand. After dispersion, the soil suspension was sieved through 2 connected sieves of 53 and 105 μm diameter. The sieves were moved back and forth and the soil retained on the top of the sieve was sprinkled with distilled water until the water in the bottom sieve was clear to the naked eye. Three fractions were obtained: i) the coarse fraction (105-2000 mm) containing coarse particulate organic carbon (POCc) and fine to coarse sands, ii) the medium fraction (53-105 mm) containing fine particulate organic carbon (POCf) and very fine sands, and iii) the fine fraction (< 53 mm) containing mineral-associated organic carbon (MOC) as well as silt and clay. The material retained in each sieve was dried, homogenized and analyzed for OC. The C content was determined by dry combustion (LECO, St. Joseph, MI).

Each soil sample was tested with hydrochloric acid to verify the presence of carbonates (Schoenenberger et al. 1998). However, no carbonates were found in any of the samples at the 0-0.20 m soil depth.

Total N concentration was determined by the semi-micro method of Kjeldahl (Bremner 1996; IRAM-SAGyP 2018).

Stratification ratios (SR) of SOC and TN were calculated based on the concentration of SOC at a depth of 0-0.10 m, divided by the concentration of that property at a depth of 0.10-0.20 m (Franzluebbers 2002). The degree of stratification of soil organic C and TN pools with soil depth, expressed as a ratio, could indicate soil quality or soil ecosystem functioning, because surface organic matter is essential to erosion control, water infiltration, and conservation of nutrients.

The fine soil fraction was subjected to acid hydrolysis with a modified procedure of the method described by Paul et al. (1997). Briefly, 0.3-0.5 g of sample was refluxed at 118 °C for 16 hours in 20 ml of 6 M HCl. If insufficient material was recovered during the physical fractionation, individual replicates were combined. The hydrolysate was discarded. The unhydrolyzed residue was washed in deionized water with repeated centrifugation and decantation, and then transferred to pre-weighed vials and dried at 60 °C to constant weight. The resistant (residue) fraction, taken as the recalcitrance pool, was analyzed for ROC and RN. It was assumed that this residue only contained the recalcitrant pool. The degree of recalcitrance was expressed as the Recalcitrance Index (RIC) proposed by Rovira and Vallejo (2002):

RIc (%) = (non-hydrolyzed C (ROC)/SOC) * 100

2.5. Carbon balance and residues decomposition

The most common model used to describe the dynamic behavior of SOC, or its fractions, is the first-order model (Six and Jastrow 2002):

$\delta SOC / \delta t = I - k \times SOC$

Where SOC: soil organic carbon; I: carbon input; k: decomposition rate.

The value of k is generally determined by isotopic techniques (Balesdent and Balabane 1996). However, approximate k-values may also be obtained from long-term experiments.

When a steady state is reached and no change in SOC or its fractions is expected over time ($\delta COS/\delta t = 0$), the k-value can be estimated as follows:

$K = I/SOC$

In this study, only the decomposition rate of POCc and POCc + POCf were calculated.

2.6. Data analysis

The statistical significance of the cover crop effect was determined using analysis of variance (ANOVA) for a split plot design. When significant differences were detected, the comparison of means test was applied using the least significant difference (LSD). When the interaction between CC and fertilization management was significant, each CC was analyzed independently.

The Pearson correlation analysis was conducted to evaluate the relationship between the chemical variables (% SOC and k) and the C input with CC residues. Contrast tests between the control and CC treatments were also applied. A Student t test was applied to analyze the differences between the 2010 and 2017 periods. All data were analyzed using Infostat statistical software (Di Rienzo et al. 2013). The significance level used for all the statistical analysis was 0.05.

3. Results and Discussion

3.1. Soil organic carbon and fractions

The effects of CC inclusion and N fertilization are shown in **Table 2**. The analysis of the results demonstrated significant differences in the SOC concentration. At the end of the CC cycle, the SOC at the 0-0.10 m depth was 16% higher than that in Ct (soil without cover crops). The SOC values were 22.3 vs. 19.2 g kg^{-1} ($p < 0.001$).

Cover crop fertilization did not show any differences between the CC, but nevertheless the SOC concentration was on average 21.0 g kg^{-1} (nf treatment) and 22.6 g kg^{-1} (f treatment), both 7.6% higher than Ct soil ($p < 0.05$). No significant interaction was found between CC inclusion and N fertilization. This result suggests that all treatments responded to N addition in the same way, therefore the average data from the treatments were used for the statistical analysis.

No significant differences were detected in SOC for the CC and fertilization effects at the 0.10-0.20 m depths. Because of this behavior, SOC and TN concentration decreased with soil depth and thus caused a natural stratification with residue accumulation on the surface. The SOC concentration was highest in the superficial layer (21.8 g kg^{-1}, average f and nf plots) followed by the 0.10-0.20 m layer (14.2 g kg^{-1}). Total N concentration showed a similar distribution at the different depths: 2.5 g kg^{-1} in the 0-0.10 m layer and 1.7 g kg^{-1} at 0.10-0.20 m depth. Other authors in other parts of the world have also reported this surface accumulation when evaluating different CC; the reduction in the intensity of soil tillage and the use of crops to maximize the amount of residue on the surface are commonly used management practices to maintain or increase SOC (Six et al. 1999).

Using contrast analysis for the 0.10-0.20 m depth, significant differences ($p < 0.05$) were observed in SOC levels between CC based on pure crops (PC) (14.5 g kg^{-1}, average of triticale, rye and vetch) compared with the vetch and VV + T mixture (13.5 g kg^{-1}). No significant differences were observed for the TN concentration with either CC inclusion or N fertilization, but the

Table 2. Distribution and concentration of soil organic carbon and total nitrogen in the control and with cover crops soils, in 0-0.10, 0.10-0.20 and 0-0.20 m depth

CC	SOC		X	TN		X	C:N
	nf	f		nf	f		
	g kg^{-1}				g kg^{-1}		
			0-0.10 m				
Ct	18.4	20.0	19.2 a	2.3	2.3	2.3	8.4
VS	21.7	21.5	21.6 b	2.5	2.6	2.6	8.3
VV	21.5	23.6	22.6 b	2.6	2.8	2.7	8.4
VV+T	21.2	23.6	22.4 b	2.6	2.5	2.6	8.6
T	21.2	23.7	22.4 b	2.4	2.7	2.6	8.6
R	21.9	23.5	22.7 b	2.1	2.3	2.2	10.3
X	21.0 A	22.6 B		2.5	2.6		8.8
Ct vs CC			***				
			0.10-0.20 m				
Ct	13.4	14.3	13.9	1.6	1.5	1.6	8.7
VS	14.8	14.3	14.6	2.0	1.9	1.9	7.7
VV	14.7	13.9	14.3	1.8	1.9	1.8	7.9
VV+T	13.1	13.8	13.5	1.7	1.8	1.7	7.9
T	14.0	15.1	14.6	1.7	1.6	1.7	8.6
R	14.3	15.1	14.7	1.6	1.4	1.5	9.8
X	14.1	14.4		1.7	1.7		8.4
VV+T vs PC			*				
L vs G						*	
			0-0.20 m				
Ct	15.9	17.2	16.6 a	1.7	1.9	1.8 a	8.5
VS	18.3	17.9	18.1 b	2.3	2.3	2.3 b	8.0
VV	18.1	18.8	18.5 b	2.2	2.3	2.3 b	8.2
VV+T	17.2	18.7	18.0 b	2.1	2.2	2.2 ab	8.3
T	17.6	19.4	18.5 b	2.1	2.2	2.1 ab	8.6
R	18.1	19.3	18.7 b	1.9	1.9	1.9 ab	10.0
X	17.6 A	18.6 B		2.1	2.1		
Ct vs CC			***			*	

Different lowercase letters in the mean column (X) indicates significant differences ($p < 0.05$) between CC. Different capital letters in the mean row (X) indicates significant differences ($p < 0.05$) between nf and f treatment. *, ***, significant differences at 0.05, 0.001 probability levels, respectively. nf and f: unfertilized and fertilized treatments. SOC: soil organic carbon. TN: total nitrogen. C:N, carbon:nitrogen rate. X: mean values. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture, control (Ct), leguminosae (L), gramineae (G), pure crops (PC).

legume species increased significantly from 1.6 to 1.8 g kg^{-1}.

Summarizing the 0-0.20 m depths, the effects of SOC and TN concentrations were detected due to the inclusion of CC of different species.

Concentration of the labile fractions (POCc and POCf) showed similar behavior to the total SOC (**Table 3**).

The concentration of SOC and its fractions decreased with soil depth, and thus caused a natural stratification with residue accumulation on the surface. Higher labile soil organic levels

Table 3. Distribution and content of labile soil organic carbon fractions and mineral-associated organic carbon, in the control and with cover crops soils, in 0-0.10 and 0.10-0.20 m

CC	POCc		X	POCf		X	MOC		X
	nf	f		nf	f		nf	f	
					g kg^{-1}				
					0-0.10 m				
Ct	4.5	5.5	5.0	2.0	2.2	2.1 a	12.0	12.4	12.2
VS	5.1	6.1	5.6	2.8	3.0	2.9 b	13.8	12.4	13.1
VV	6.1	7.9	7.0	2.7	3.3	3.0 b	12.6	12.3	12.5
VV+T	5.3	7.4	6.4	3.2	3.3	3.3 b	12.7	12.8	12.8
T	4.9	6.5	5.7	2.8	3.7	3.3 b	13.4	13.4	13.4
R	5.7	6.2	6.0	2.8	3.3	3.0 b	13.4	14.1	13.7
X	5.3 A	6.6 B		2.7 A	3.1 B		13.0	12.9	
Ct vs CC						***			
					0.10-0.20 m				
Ct	2.3	2.8	2.6	0.7	0.8	0.8	10.4	10.7	10.6
VS	1.6	2.6	2.1	0.8	0.8	0.8	12.3	11.0	11.7
VV	2.4	3.3	2.8	0.8	0.8	0.8	11.4	9.9	10.7
VV+T	2.3	3.6	3.0	0.8	0.8	0.8	10.1	9.4	9.8
T	2.7	4.2	3.5	0.9	1.0	0.9	10.5	9.9	10.2
R	1.8	1.5	1.7	1.8	1.0	1.4	11.3	12.7	12.0
X	2.2	3.0		1.0	0.8		11.0	10.6	
VV+T vs PC									*
L vs G						*			

Different lowercase letters in the mean column (X) indicates significant differences ($p < 0.05$) between CC. Different capital letters in the mean row (X) indicates significant differences ($p < 0.05$) between nf and f treatment. *, ***, significant differences at 0.05, 0.001 probability levels, respectively. nf and f: unfertilized and fertilized treatments. POCc: coarse particulate organic carbon, POCf: fine particulate organic carbon, MOC: mineral-associated organic carbon. X: mean values. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T), mixture, control (Ct), leguminosae (L), gramineae (G), pure crops (PC).

were observed in the 0-0.10 m layer than at the 0.10-0.20 m depth. The distribution and quality of the POCc labile fraction showed no differences between the control soil (Ct) and CC, therefore this fraction was analyzed as a whole. The results suggested that 9 years of C-input from cover crops were insufficient to affect the most dynamic and labile fractions of soil organic matter, despite the differences in C-input between the species. These effects agree with those reported by Sainju et al. (2003), who found no CC effects on the labile soil organic fractions after 2 years of cumulative effects.

Fertilization enhanced the POCc fraction significantly (24.5%), increasing from 5.3 to 6.6 g kg^{-1}, whereas POCf levels rose from 2.7 to 3.1 g kg^{-1} (14.0%) ($p < 0.05$). In this labile fraction the contrast analysis detected significant differences between CC inclusion and Ct ($p < 0.001$). Cover crops enhanced POCf concentration by 47% (3.3 vs 2.1 g kg^{-1}). This difference may have resulted from the quality of the gramineae and leguminosae species used as CC. It is to be noted that soil fractioning by particle size may reflect the changes in soil use and agricultural practices in more detail. The mineral-associated organic carbon fraction (MOC) showed minor variations due to soil depth.

Some authors found similar conclusions in labile CO fractions at all the depths analyzed, with the highest level in the 0-0.05 m surface layer, corroborating that this fraction is a more sensitive indicator and would be able to detect management practice effects (Eiza 2005).

Cazorla et al. (2013) found increments in the same fractions by CC inclusion at all the depths studied in a Typic Argiudoll. The effect on the labile fraction of C could be in part due to greater addition of residues, higher input of crop roots and greater stability of aggregates in the system (Liu et al. 2005).

Cover crop fertilization did not show any changes in the CO fractions - neither the labile nor the stable ones - at 0.10-0.20 m depth. Concentration of fine labile soil organic carbon (POCf) increased significantly ($p < 0.05$) when G was used as a CC, compared with L (1.1 vs 0.8 g kg^{-1}), whereas pure crops increased the MOC concentration compared with that of a mixture (11.1 vs 9.8 g kg^{-1}).

Stratification of SOC showed similar values for the control (Ct) and CC soils. The stratification rate (SR) was 1.38 for the natural soil, lower than for CC, which had values varying from 1.53 to 1.66 (**Figure 3**). Regarding the SOC, stratification values higher than 2.0 are essential for soil quality maintenance (Franzluebbers 2002; Fernández-Romero et al. 2016). The degree of stratification with soil depth, expressed as a ratio, might be related to soil quality or soil ecosystem functioning. A value of 2 for SR is considered the limit for good-quality agricultural soils. In this study, SR < 2 would indicate the negative effect of a soybean-maize sequence on the soil quality, although this management practice was slightly enhanced with respect to the control soil.

For TN pools, soils with CC inclusion showed a homogeneous N distribution for all treatments. The SR value was less than 2 (1.44), showing a fast decomposition of the superficial residue, in comparison with the SR of SOC.

3.2. C balance

During the 2008-2017 period, CC dry matter-input in the soil differed between years. In all years, VS supplied the lowest dry matter-input to the soil (4000 kg ha^{-1} year^{-1} on average) and T the highest (8700 kg ha^{-1} year^{-1}) due to a larger biomass accumulation. Cover crop fertilization did not reflect any significant differences except in 2012 with the VV+T mixture. The C-input into soil was estimated from this data. C-input by VS was 1600 kg ha^{-1} year^{-1} and by T was 3550 kg ha^{-1} year^{-1}, and the rest of CC supplied intermediate values. These results are consistent with other observations reported by Galantini et al. (2002),

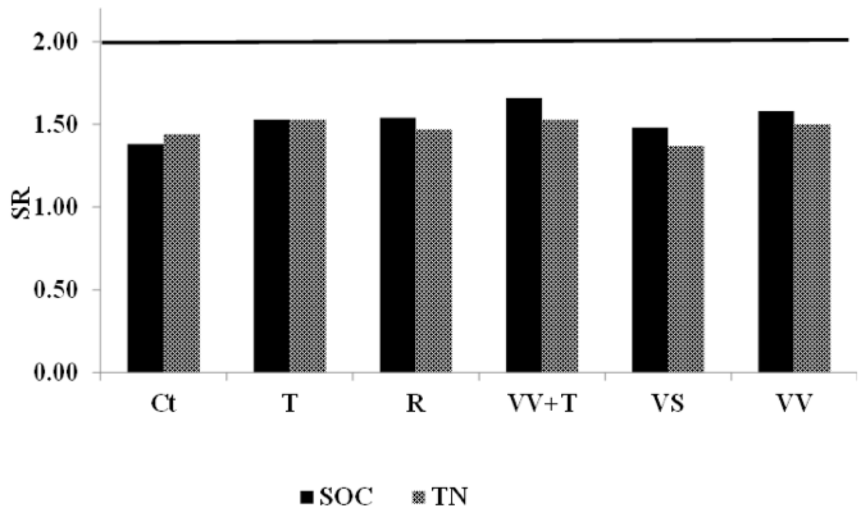

Figure 3. Stratification ratio (SR) of soil organic carbon (SOC) and total nitrogen (TN) concentrations. The horizontal line is the stratification rate threshold. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture.

which revealed that variations in the amount of C supplied by CC were caused by differences in water-use efficiency during the fallow period (in SOC decomposition) and during the crop cycle (in dry matter production and C-input into the soil).

The amount of residue C-input into soil from the crop harvest was estimated by taking account of a harvest index of 0.47 for soybean and 0.48 for maize (Duval et al. 2016). The biomass C-harvest crop (soybean and maize) input sequence was VS > VV > VV + T > R > T (Figure 4) and the C-CC input sequence was T > VV + T > R > VV > VS. The total C-input into the soil from both the harvest crop and CC showed the same sequence T > VV + T > R > VV > VS, corroborating that triticale improved the C balance whereas legume species supplied the smallest biomass residues. Differences in SOC and organic fraction concentrations could be due to the presence of different C-input quantities.

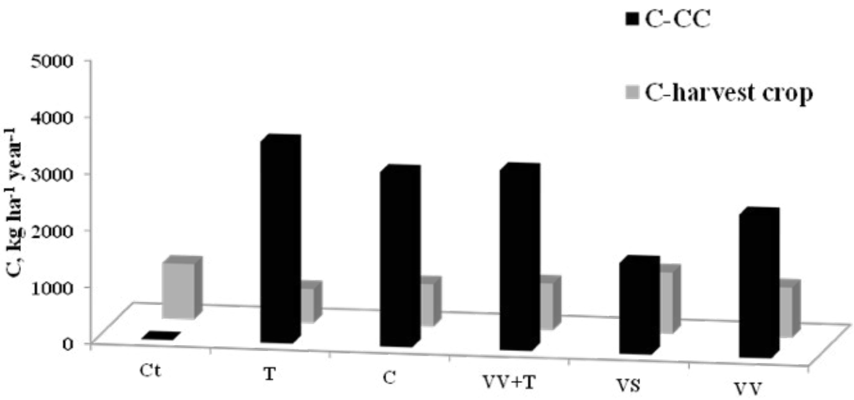

Figure 4. Relationship between C-input by cover crops and C-input by harvest crops and annually soil C content. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T), hairy vetch (VV) + triticale (T) mixture.

Since the initial SOC in the control (Ct) plot in the experimental site was 16.4 g kg^{-1}, the CC treatments ranged from 18.1 g kg^{-1} with VS to 18.7 g kg^{-1} with R. The other CC showed medium values (Figure 5). The C-input into the soil showed an association with the TOC concentration (R^2 = 0.59, $p < 0.001$), according to previous information. The Ct plot needed at least 2.9 Mg C-input ha^{-1} year^{-1} to avoid a decrease in SOC at the 0-0.20 m depth.

This strong association was greater with the fine labile carbon fraction (POCf) (R^2 = 0.91, $p < 0.05$), than with the coarse labile carbon fraction (POCc) (R^2 = 0.2948, ns) and MOC (R^2 = 0.0748, ns) (Figure 6). The absence of association with the latter fractions could be due to fast residue decomposition that accumulated in POCf and is probably related to the low C:N rate and the low N stratification.

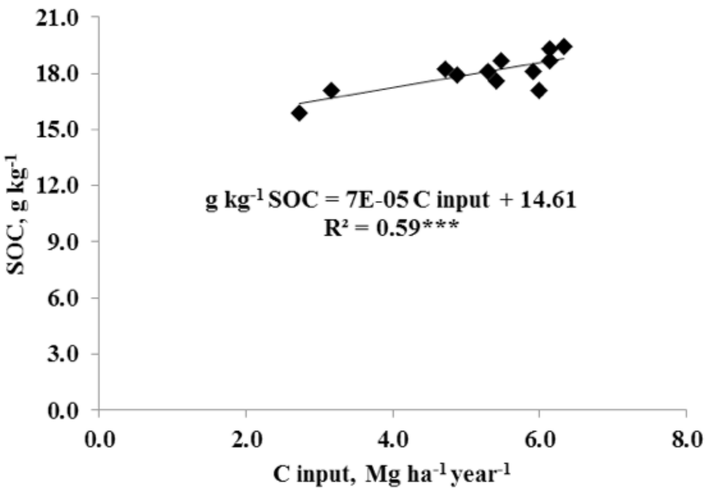

Figure 5. Relationship between C input by cover crops and concentration of soil organic carbon (SOC) at 0-0.20 m depth. Significant level, *** ($p < 0.001$).

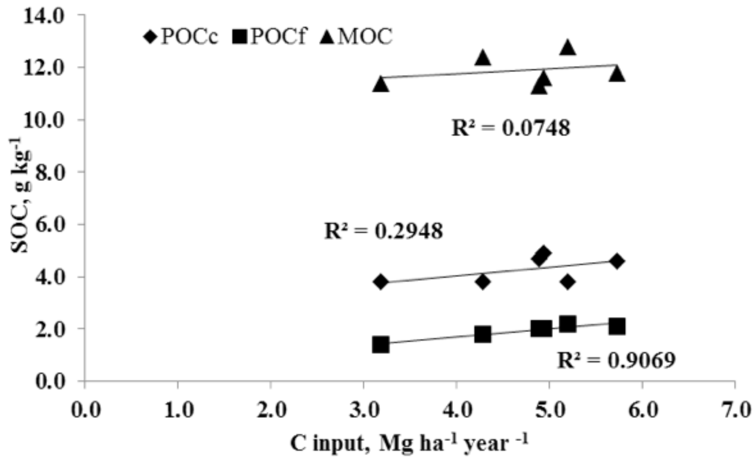

Figure 6. Relationship between C-input by cover crops, labile carbon fractions (coarse and fine) and the mineral-associated organic carbon (MOC) concentrations.

3.3. Residue decomposition

It is known that labile soil organic carbon fractions reach an equilibrium or steady state in a shorter period of time (5-10 years) than the recalcitrant fraction (decades or centuries) (Duval et al. 2015). The transformation rate (k) can thus be estimated from the average annual C-input and C content in the soil. Increase in TOC content (Δ) was significantly related to the amount of C supplied by the cover crops and harvested crops ($R^2 = 0.93$, $p < 0.0001$) (**Figure 7a**). In the 0-0.20 m layer the C-input into the soil (mostly by CC) improved the C-balance, which was the main goal of this management practice for the area under study. The content of labile soil organic carbon (POCc+f) showed less association with the soil carbon changes and C-input into the soil ($R^2 = 0.77$, $p < 0.05$) (**Figure 8a**).

The k values estimated ranged from 0.02 to 0.09 for SOC and from 0.07 to 0.24 for POCc+f. This variation was associated with the C-input (**Figures 7b, 8b**). This was also found by Duval et al. (2016) and indicates that higher C-amounts, mainly supplied by the cover crops, enhanced the decomposition rate or the "priming effect". The more labile soil organic carbon fraction (POCc) with rye as the CC showed a faster decomposition rate with a value of 0.38 (**Table 4**).

a)
b)

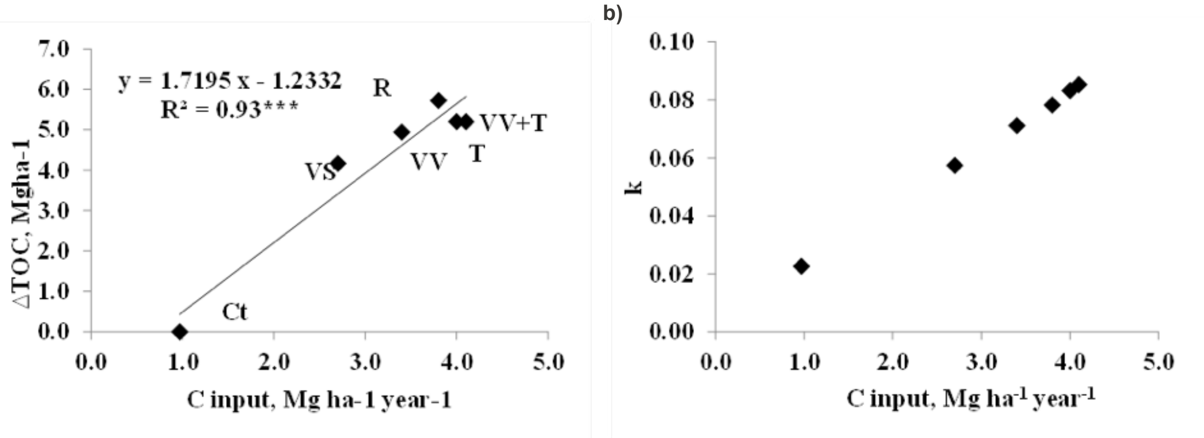

Figure 7. a) Relationship between annual C-input, changes in the content of total organic carbon (TOC). b) Decomposition rate (k). Significant level, *** (p < 0.001).

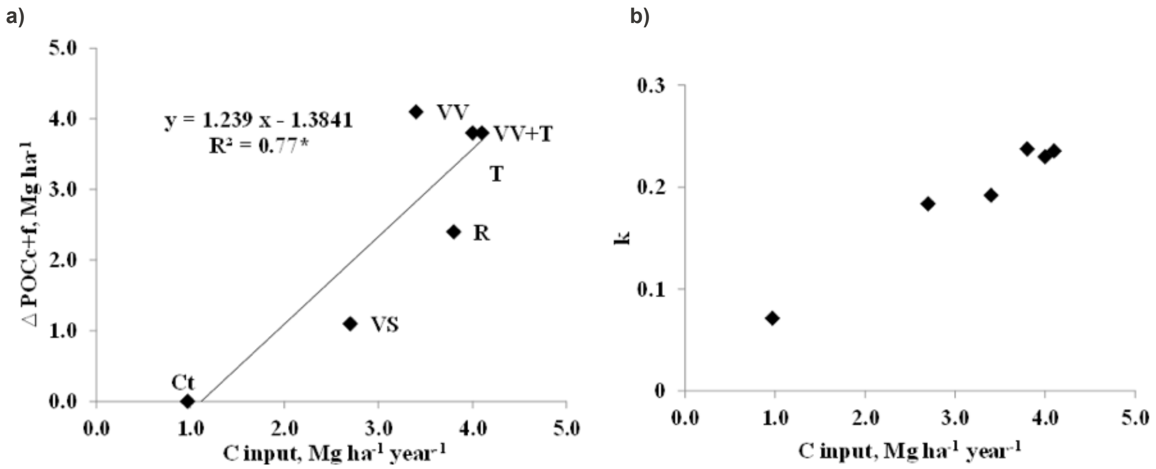

Figure 8. a) Relationship between annual C-input and changes in the content of labile organic carbon fractions (POCc+f). b) Decomposition rate (k). Significant level, * ($p < 0.05$).

In both cases, the greater k values corresponded to gramineous species, such as triticale and rye. The slight differences between treatments in the sampling period (9 years) could be associated with the immediate use of C by soil microorganisms and the fast residue decomposition accumulating in the labile organic carbon fraction.

Table 4. Decomposition rate of soil organic carbon and labile fractions under cover crops treatments

CC	k		
	SOC	POCc	POCc+f
Ct	0.02	0.10	0.07
VS	0.06	0.27	0.18
VV	0.07	0.27	0.19
VV+T	0.08	0.33	0.23
T	0.08	0.34	0.24
R	0.08	0.38	0.24

k: decomposition rate. SOC: soil organic carbon, POCc: coarse particulate organic carbon, POCf: fine particulate organic carbon. Common vetch (VS), hairy vetch (VV), rye (R), triticale (T) hairy vetch (VV) + triticale (T) mixture, Control (Ct).

3.4. Changes in SOC after 7 years under gramineae species

After the 2010-2017 period of using gramineae (G) (triticale and rye) as the CC in a soybean-maize sequence, the levels of SOC and its fractions (labile organic carbon and mineral-associated organic carbon) at the 0-0.20 m depth generally increased, probably due to the surface accumulation of G residues (Table 5). Other authors in other parts of the world (Bella 2015) have also reported this surface effect when evaluating different cover crop species. During this period, the maize yields were on average 8300 kg ha^{-1} less with G-CC compared with Ct, but nevertheless the soybean grain yields did not show any significant differences between the control and the CC treatments, with a production of 3500 kg ha^{-1} (data not showed) (Bella 2015).

The SOC concentration increased 20 and 22% ($p < 0.05$) with the inclusion of a triticale (f and nf) cover crop. This species had the highest production potential (annual average of 9000 kg ha^{-1}) with a fertilization response.

The labile carbon fraction (COPc and COPf) concentrations showed variable balances. The POCc fraction increased from 58 to 192% ($p < 0.05$), contrary to that observed with the Ct which decreased by 5%. Gramineae cover crop effects on the POCf concentration showed an increase of 64% when triticale f was used ($p < 0.001$). In the MOC fraction, these changes were observed less and they ranged from increases of 3.3% to losses of 7.1%.

In this study, POCc seemed to be more sensitive to a gramineae CC effect; the cover crops also increased SOC lability, as revealed by the increased proportion, especially of POCc in the 0-0.20 m layer soil compared with MOC.

Table 5. Soil organic carbon, coarse particulate organic carbon, fine particulate organic carbon, and mineral associated organic carbon contents under cover crops treatments at 0-0.20 m depth

CC	Year	SOC	POCc	POCf	MOC
		g kg^{-1}			
Ct	2010	16.3	4.0	1.4	10.9
	2017	16.5	3.8	1.4	11.4
	Δ	+ 1.2	-5.0	0	+ 4.5
R f	2010	17.5	2.4	1.8	13.3
	2017	19.3	3.8	2.1	13.4
	Δ	+ 10.3	+ 58.3	+ 16.7	+ 0.7
T nf	2010	14.4	1.3	1.8	11.2
	2017	17.6	3.8	1.8	12.0
	Δ	+ 22.2*	+ 192*	0	+ 7.1
T f	2010	16.1	2.7	1.4	12.0
	2017	19.4	5.4	2.3	11.6
		+ 20.5	+ 100	+ 64.3**	- 3.3

*: significant differences at 0.05; 0.001 probability levels. nf and f: unfertilized and fertilized treatment. SOC: soil organic carbon, POCc: coarse particulate organic carbon, POCf: fine particulate organic carbon, MOC: mineral-associated organic carbon. Rye (R), Triticale (T), Control (Ct).

3.5. Quality of the recalcitrant organic matter

No statistical differences for the mineral-associated organic carbon (MOC) fraction between treatments were observed. In order to verify if there were any changes in their quality, the recalcitrant fraction (ROC) and the Recalcitrance Index (RIc) of this fraction were determined.

The recalcitrant C behaved differently from SOC and did not show any significant increases with the use of CC (9.1 g kg^{-1} on average) (**Table 6**). This behavior could indicate that stabilization of carbon in a recalcitrant form may be limited by the organic matter input (Pandey et al. 2014). The gramineae species would produce slight increases in organic materials and resistant fractions, due to their high C: N ratio (9.8 g kg^{-1} on average). The soil recalcitrant pool obtained by acid hydrolysis is composed of organic molecules that are more difficult to be degraded naturally, hence they are stable over time and may be considered the lower limit of the carbon stock. It could be inferred from this chemical method that it would be possible to estimate the size of the labile C pool.

On average, the Recalcitrant Index of C (RIc) in the upper horizon was 46% compared with 71% in the sub-superficial layer, suggesting that the non-hydrolysable pool, such as the higher C stable fraction, would accumulate at depth. Generally, the greater biomass supplied by the residue input, such as CC, would contribute to OC accumulation, mostly in labile compounds.

Table 6. Soil organic carbon, recalcitrant carbon, total nitrogen, recalcitrant nitrogen contents and recalcitrance indices under cover crop treatments at the 0-0.10, 0.10-0.20 and 0-0.20 m depths

CC	CARBON				NITROGEN			
	SOC	MOC	ROC	RIc	TN	RN	RIn	ROC:RN
	g kg^{-1}							
	0-0.10 m							
Ct	18.4 a	11.4	9.0	48.9	2.5	0.4	17.2 a	22.5
T	21.2 b	13.4	10.2	48.0	2.4	0.6	24.1 b	17.0
R	21.9 b	13.4	9.6	43.9	2.2	0.5	25.1 b	19.2
	0.10-0.20 m							
Ct	13.5	10.5	9.2	68.2	1.7	0.6	37.6	15.3
T	14.0	10.5	9.4	75.4	1.7	0.5	33.1	18.8
R	14.3	11.3	10.2	70.8	1.6	0.6	36.8	17.0
	0-0.20 m							
Ct	15.9	10.9	9.1	58.5	2.1	0.5	27.4	18.9
T	17.6	11.9	9.8	61.7	2.0	0.5	28.6	17.9
R	18.1	12.3	9.9	57.3	1.9	0.5	30.9	18.1

Different lowercase letters in the column indicate significant differences ($p < 0.05$) between CC. SOC: soil organic carbon, MOC: mineral-associated organic carbon, ROC: recalcitrant organic carbon. RIc: recalcitrance index of carbon, TN: total nitrogen, RN: recalcitrant nitrogen, RIn: recalcitrance index of nitrogen.

This is especially important because the content of this fraction is associated with potential soil fertility. This index showed that the surface layer (0-0.10 m) had more labile material contents compared with the 0.10-0.20 m depth, where the most stable fractions of OC were accumulated. These results are consistent with other observations reported in the literature (Rovira and Vallejo 2007), which confirmed the extended concept that SOC stabilizes in deep horizons because it is more physically protected and it is in a more advanced state of biochemical stabilization relative to the SOC in surface horizons.

Unlike the ROC, the RN showed similar values for all the treatments and sampling depths (**Table 6**). RIn increased with soil depth; in the 0-0.10 m layer the control value was significantly lower than the gramineae ($p < 0.05$), but the trend was not observed in the 0.10-0.20 m depth.

There are conflicting results in the literature for changes in RIn values. In soils of the Argentinian pampas, N recalcitrance decreased with depth in most cases, in both burnt and unburnt soils (Sánchez and Lázzari 1999); N recalcitrance was more or less constant throughout the soil profile except for some N-poor horizons, in which the RIn values were much higher (Tan et al. 2004).

4. Conclusions

This study demonstrated that the effect of cover crops on SOC and the labile fraction (POCf) in the upper soil layer was strongly related with high residue production. However, it was insufficient to increase three physical fractions of soil organic carbon values in deep soil. No changes in soil organic carbon concentrations in the surface soil and in the stratification index were shown after 9-year long studied period with a summer soybean-maize sequence. Gramineae crops were more efficient in biomass production than the legume crops, with more C input into soils. Triticale was the most suitable cover crop showing a high positive C balance in both SOC and the labile fraction. An increase in the residue decomposition rate was also detected. CC had a positive impact on recalcitrant C and the recalcitrance carbon index in the sub-superficial layer, showing more stable C stock and less labile C than in the superficial layer.

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Soil contribution to CO_2 fluxes in *Chinampa* ecosystems, Mexico

Contribución de los flujos de CO_2 de suelos en ecosistemas de Chinampa*, México*
Contribuição do solo para os fluxos de CO_2 nos ecossistemas Chinampa*, México*

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ABSTRACT

Since soil CO_2 flux is a key component of ecosystem carbon balance, quantifying its contribution to the ecosystem carbon flux and understanding the factors that underlie its temporal variation is crucial for a better comprehension of ecosystem carbon dynamics under climate change and for optimal ecosystem use and management. Our objectives were to quantify the contributions of total soil CO_2 efflux (F_S) to ecosystem respiration (R_E) and heterotrophic soil CO_2 efflux (F_H) to F_S in two *chinampa* ecosystems with different natural grass covers. We also aimed to identify the main environmental drivers of seasonal variability of these contributions. The CO_2 fluxes were measured on each site about every 14 days from September 2008 to August 2009 in the Xochimilco Ecological Park in Mexico City using dark chamber techniques. For two studied sites, R_E, F_S and F_H were estimated on average as 94.1 ± 8.5, 34.7 ± 3.5 and 16.5 ± 1.7 (± S.E.) mg C-CO_2 m^{-2} h^{-1}, respectively. On average over the study period and sites, the annual cumulative R_E, F_S and F_H fluxes were 824 ± 74, 304 ± 31 and 145 ± 15 g C m^{-2} year, respectively. The R_E, F_S and F_H varied between the winter and summer seasons; this variation was explained mostly by seasonal variations of soil temperature, soil water content and shoot plant biomass. Temperature sensitivity of CO_2 fluxes depended on vegetation type and plant growth differences among the sites and decreased in the following order: $R_E > R_s > R_H$. The contribution of F_S to R_E and F_H to F_S for the two studied sites and period averaged about 38% and 50%, respectively regardless of the site vegetation type, but the degree of F_S/R_E and F_H/F_S variability depended on the differences in seasonal dynamics of plant cover. The contribution of F_H to F_S varied from 37% in summer to 73% in winter at the site without a seasonal shift in dominant plant species, but F_H/F_S was close to constant during the year at the site with a seasonal change in dominant plant species. During the cold period, the contribution of F_H to F_S increased following plant growth decrease. The linear regression analysis showed that plant biomass was the dominant factor controlling the seasonal variation of F_H/F_S ratios, whereas the plant biomass dynamic followed the dynamics of soil water content, water table depth, and soil temperature. Our results suggest that seasonal variation of soil contribution to total fluxes from the *chinampa* ecosystem is locally differentiated. These differences were related to differences in seasonal dynamics of cover productivity which has been associated with localization of soil water content. This finding has important implications for assessing the contribution of the *chinampa* ecosystem to the global carbon budget.

RESUMEN

Dado que el flujo de CO_2 del suelo es un componente clave del balance de carbono del ecosistema, la cuantificación de su contribución a las pérdidas de carbono del ecosistema y la comprensión de los factores que subyacen a la variación temporal de la magnitude de los flujos es crucial para una mayor comprensión de la dinámica del carbono del ecosistema conforme a los cambios climáticos y para planificar el uso y la gestión óptima de los ecosistemas. Nuestros objetivos fueron cuantificar la contribución del flujo de CO_2 total del suelo (F_S) a la respiración del ecosistema (R_E) y el flujo de CO_2 heterótrofo del suelo (F_H) con respect a F_S en dos ecosistemas de chinampa *con cobertura de*

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pastizal de diferente tipo. Además se trataron de identificar los factores principales de la variabilidad estacional de estas contribuciones. Los flujos de CO_2 se midieron en cada sitio cada 14 días desde septiembre de 2008 a agosto de 2009 en el Parque Ecológico de Xochimilco de la Ciudad de México, usando la técnica de cámaras obscuras. En promedio, para los dos sitios estudiados, R_E, F_S y F_H fueron, respectivamente, 94,1 ± 8,5, 34,7 ± 3,5 y 16,5 ± 1,7 (± S.E.) mg C-CO_2 m^{-2} h^{-1}. En promedio, los flujos anuales acumulativos R_E, F_S y F_H durante el periodo de estudio en los dos sitios fueron 824 ± 74, 304 ± 31 y 145 ± 15 g C m^{-2} por año, respectivamente. La variación entre las estaciones de verano e invierno de R_E, F_S y F_H se explicó principalmente por las variaciones estacionales de temperatura del suelo, contenido de agua en el suelo y biomasa de los brotes de las plantas. La sensibilidad a la temperatura de los flujos de CO_2 depende del tipo de vegetación y de las diferencias en el crecimiento de las plantas entre los sitios y disminuye en el orden siguiente: $R_E > R_S > R_H$. La contribución de F_S a R_E y F_H a Fs para los dos sitios estudiados y el periodo promedió cerca de 38% y 50% respectivamente, sin importar el tipo de vegetación del sitio; el grado de variabilidad de F_S/R_E y F_H/F_S dependió de las diferencias en la dinámica estacional de la cobertura de la vegetación. La contribución de F_H a F_S varió de un 37% en verano a un 73% en invierno en el sitio sin cambio estacional de plantas dominantes, pero fue casi constante durante el año para F_H/F_S en el sitio con cambio estacional de especies de plantas dominantes. Durante el period frío la contribución de F_H a F_S aumentó conforme a la disminución del crecimiento de las plantas. El análisis de regresión lineal mostró que la biomasa de las plantas fue el factor dominante que controlaba la variación estacional de las relaciones F_H/F_S, mientras que la dinámica de la biomasa de las plantas siguió la dinámica del contenido de agua en el suelo, la profundidad del manto freático y la temperatura del suelo. Nuestros resultados sugieren que la variación estacional de la contribución del suelo a los flujos totales del ecosistema chinampa *se diferencia localmente. Estas diferencias están relacionadas con las diferencias en la dinámica estacional de la productividad de la vegetación que se asocia con la localización del contenido de agua del suelo. Este hallazgo puede tener implicaciones importantes para evaluar la contribución del ecosistema de* chinampa *al contenido global de carbono.*

RESUMO

Dado que o fluxo de CO_2 do solo é um componente chave do balanço de carbono nos ecossistemas, a quantificação da sua contribuição para o fluxo de carbono no ecossistema e a compreensão dos fatores subjacentes à sua variação temporal são cruciais para uma melhor compreensão da dinâmica do carbono no ecossistema no âmbito das alterações climáticas e para planear o uso e a mais correta gestão dos ecossistemas. Os nossos objetivos foram quantificar as contribuições do fluxo total de CO_2 do solo (F_S) para a respiração do ecossistema (R_E) e do fluxo heterotrófico de CO_2 do solo (F_H) relativamente a F_S em dois ecossistemas chinampa *com diferentes coberturas naturais de pastagem. Também se pretendeu identificar os principais fatores da variabilidade sazonal dessas contribuições. Os fluxos de CO_2 foram medidos em cada local cada 14 dias de setembro de 2008 a agosto de 2009 no Parque Ecológico Xochimilco na cidade do México usando técnicas de câmara escura. Em média, para os dois sítios estudados, R_E, F_S e F_H foram, respetivamente, 94,1 ± 8,5, 34,7 ± 3,5 e 16,5 ± 1,7 (± S.E.) mg C-CO_2 m^{-2} h^{-1}. Os fluxos anuais cumulativos de R_E, F_S e F_H durante o período de estudo e nos dois sítios foram em média, respetivamente, 824 ± 74, 304 ± 31 e 145 ± 15 g C m^{-2} por ano. A variação entre as estações de verão e inverno de R_E, F_S e F_H explicou-se, principalmente, pelas variações sazonais da temperatura do solo, conteúdo de água no solo e biomassa da parte aérea das plantas. A sensibilidade à temperatura dos fluxos de CO_2 depende do tipo de vegetação e das diferenças no crescimento das plantas entre os sítios e diminuiu de cordo com a ordem seguinte: $R_E > R_S > R_H$. A contribuição de F_S para R_E e F_H para F_S, para os dois sítios e período estudados, foi em média de cerca de 38% e 50%, respetivamente, independentemente da vegetação do sítio; o grau de variabilidade de F_S/R_E e F_H/F_S dependeu das diferenças na dinâmica sazonal da cobertura vegetal. A contribuição de F_H para F_S variou de 37% no verão a 73% no inverno no sítio sem alteração sazonal das espécies de plantas dominantes, tendo sido quase constante durante o ano para F_H/F_S no sítio com alteração sazonal de espécies de plantas dominantes. Durante o período frio a contribuição de F_H para F_S aumentou com a diminuição do crescimento das plantas. A análise de regressão linear mostrou que a biomassa das plantas foi o fator dominante no controlo da variação sazonal das relações F_H/F_S, enquanto a dinâmica da biomassa das plantas seguiu a dinâmica do conteúdo de água no solo, a profundidade do aquífero e a temperatura do solo. Os nossos resultados sugerem que a variação sazonal da contribuição do solo para os fluxos totais do ecossistema* chinampa *se diferencia localmente. Estas diferenças estão relacionadas com as diferenças na dinâmica sazonal da produtividade da vegetação que está associada à localização do conteúdo de água do solo. Este resultado tem implicações importantes para avaliar a contribuição do ecossistema de* chinampa *para o conteúdo global de carbono.*

KEY WORDS
Ecosystem respiration, total soil CO_2 efflux, heterotrophic soil CO_2 efflux.

PALABRAS CLAVE
Respiración del ecosistema, flujo total de CO_2 del suelo, flujo heterotrófo de CO_2 del suelo.

PALAVRAS-CHAVE
Respiração do ecossistema, fluxo total de CO_2 do solo, fluxo heterotrófico de CO_2 do solo.

1. Introduction

Ecosystem respiration (R_E) including aboveground plant respiration and total soil CO_2 flux (F_S) is commonly regarded as the most critical component determining large-scale spatial and temporal variation in ecosystem net carbon balance (Grogan and Jonasson 2005). For characterizing spatial and temporal variability in ecosystem carbon balance and for correct prediction of carbon cycling and sequestration under changing climatic conditions, the controlling factors, component fluxes and their contribution to R_E should be well understood (Kuzyakov and Cheng 2001). The heterotrophic soil CO_2 flux (F_H) (i.e. the decomposition of soil organic matter by the soil microbial community) and autotrophic root respiration are the main contributors to soil CO_2 production and consequently, to F_S (Kuzyakov and Cheng 2001). For grasslands, the contribution of F_H to F_S was reported to range between 40 and 70% (Subke et al. 2006). Considerable variations of the contributions might be connected with large differences among site types and vegetation characteristics (Xavier et al. 2019; Santos et al. 2019). Climate may drive variation of CO_2 fluxes on seasonal and annual time scales (Martin and Bolstad 2005). Since autotrophic and heterotrophic respirations have a differential response to environmental drivers such as temperature, moisture and substrate supply (Hartley et al. 2006; López et al. 2018; Santos et al. 2019) or water table depth (Juszczak et al. 2013), high temporal variability of contributions of each flux component to the ecosystem flux can be expected. Furthermore, root respiration exhibits greater seasonality than heterotrophic respiration (Widén and Majdi 2001) and the seasonal variation of plant biomass could increase temporal variability of the contribution of CO_2 flux components to R_E.

R_E and F_S are widely documented to depend on temperature (Lloyd and Taylor 1994; Alm et al. 1997; Almagro et al. 2009; Mahecha et al. 2010; López et al. 2018; Silva et al. 2019). The temperature sensitivity is expressed as Q_{10}, which is the rate of respiration increase as a consequence of increasing the temperature by 10 ºC. The Q_{10} values of autotrophic and heterotrophic respiration varies widely with environmental conditions and terrestrial ecosystem types (Atkin et al. 2005; Ikkonen et al. 2012a). On an annual time scale, the Q_{10} values could be affected by climatic factors such as temperature (Vanhala et al. 2008), soil water content (Ikkonen et al. 2012a), or plant activity (Wang et al. 2010). The differences in temperature sensitivities and their responses to varying external drivers could be partly responsible for temporal variability of the contribution of different components to ecosystem fluxes.

Chinampas are unique anthropogenic wetland ecosystems used for agriculture in the Valley of Mexico since pre-Hispanic times. *Chinampas* were made by hand from the wetland around the lakes in the Valley of Mexico by forming raised fields separated by a system of canals (Jiménez-Osornio and Gómez-Pompa 1987; Blanco-Jarvio et al. 2011). The fields constructed by the accumulation of lacustrine organic loamy sediments were typically characterized by exceptionally high yields (Jiménez-Osornio et al. 1995). In pre-Hispanic times this intensive agricultural system used for growing foodstuffs occupied large areas, but to date, the *chinampa* land area has been greatly reduced due to the expansion of Mexico City. A part of the remaining *chinampas* was reserved in Xochimilco Ecological Park to restore these unique ecosystems without soil cultivation and fertilization but with the development of native vegetation.

Although many studies focused on greenhouse gas production and emission from soil of the Valley of Mexico (Beltrán-Hernández et al. 2007; Silva et al. 2008; Dendooven et al. 2010; Dendooven et al. 2012a, b), little is known about temporal dynamic of CO_2 fluxes from *chinampas* soils (Ortiz-Cornejo et al. 2015) and no information concerning soil contribution to *chinampa* ecosystem fluxes are available. We hypothesized that the magnitude of the F_S contribution to the total *chinampas* flux varies depending on soil water and temperature regime as well as the seasonal dynamic of plant cover. The objective of the study was to evaluate the contribution of F_S to R_E and F_H to F_S, their seasonal variability and dependence on driving factors in *chinampa* ecosystems.

2. Material and Methods

2.1. Study sites

We studied two *chinampas* ecosystems within the Xochimilco municipality of Mexico City, DF, at the geographical coordinates 19°17’45" N and 99°05’34" W and at the altitude of 2240 m above sea level (**Figure 1**). The climate of the study area is temperate subhumid. The area is characterized by an alternation of the dry season from November to March and the rainy season from April to October. The mean annual precipitation for the 1996-2009 period was 686.1 mm, according to the data of the Escuela Nacional Preparatoria meteorological station, Plantel 1 "Gabino Barreda" UNAM (Xochimilco, Mexico City, D.F.) located close to the sampling site. The mean air temperature was 17 °C with a minimum of 13.5 °C in January and a maximum of 19.3 ºC in July as reported by the same meteorological station. The soil of *chinampas* was classified by its origin as Terric Anthrosol (IUSS Working Group WRB 2006) because the surficial layers of the soil were known to be constructed of excavated lacustrine sediments. The soil texture varied from silty loam to clay and the morphology of the soil profile is relatively uniform and the horizons are hardly distinguishable (Ramos-Bello et al. 2011). The morphology of *chinampa*’s soils resembles that of deep organic soils (Histosols), but the organic matter content is lower (García et al. 1994). High salinity and sodicity of groundwater and soils have been reported for the area (Ramos-Bello et al. 2011). The pH of the *chinampas* soils, as was reported by N. Ortiz-Cornejo et al. (2015), is alkaline with total N ranges from 5.9 to 6.2 g kg^{-1} and organic carbon content equals 28.4 t ha^{-1} at the 20-40 cm depth. High organic matter content throughout the topsoil and irregular vertical distribution of organic carbon and bulk density has been reported for the *chinampas* area (Ikkonen et al. 2012b).

Two study sites were located in the Xochimilco Ecological Park in Mexico City (**Figure 1**). The uniqueness of this area is connected with the peculiarity of anthropogenic *chinampas* soils through a specific agricultural practice referred to the Pre-Hispanic period. The distance between the study sites did not exceed 100 m, but they varied in groundwater table depth and dominant plant species composition. The vegetative community of first study site (S1) was dominated by rushes (*Juncus* spp.) and broadleaf cattail (*Typha latifolia* L.). The plant cover of the second site (S2) varied between the seasons: in the dry period the dominant species were bristly oxtongue (*Picris echioides* L.) and greater plantain (*Plantago major* L.), and in the rainy season the vegetation was represented mainly by rushes (*Juncus* spp.) and common reed (*Phragmites australis* (Cav) Trin. ex. Stend.). Single specimens of seashore saltgrass (*Distichlis spicata* L.) were found on both sites. The highest root density was found in the layer of 0-7 cm depth, but it decreased sharply with the depth (Ikkonen et al. 2012a).

Figure 1. Location of the study sites in the Xochimilco Ecological Park in Mexico City, Mexico.

2.2. CO_2 flux measurements

The R_E, F_S and F_H fluxes were measured using static chamber techniques (Alm et al. 1997) every two-three weeks from September 2008 to August 2009. The measurements were performed between 8:00 and 12:00. All chambers were opaque in order to prevent photosynthesis and ensure that only respiration was measured. For ecosystem respiration measurements four stainless steel collars (length × width × height = 40 cm x 30 cm x 10 cm) were inserted into the soil six months prior to the first flux measurement. Locations for collars were selected using the presence of photosynthesizing plants as the main criterion. A steel removable chamber 40 cm x 30 cm x 50 cm (length × width × height) in size was placed and sealed over the collars using water for capsulation of chamber air during each flux campaign. An internal electric fan was used to homogenize air temperature and humidity within the chamber. An aluminum cover was added outside of the chamber to reduce the impact of direct irradiative heating during sampling. The measurement of F_S flux was made with four replicate cylindrical chambers (diameter × height = 10 cm x 15 cm) directly inserted into the soil, about 5 cm below the surface. The locations for the cylindrical chambers were selected between individual plants without their presence inside the chambers. In order to collect the data on F_H, the same cylindrical chambers were inserted into the soil of surface section fenced off by PVC tubes that were inserted 30 cm into the soil six months prior to the measurements. The sections for F_S measurements contained plant litter but did not include photosynthesizing plants and the sections for F_H measurements did not include plant roots.

The headspace gas samples were collected through a sampling port to 20 ml vessels (Corning System, USA) using a double-sided needle every 10 min from 0 to 30 min. The vessels were vacuum sealed with a rubber stopper and metal cap. All air samples were transported to a laboratory and analyzed within 24 h following gas collection. The CO_2 molar fraction were recorded by HP Agilent 6890 GC System gas chromatographer (GMI, USA) with a Poropac-Q column (35 ºC of column's temperature and 300 ºC of detector's temperature; argon was used as carrier gas). The flux was calculated by estimating the slope of the increase in CO_2 molar fraction in the chamber, adjusted for air temperature and pressure.

2.3. Environmental measurements and shoot plant biomass

Air temperatures, air pressure and water table depth (WTD) were recorded on each sampling occasion immediately after the flux measurements. The ambient air temperature, atmospheric pressure and humidity were simultaneously registered with a weather station (Crosse Technology, USA). Soil temperatures at 5-cm depth in the organic soil layer were recorded using a portable temperature probe (WIKA, USA) immediately after the flux measurements at both S1 and S2 sites and at 30-min intervals using a 5TM sensor with an E50-series data logger (Decagon, USA) at the S1 site from September 2008 to August 2009. The soil water content at 5-10 cm depth was determined gravimetrically in four replicates by oven drying the samples at 105 ºC for 24 h. WTD was measured in soil profile pits.

Shoot biomass was measured four times during the flux measuring period in 2008-2009: September 18, November 18, January 27, and April 13 for Site 1 and September 4, November 18, January 27, and April 13 for Site 2. We quantified shoot plant biomass by clipping green vegetation and litter within four randomly located 20 x 20 cm plots outside permanently designated locations for CO_2 flux measurement. The clipped material was separated into plant species and total litter, dried at 105 ºC and weighed after drying.

2.4. Statistical analysis

The C-CO_2 flux results are presented as means ± SE. We defined the proportion of soil respiration (F_S) in the total ecosystem respiration (R_E) as a percentage F_S/R_E and the proportion of heterotrophic respiration (F_H) in total soil respiration as a percentage F_H/F_S. Data were tested for normality and homogeneity of variance using the Chi-Square test and Levene's test in Statistica (v.8.0.550.0, StatSoft, Inc). Differences of means between the two studied sites were tested with one-way ANOVA followed the least significance difference (LSD) test. The correlation coefficients were calculated to examine the relationships between R_E, F_S, F_H, F_S/R_E, F_H/F_S, soil temperature, soil water content, water table depth and shoot biomass. The statistical significance was judged at the 5% probability level and the statistical analyses were performed using Statistica (v.8.0.550.0, StatSoft, Inc). The sensitivities of CO_2 fluxes to variations in soil temperature were calculated in the form of Q_{10} values according to Meyer et al. (2018). Annual cumulative C-CO_2 fluxes were defined using mean flux values for the measuring period.

3. Results

3.1. Environmental variables and shoot plant biomass

According to the data of the "Gabino Barreda" meteorological station, the total precipitation from September 2008 to August 2009 was close to 450 mm, which was lower than the mean annual value for the same period (**Figure 2A**). During the dry season (from October to April) the area received only 10% of the total precipitation. At both sites WTD followed precipitation pattern, but the temporal variations in WTD were much higher at S2 than at S1 (**Figure 2B, Table 1**). WTD at S1 remained only a few cms below the surface throughout the measuring period. WTD was nearly 10 cm deeper at S2 compared with S1 for the August-February period and more than 30 cm deeper at the end of the dry season (February-April). From May to August, WTD trends showed only small differences between the study sites. Following the patterns of precipitation, soil water content was high from May to September and low from October to April at both sites, but the values were 1.5-2.5 times higher at S1 (**Figure 2C**). The lowest soil water content values were recorded from November to April and the highest ones immediately after the beginning of the May to August rainy season. Mean soil temperature at 5 cm depth did not differ significantly between S1 and S2 sites (**Table 1**). During the study period, the mean annual soil temperature was about 15.0 °C (**Figure 2D**).

Table 1. Mean ± standard error of measured values at the S1 and S2 sites of *chinampa* ecosystem for the study period

Parameter	S1	S2
Soil water content, %	322 ± 24^{a}	145 ± 22^{b}
WTD, cm	17.7 ± 1.9^{b}	27.4 ± 3.4^{a}
Soil temperature, °C	14.8 ± 0.6^{a}	16.1 ± 0.5^{a}
Total shoot biomass, g m^{-2}	406 ± 151^{b}	706 ± 107^{a}
R_E, mg C-CO_2 m^{-2} h^{-1}	78.0 ± 13.1^{a}	108.1 ± 10.3^{a}
F_S, mg C-CO_2 m^{-2} h^{-1}	28.0 ± 5.4^{a}	40.5 ± 4.3^{a}
F_H, mg C-CO_2 m^{-2} h^{-1}	13.0 ± 2.9^{b}	19.6 ± 1.8^{a}
F_S/R_E, %	36.7 ± 3.0^{a}	39.4 ± 3.2^{a}
F_H/F_S, %	50.0 ± 5.6^{a}	50.1 ± 1.7^{a}
Winter season		
F_S/R_E, %	42.9 ± 3.6^{a}	36.1 ± 3.4^{a}
F_H/F_S, %	72.6 ± 4.6^{a}	50.7 ± 1.6^{b}
Summer season		
F_S/R_E, %	32.2 ± 1.6^{a}	36.1 ± 3.4^{a}
F_H/F_S, %	36.8 ± 2.6^{b}	49.8 ± 1.8^{a}

WTD – water table depth, R_E – ecosystem respiration, F_S – total soil CO_2 flux, F_H – heterotrophic soil CO_2 flux, F_S/R_E – contribution of F_S to R_E, F_H/F_S – contribution of F_H to F_S. Means followed by different letters in the same line are significantly different (LSD test). N = 76 and 85 (Soil temperature, R_E, R_s, R_H) 19 and 22 (Soil water content, WTD, F_S/R_E, F_H/F_S), 16 and 16 (Total shoot biomass) for S1 and S2, respectively.

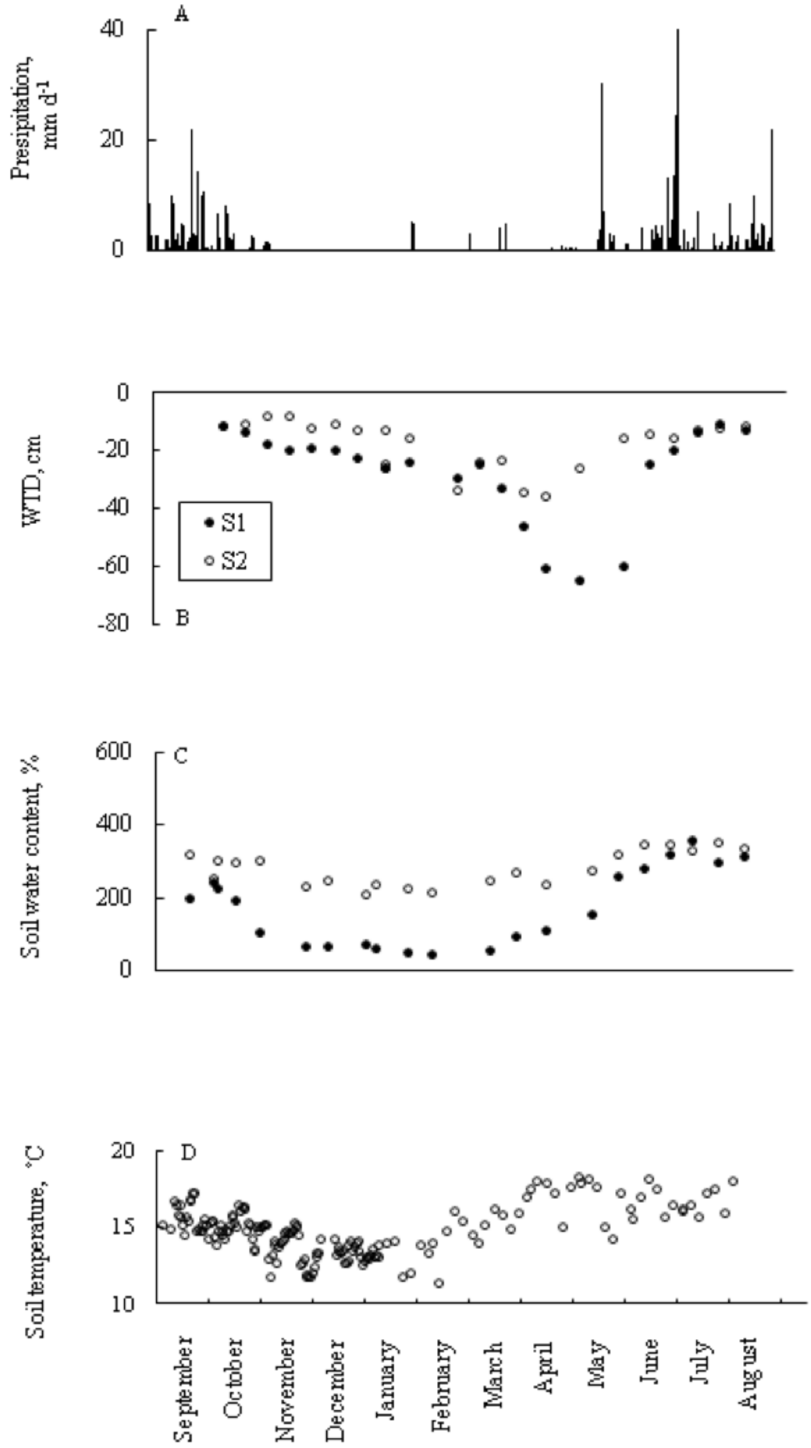

Figure 2. Precipitation (A), water table depth (WTD) (B), soil water content within 5-10 cm depth (C) and daily mean soil temperature at 5-cm depth (D) during the study period at the S1 and S2 sites.

The shoot plant biomass differed between the study sites (**Figure 3, Table 1**). The site S2, where the water table was lower during winter, had higher total plant biomass and litter than S1. In the rainy summer season, the S2 vegetation was dominated by rushes and common reed, and in winter it was replaced mainly by bristly oxtongue and seashore saltgrass. This replacement was connected with the lowering of WTD. For the S1 site, the seasonal change of dominant plant species was not observed. The decreases of shoot plant biomass started in November at both sites, but at the S2 site it quickly recovered due to active growth of the winter period vegetation ('dry season type'). Unlike the S1 site, litter mass of S2 was high during the winter and close to zero during the summer. The decomposition rate of S1 litter was slow probably because WTD remained only a few cm below the surface during the measurement period. The results indicated significant positive relations between total shoot biomass and soil water content and a negative relation between biomass and WTD in S1. For the S2 site, shoot plant biomass was positively related to soil water content and temperature (**Table 2**).

3.2. Seasonal variation in CO_2 fluxes

The mean C-CO_2 fluxes for the measuring period are shown in **Table 1**. Although R_E, F_S and F_H were

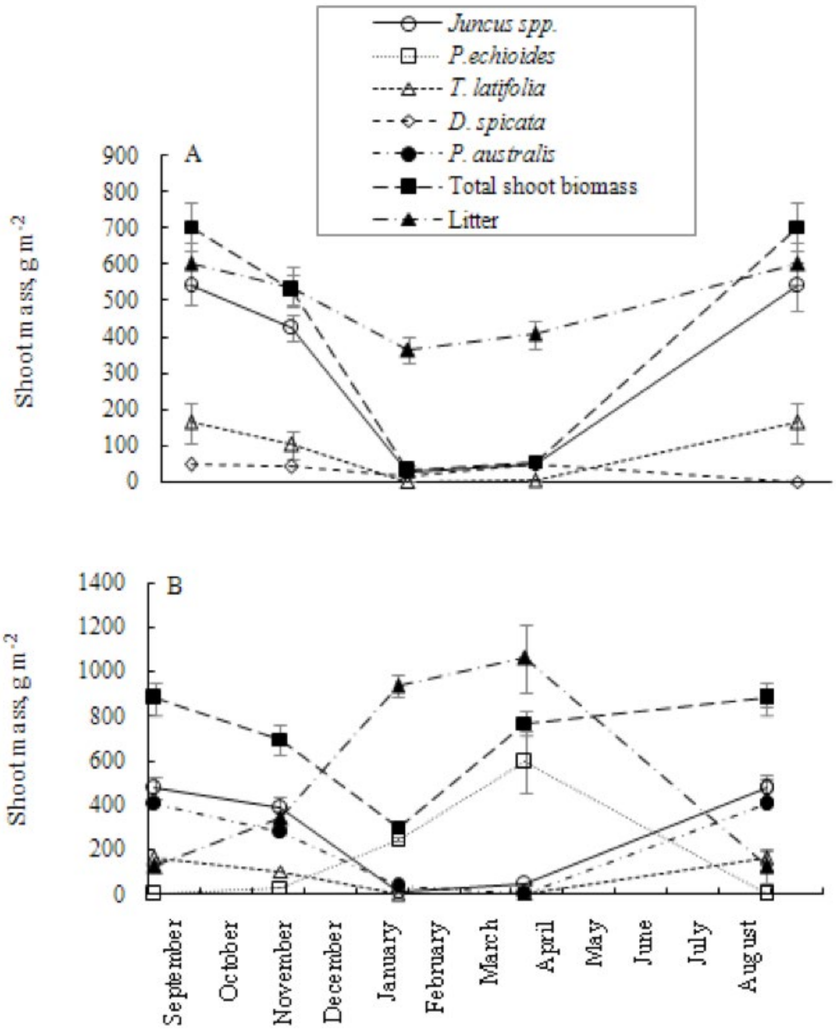

Figure 3. The dynamics of shoot plant biomass for the S1 (A) and S2 (B) sites during the study period.

Table 2. Statistical data (*P* value, R^2) indicating relationships between measured CO_2 fluxes, F_S/R_E, F_H/F_S and environmental variables at the S1 and S2 sites of *chinampa* ecosystem

	S1		S2	
Variable	*P* value	R^2	*P* value	R^2
R_E				
Soil water content	< 0.001	0.63	< 0.001	0.41
WTD	0.028	-0.09	0.285	0.02
Soil temperature	< 0.001	0.59	0.112	0.04
Total shoot biomass	0.002	0.18	< 0.001	0.18
F_S				
Soil water content	< 0.001	0.49	0.002	0.21
WTD	0.440	-0.01	< 0.001	0.36
Soil temperature	< 0.001	0.49	0.001	0.17
Total shoot biomass	0.616	0.00	< 0.001	0.30
F_H				
Soil water content	0.239	0.04	< 0.001	0.34
WTD	0.470	0.01	0.680	0.00
Soil temperature	< 0.001	0.46	0.020	0.09
Total shoot biomass	0.206	-0.03	< 0.001	0.30
F_S/R_E				
Soil water content	< 0.001	-0.26	0.030	-0.10
WTD	0.002	0.17	0.224	0.02
Soil temperature	0.704	-0.00	0.006	0.12
Total shoot biomass	< 0.001	-0.41	0.154	0.03
F_H/F_S				
Soil water content	< 0.001	-0.48	< 0.001	0.28
WTD	< 0.001	0.56	0.003	-0.13
Soil temperature	0.031	-0.09	0.081	-0.05
Total shoot biomass	< 0.001	-0.85	0.917	0.00
Total shoot biomass				
Soil water content	< 0.001	0.65	< 0.001	0.69
WTD	< 0.001	-0.53	0.019	0.09
Soil temperature	0.04	0.08	< 0.001	0.36

WTD – water table depth, R_E – ecosystem respiration, F_S – total soil CO_2 flux, F_H – heterotrophic soil CO_2 flux, F_S/R_E – contribution of F_S to R_E, F_H/F_S – contribution of F_H to F_S. N = 76 (S1) and 85 (S2).

higher at the S2 than at the S1 site, no significant differences in mean seasonal fluxes were found among the sites ($P > 0.05$). Regardless of the flux and vegetation type, all the CO_2 fluxes exhibited a pronounced seasonal variation with the lowest values during the cold period from November to February and the highest values during the warm period (**Figure 4**), indicating the strong positive correlation of the flux with the temperature (**Table 2**). After the winter period, when the R_E values were minimal, an increase of R_E was recorded at the S2 earlier than at the S1. The results did not indicate a significant relationship between F_S or F_H and soil water content or WTD ($P > 0.05$) especially at the S1 site characterized by low seasonal variability of WTD. The annual cumulative R_E, F_S and F_H fluxes were higher by about 30% at the S2 than at the S1 (**Table 2**). For the site S1, the annual cumulative R_E, F_S and F_H fluxes were 683 ± 109, 246 ± 47 and 114 ± 25 C m^{-2} year, respectively, and for the S2 site, these parameters were 947 ± 85, 355 ± 36 and 172 ± 15 C m^{-2} year, respectively.

3.3. Seasonal variation in the contribution of F_S to R_E and F_H to F_S

The contribution of F_S to R_E or F_H to F_S did not differ significantly between the study sites ($P > 0.05$), however, the F_S/R_E and F_H/F_S values of the S1 site, in contrast to the S2, showed a

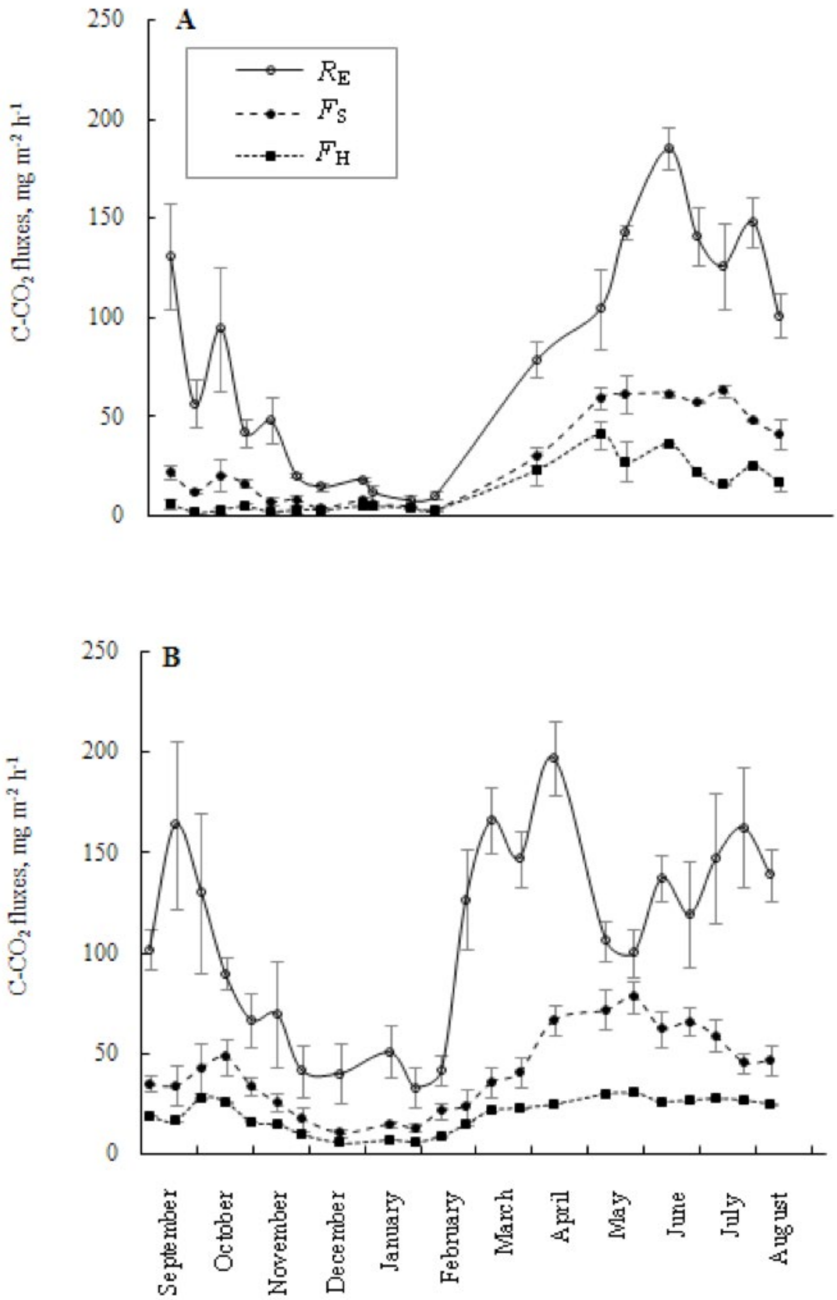

Figure 4. Mean ± standard error of observed values of the ecosystem respiration (R_E) rate, total soil CO_2 flux (F_S) and heterotrophic soil CO_2 flux (F_H) for the S1 (A) and S2 (B) sites.

trend that varied between seasons (**Figure 5**). While the daily F_S/R_E and F_H/F_S values in the S2 were close to the means for the measuring period, they were lower than the mean values during the warm season and higher than ones during the cold season in the S1 (**Table 1**).

3.4. Environmental factors controlling CO_2 fluxes

The variations in CO_2 fluxes in the *chinampas* area were clearly associated with the variations of all measured independent parameters: soil water content and temperature, WTD, and plant biomass (**Table 2**). However, the degree

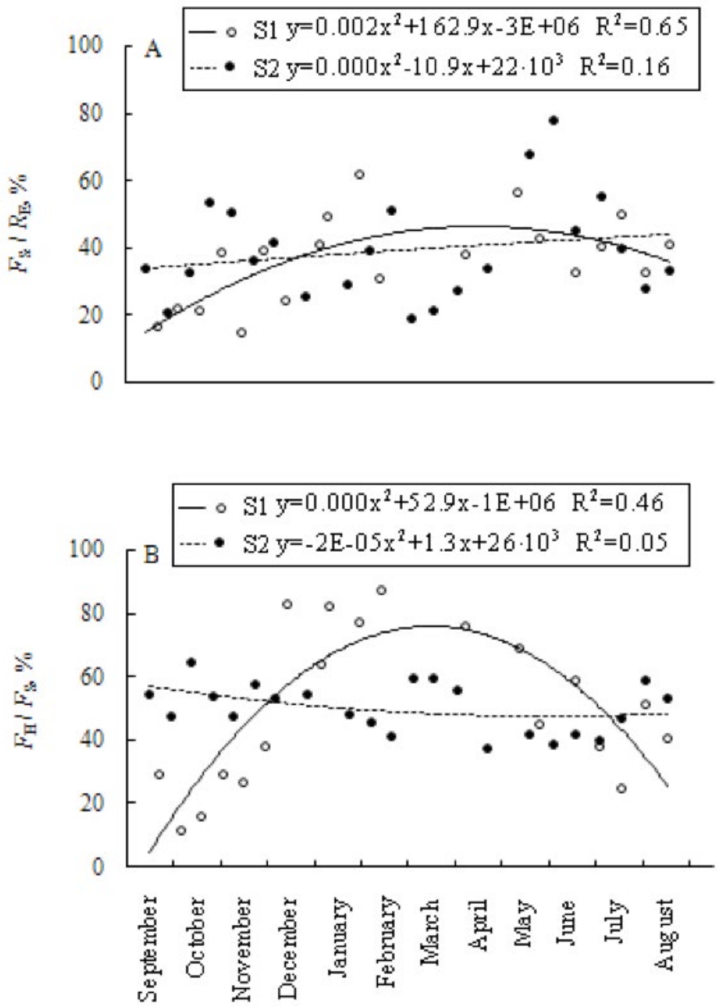

Figure 5. The contribution of F_S to R_E (F_S/R_E, A) and F_H to F_S (F_H/F_S, B) for the S1 and S2 sites during the study period.

of influence of environmental variables and biomass on the CO_2 fluxes differed depending on flux and vegetation type. The regression analysis of the results of the S1 site indicated that R_E was significantly positively correlated with soil water content and soil temperature, whereas at the S2 site the variation in R_E was explained by the dependence on soil water content alone. A positive correlation of R_E with total shoot biomass was found for both study sites. Whereas soil respiration at the S1 could be explained by the relationships with soil water content and temperature, for the S2 the F_S flux was mostly affected by WTD and plant biomass. The F_H variation was explained by soil temperature alone at the S1 and by the combined influence of soil water content, soil temperature and shoot biomass at the S2. Total shoot biomass had the strongest negative effect on F_S/R_E and F_H/F_S at the S1, but not at the S2. Soil water content was negatively related with F_H/F_S at the S1 and positively related with F_H/F_S at the S2. A combined effect of soil water content, WTD and soil temperature on total shoot biomass was found for both study sites.

4. Discussion

This study was carried out to partition the components of ecosystem respiration and their individual responses to environmental factors, such as temperature, soil water content, and vegetation type in the *chinampa* ecosystem. The mean and annual cumulative CO_2 flux observed in this study (**Table 1**) were lower than respective values shown for a broad range of grassland ecosystems (Wang and Fang 2009; Hu et al. 2016). Low soil CO_2 fluxes can be explained by a high salt content in soils in the Valley of Mexico (Dendooven et al. 2010), where the *chinampas* area is located. The bottom sediments of the former lake Texcoco located in the center of the Valley of Mexico were enriched with pyroclastic deposits. Weathering of the volcanic ash resulted in the release of sodium, and, consequently, in the sodicity of the lake and soils formed later in the exposed lacustrine sediments when the lake Texcoco was drained in the 17th century (Luna-Guido et al. 2000). In the soils of the former lake of Texcoco the pH values can range from 9.8 to 11.7 and electrolytic conductivities in saturation extracts vary from 22 to 150 dS m^{-2} (Beltrán-Hernández et al. 2007). Excessive amounts of salts result in poor soil structure and inhibit biological processes (Dendooven et al. 2010). The production of CO_2 decreases with increased electrolytic conductivity (Beltrán-Hernández et al. 2007), which depends on salt concentration and thus high salinity and sodicity of soils reported for the *chinampas* area (Ramos-Bello et al. 2011) might be one of the reasons for the low soil CO_2 fluxes observed in this study. Moreover, high soil water content especially can be at least partly responsible for the depressed CO_2 fluxes in the *chinampas* area due to the limitation of O_2 diffusion into the soil and, thus, aerobic decomposition of organic carbon (Alm et al. 1997). In our study a significant positive soil water content effect was generally observed for all sites (**Table 2**). The soil water content explained about 40-60% of the variability of R_E and 20-50% of the variability of F_S, with the effect especially well marked at the S1 site. It should be noted that WTD had less impact on CO_2 fluxes than soil water content, but in general, the CO_2 fluxes decreased with increased WTD at the S1 and increased with decreased WTD at the S2.

Temperature is commonly shown to be the major driver of ecosystem or soil CO_2 fluxes, as temperature rise can stimulate biological activity resulting in increased gas fluxes (Silvola et al. 1996). In agreement with a number of previous reports (Silvola et al. 1996; Hu et al. 2016) we also found that CO_2 fluxes in the *chinampa* ecosystem show a positive relationship with soil temperature explaining about 50-60% of flux variations at the S1 site, but much less at the S2 (**Table 2**). For the *chinampa* ecosystem, the temperature sensitivity of all types of CO_2 fluxes, especially for R_E, were higher at the S1 site than at the S2 site (**Table 3**), which can be due to the differences in the seasonal dynamics of plant cover. The vegetation cover of the S2, in contrast to the S1, varies between seasons (**Figure 3**), changing from 'wet season type' to 'dry season type' in November. During the dry period, while the green shoot biomass of the S1 is close to zero, bristly oxtongue and greater plantain plants actively grow at the S2 thereby increasing not only R_E, but also F_S via root and rhizosphere respiration and F_H via the rhizosphere priming effect (Kuzyakov and Cheng 2001). The dry and cold periods are closely related to each other in the study area, thus the development of new vegetation cover under low temperature could modify temperature sensitivity of CO_2 fluxes and cause a decline in annual Q_{10} values at the S2 (**Table 3**). This is consistent with the statement of Song et al. (2014) that temperature sensitivity of R_E reflects the ecosystem's structure and related biotic and abiotic factors that can closely interact with each other. Temperature sensitivity of soil CO_2 fluxes can be modulated by soil water content (Almagro et al. 2009). In our case, soil water content influenced the temperature sensitivity of CO_2 fluxes via the shift of dominant plants during the year. Thus, seasonal vegetation trend effects on the temperature response of R_E, F_S and F_H should not be ignored for a correct prediction of CO_2 fluxes under climatic changes.

The mean values of the contributions of F_S to R_E for the studied *chinampa* sites are significantly lower than those reported in the literature (Zobitz et al. 2008). Due to low values of F_S, presumably caused by high salts content in soils and consequent low microbiological activity, F_S was not the main contributor to R_E at both sites of *chinampa* ecosystem. Recent studies demonstrated that the F_S contribution to R_E and F_H contribution to F_S can vary between seasons with a large proportion of soil microbial respiration during the winter period (Zobitz et al. 2008). The component contribution variability could be due to seasonal variation in microbial community composition and plant physiological and growth processes (Grogan and Jonasson 2005). Moreover, above- and belowground vegetation parts or microbial biomass could differ in their respiration response to changes in environmental factors (Hartley et al. 2006), causing variability in the contribution of components to soil and ecosystem CO_2 fluxes. The two studied sites of *chinampa* ecosystem were different in their seasonal trend of the F_S/R_E and F_H/F_S ratios (**Figure 5**). In contrast to the S1 site, at the S2 site, the mean contribution of F_S to R_E and F_H to F_S did not vary significantly between the winter and summer seasons (**Table 1**) despite the correlation with the soil water content or temperature (**Table 2**). We assume that the seasonal shift in the abundance of dominant species and active plant growth during the winter period caused an increase in the contribution of autotrophic respiration to winter CO_2 fluxes and a decrease in variability of the F_S/R_E and F_H/F_S ratios at the S2. At the S1, where plant growth was depressed during the cold season, the contribution of F_S to R_E and R_H to F_S was higher in winter and lower in summer (**Table 1**). This confirms the fact that changes in the relative contributions of individual component fluxes to R_E are regulated by a shift in plant internal allocation of recently fixed plant-associated carbon in an ecosystem (Grogan and Jonasson 2005). The linear regression results showed that plant biomass was the dominant factor controlling the seasonal variation of F_H/F_S ratios at the S1 (**Table 2**), wherein plant biomass dynamics followed the dynamics of soil water content, WTD and soil temperature. During the winter period with low soil temperature and soil water content, the contribution of heterotrophic respiration to soil respiration increased following plant growth depression at the S1. In addition, the winter increase in the F_H/F_S ratios at the S1 appeared to be the result of lower temperature sensitivity of heterotrophic compared to autotrophic respiration (**Table 3**).

Table 3. Q_{10} values of measured CO_2 fluxes at the S1 and S2 sites of *chinampa* ecosystem

CO_2 flux	S1	S2
R_E	3.0	0.9
F_S	2.4	1.6
F_H	1.8	1.4

R_E – ecosystem respiration, F_S – total soil CO_2 flux, F_H – heterotrophic soil CO_2 flux. N = 76 (S1) and 85 (S2).

5. Conclusions

The studied *chinampa* ecosystem sites with different vegetation characteristics demonstrated that R_E, F_S and F_H were lower than those reported for most grasslands. This presumably could be caused by the high salinity and sodicity of *chinampa* soils. Despite their low values, R_E, F_S and F_H showed seasonal variations followed by variations in shoot biomass, soil water content and temperature, whereas temperature sensitivity of CO_2 fluxes reflected the vegetation-type and plant growth differences among the sites. The seasonal variation of soil contribution to total CO_2 fluxes was locally differentiated for the *chinampa* ecosystem. These differences were related to differences in seasonal dynamics of cover productivity which has been associated with localization of soil water content. The variability of the contributions of F_S to R_E and F_H to F_S was clearly expressed at the site without a seasonal change of plant dominant species, while the component contribution did not vary between the seasons at the site where the plant dominants of 'dry season type' and 'wet season type' changed. This confirms the fact that soil contribution to CO_2 fluxes may depend on the vegetation type and seasonal dynamics of the plant cover. Further studies are needed to improve management of the *chinampas* area in Mexico in order to reduce their carbon losses. These ecosystems contain huge amounts of organic carbon, which may be released to the atmosphere in the case of their improper use. In this respect, the research should be focused on the balance of carbon in the *chinampas* under different land uses and management practices, and on the response of these ecosystems to the global climatic change.

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Stabilization of organic material from soils and soil-like bodies in the Lena River Delta (^{13}C-NMR spectroscopy analysis)

Estabilización de la materia orgánica de suelos y cuerpos similares al suelo en el Delta del Río Lena (Análisis de espectroscopía ^{13}C-NMR)
Estabilização da matéria orgânica do solo e corpos semelhantes ao solo no Delta do Rio Lena (Análise por espectroscopia ^{13}C-NMR)

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ABSTRACT

The Arctic ecosystem has a huge reservoir of soil organic carbon stored in permafrost-affected soils and biosediments. During the short vegetation season, humification and mineralization processes in the active soil layer result in the formation of specific soil organic substances – humic substances. Humic acids are high molecular, specific, thermodynamically stable macromolecules. The study was conducted in the Lena River Delta, the largest river delta located in the Arctic. Cryosol-type soils on alluvial deposits of the river form an area of about 45 thousand km^2 under permafrost conditions. The vegetation cover is represented by moss-lichen communities with the presence of *Salix glauca* in the flooded areas, as well as *Betula nana* in the areas not subject to flooding. The paper presents the elemental and molecular composition of humic acids isolated from soils, integral indicators of humification (stabilization) of organic matter in the soils of the Lena River Delta. The study was conducted using the ^{13}C (CP/MAS) NMR spectroscopy method. In the work, it was revealed that up to 33% of aromatic and up to 15% COOR fragments are accumulated in humic acids. The AR/AL ratio ranged from 0.69 to 0.89. The studied soils are variants of modern soil formation (not subjected to alluvial processes) and soil-like bodies that melted from the IC of the river delta. A relatively high degree of condensation of humic acid macromolecules in comparison with other polar regions of the Arctic and Antarctic was noted.

RESUMEN

El ecosistema ártico constituye una enorme reserva de carbono orgánico que se encuentra almacenado en suelos afectados por permafrost y biosedimentos. Durante la corta estación vegetativa, los procesos de humificación y mineralización en la capa activa del suelo dan lugar a la formación de sustancias orgánicas específicas en el suelo, las sustancias húmicas. Los ácidos húmicos son macromoléculas de alto peso molecular, específicas y termodinámicamente estables. Este estudio se llevó a cabo en el Delta del Río Lena, el mayor delta de río situado en el Ártico. Allí se encuentran Criosoles formados sobre depósitos aluviales de río que ocupan un área aproximada de 45.000 km^2 bajo condiciones de permafrost. La cobertura vegetal está representada por comunidades de líquenes y musgos con la presencia de Salix glauca *en las zonas inundadas, así como* Betula nana *en las zonas no inundadas. Este trabajo presenta la composición elemental y molecular de los ácidos húmicos aislados de los suelos, indicadores integrales de*

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la humificación (estabilización) de la materia orgánica en los suelos del Delta del río Lena. El estudio fue realizado mediante el método de espectroscopía ^{13}C (CP/MAS) NMR. Así, se obtuvo que en los ácidos húmicos se acumulaban hasta un 33% de fragmentos aromáticos y un 15% de fragmentos COOR. La relación AR/AL osciló entre 0,69 y 0,89. Los suelos estudiados son variantes de la formación de suelos modernos (no sometidos a procesos aluviales) y cuerpos similares al suelo que se derritieron a partir del Complejo de Hielo (IC) del delta del río. Se observó un grado relativamente alto de condensación de macromoléculas de ácidos húmicos en comparación con otras regiones polares del Ártico y Antártico.

RESUMO

O ecossistema Ártico é uma enorme reserva de carbono orgânico, que está armazenado em solos afetados por permafrost e em biosedimentos. Durante a curta estação vegetativa, os processos de humificação e mineralização na camada ativa do solo dão origem à formação de substâncias orgânicas específicas do solo – as substâncias húmicas. Os ácidos húmicos são macromoléculas de elevada massa molecular, específicas e estáveis em termos termodinâmicos. Este estudo foi realizado no Delta do Rio Lena, o maior delta fluvial localizado no Ártico. Numa área de aproximadamente 45 000 km^2, ocorrem Criossolos formados a partir de depósitos aluviais do rio Lena em condições de permafrost. A cobertura vegetal é representada por comunidades de líquenes e musgos com a presença de Salix glauca *nas zonas inundadas, bem como* Betula nana *nas zonas não inundadas. O artigo apresenta a composição elementar e molecular dos ácidos húmicos isolados dos solos, indicadores integrais da humificação (estabilização) da matéria orgânica nos solos do Delta do Rio Lena. O estudo foi realizado utilizando o método de espectroscopia ^{13}C (CP/MAS) NMR. Nos ácidos húmicos acumulam-se até 33% dos fragmentos aromáticos e 15% dos fragmentos de COOR. A relação AR/AL variou entre 0,69 e 0,89. Os solos estudados são variantes da formação moderna dos solos (não sujeitos a processos aluviais) e corpos semelhantes a solo que resultaram da fusão do Complexo de Gelo (IC) do delta do rio. Foi observado um grau relativamente elevado de condensação de macromoléculas de ácidos húmicos em comparação com outras regiões polares do Ártico e do Antártico.*

KEYS WORDS
Permafrost soils, SOM, Arctica, CP/MAS, humic acids.

PALABRAS CLAVE
Suelos del permafrost, SOM, Arctica, CP/MAS, ácidos húmicos.

PALAVRAS-CHAVE
Solos de permafrost, SOM, Ártica, CP/MAS, ácidos húmicos.

1. Introduction

Soil organic carbon (SOC) is a product that accumulates in the soil after the partial decomposition of diverse types of materials, derived from microorganisms and plant remnants. This constitutes a key element of the global carbon cycle through the atmosphere, vegetation, soils, rivers and the ocean (Davis 2001; Dutta et al. 2006; Schimel 1995). The soil organic matter (SOM) supports the key functions of the soil and ecosystem services, as it is crucial for stabilizing the structure of the soil, retaining, releasing nutrients for plants, and for ensuring the penetration of water and its storage in the soil. Loss of SOC indicates a degree of soil dehumification and degradation. Soils represent the largest surface reservoir of organic carbon in the Earth. Due to the local geogenic features, climatic conditions and land use, and management (among other environmental factors), soils retain differing amounts of SOC (Boike et al. 2013; Dai et al. 2002; Kutzbach et al. 2004). It is estimated that the largest amount of SOC is stored in the northern permafrost region with over 1024 Pg (1 Pg = 10^{13} kg) of organic carbon in the soil in a layer of up to 3 m, as well as 34 Pg of nitrogen (Jones et al. 2010; Zubrzycki et al. 2013; Zubrzycki et al. 2014) mainly in peat soil. The permafrost-affected zone occupies an area of more than 8.6 million km^2, which is about 27% of all land areas above 50°N. They accumulate in themselves a huge amount of organic carbon, so they are considered one of the most important elements of the cryosphere. There, carbon accumulates in soils in huge quantities due to low temperatures, leading to low biological activity and slow decomposition of SOM (Cauwet and Sidorov 1996; Ejarque and Abakumov 2016). The corresponding soil type is called Histosol (IUSS Working Group WRB 2015) and is characterized by SOC content of 12 to 50%. The presence of permafrost and long-term freezing of soils has a strong influence on the processes of ion exchange, the water-physical regime, the solubility of nutrients and their availability for plants, and on bioproductivity in general. The loss of SOC negatively affects not only soil health and food production, but also exacerbates climate change. When SOM decomposes, carbon-based greenhouse gases are released into the atmosphere. If this happens too fast, soils can contribute to the warming of our planet. On the other hand, many soils have the potential to increase SOC reserves, thus mitigating climate change by reducing atmospheric CO_2 (Knoblauch et al. 2013; Lara et al. 1998).

Cryoturbation and cryogenic mass exchange lead to the translocation and further accumulation of organic matter into deeper soil horizons. Another process is the movement of organic matter in a dissolved state and its accumulation on the border with the permafrost table (Dutta et al. 2006; Schimel 1995). During to cryoturbation processes, small fragments of organic matter separate from the lower parts of the surface horizons under the influence of ice penetration, move inside the profile, and mix with the mineral part of the underlying horizons. Such movement of organic masses along the profile leads to its compaction, homogenization, and destruction of plant remnants (Davidson and Janssens 2006).

The Lena River has a great influence on the biogeochemistry of the Arctic Ocean. Arctic rivers are the main suppliers of organic and inorganic carbon and largely determines the organic carbon cycle in the Arctic basin (Boike et al. 2013; Dobrovolsky 2005; Kutzbach et al. 2004). The soils of the Lena River Delta are formed in conditions of seasonal freezing/thawing processes and annual flooding. The annual supply of nutrients by the river and a mild climate cause a high level of microbiological processes in the soil, which contributes to the relatively high rate of humification of organic matter in the soil (Bolshiyanov et al. 2013).

The SOM of Arctic soils is very specific in comparison with soils are not affected by permafrost, and modern instrumental methods and approaches are needed to study it. The intensity of mineralization and humification of soil organic matter is extremely low due to the long duration of the frozen state of soils and the small sum of positive temperatures (Ejarque and Abakumov 2016; Lodygin et al. 2017; Lodygin and Beznosikov 2010). The soils of the Lena Delta are characterized by humus and peat soils, and there are also buried organic residues associated with both cryogenic processes and the specifics of sedimentation in the deltas of large rivers (Polyakov et al. 2018). Due to the widespread development of the lake system, silty particles and organic residues are deposited at the bottom of the lakes due to permafrost lateral mass transfer (Boike et al. 2013; Bolshiyanov et al. 2013).

For specific conditions of soil formation, in particular humus accumulation, in addition to traditional methods for the analysis of organic matter, the recent method of the study of organic matter based on its molecular composition has an advantage. The content of molecular fragments of HAs determined by CP/MAS ^{13}C–NMR spectroscopy contributes to the understanding of the fundamental processes of humus formation and the composition of natural high-molecular weight HAs in soils subjected to the influence of cryogenesis (Chukov et al. 2015; Dai et al. 2002; Ejarque and Abakumov 2016; Lodygin et al. 2014; Lupachev et al. 2017).

The advantage of the nuclear magnetic resonance spectroscopy method is the ability to quantify the content of groups of individual and structural fragments in humic acid (HAs) molecules. This method is also used to assess changes in SOM during decomposition and humification. So far, studies of the quality of SOM from polar environments have revealed a generalized, slightly degraded nature of organic molecules that retain most of the chemical nature of their precursor material due to the low progress of humification (Abakumov et al. 2015; Davidson and Janssens 2006; Dziadowiec et al. 1994).

Arctic soils, according to studies by various scientists, have a high proportion of aliphatic compounds in the composition of the HAs

(Abakumov et al. 2015; Chukov et al. 2015; Lodygin et al. 2017; Polyakov et al. 2019b; Vasilevich et al. 2018; Vasilevich et al. 2019). The low content of aromatic compounds of HAs is primarily associated with vegetation (the precursors of humification), as well as soil-climatic conditions, a low degree of aeration, cryogenic processes, and low microbiological activity that lead to soil mineralization and store of organic matter in permafrost. Russian scientists have well studied permafrost peat soils in north-west Russia, where they note a low proportion of aromatic compounds. Upon transition from the Arctic zone to the boreal zone, an increase in the proportion of aromatic compounds occurs, which is associated with an increase in microbiological activity and a change in plant communities.

The vegetation cover of the Arctic ecosystem is represented mainly by moss-lichen communities. The content of aromatic hydrocarbons in them, in particular lignin, tannin, and flavonoids, is rather low or close to zero. The content of proteins and nitrogen-containing fragments is also extremely low at 2-10%. Thus, the composition of precursors plays a key role in the composition of HAs. The predominantly aliphatic character of the HAs is associated with a high proportion of carbohydrates among the humification precursors (Orlov 1990). Antarctic soil-like bodies are also similar in relation to the aliphatic and aromatic fragments in the composition of the HAs by ^{13}C (CP/MAS) NMR and ^{1}H-^{13}C HETCORE NMR spectroscopy. The spectra obtained are very homogeneous, due to the low diversity of the vegetation cover (Abakumov et al. 2015; Abakumov et al. 2019; Lodygin et al. 2017; Lodygin and Beznosikov 2010; Lodygin et al. 2014; Lupachev et al. 2017).

Humic acids are heterogeneous systems of high-molecular condensed compounds formed from the decay of plant and animal remnants in terrestrial and aquatic ecosystems. Climatic parameters, precursors of humification, and local position in the landscape determine the diversity of the composition and properties of HAs (Chukov et al. 2015; Ejarque and Abakumov 2016; Lodygin et al. 2014). The stabilization of organic material is defined as the transformation of organic matter into a state inaccessible to soil microorganisms, and the stabilization property itself is a characteristic stage of carbon dynamics (Semenov et al. 2009). Using ^{13}C–NMR spectroscopy we identify the proportion of aromatic compounds in the composition of HAs, to assess the stabilization of organic matter in soils.

This work is the continuation of a long-term study of the molecular composition of Arctic soils, using the example of alluvial soils of the Lena River Delta. Thus, the aim of this work is to study HAs by ^{13}C–NMR spectroscopy of permafrost soils of the Lena River Delta, buried organic matter, and melted soil-like bodies from the Ice Complex of the delta. To achieve aim of work, the following objectives were set:

1. to find out the molecular composition of HAs from study soils
2. to investigate elemental composition of HAs
3. to determine the stabilization rates of HAs isolated from study soils

2. Materials and Methods

2.1. The study sites

The Lena River Delta, the largest northern delta in the world, is located in the Arctic zone and has an area of about 29,630 km^2. Due to such a huge area and location, it has a significant impact on the hydrological regime of the Arctic Ocean, since a large amount of fresh water flows from the delta into the least salty ocean of our planet. The delta was formed as a result of river activity: sediment removal, erosion, and abrasion under the influence of sea level fluctuations and the movement of the Earth's crust (Bolshiyanov et al. 2013) (**Figure 1**).

The Lena River Delta is located in the area with an Arctic continental climate. The climatic characteristics of the area are presented in **Table 1**.

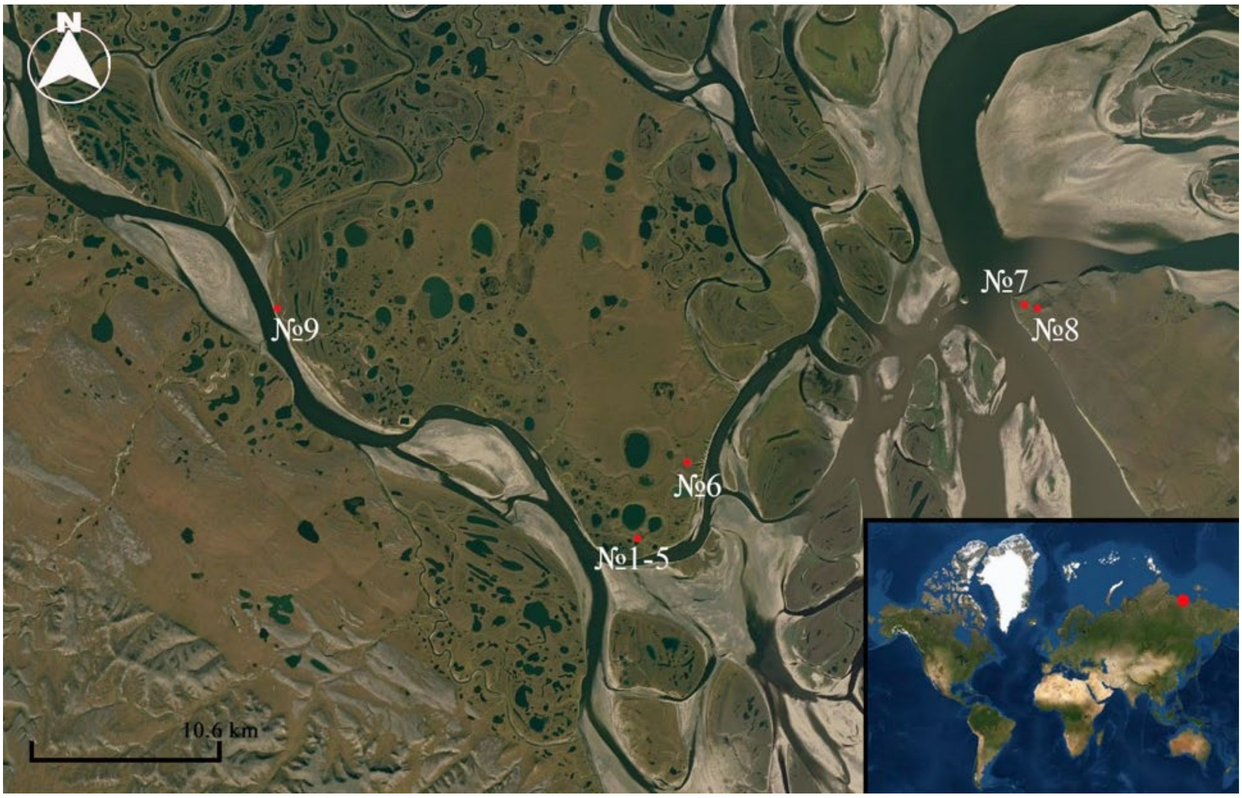

Figure 1. The Lena River Delta. Study area. Sample numbers correspond to **Table 2**.

Table 1. Climate parameters of the study region (Data obtained from the station «Samoylov Island»)

Climate parameters	Lena River Delta
Mean annual air temperature (°C)	-13
Mean air temperature (ºC):	
of the warmest month (July)	6.5
of the coldest month (January)	-32
Number of days with mean daily Air temperature:	
above 0 °C	73
above 5 °C	35
above 10 °C	11
Freezing depth (cm)	30-50
Snow thickness (cm)	23
Annual precipitation (mm)	323
In summer (mm)	125

Most of the land is characterized by the presence of a permafrost table at a depth of about 1 meter. The depth of the active layer varies: on loamy soils it can reach 30 cm at the end of August, and on sand soils it can reach 1 meter.

The Lena River Delta is covered with various types of tundra vegetation. The main components are lichens, mosses, grasses (cereals and sedges) and some types of shrubs. Here, cereal-sedge-moss coenoses predominate in relief depressions of the-hypno-sedge polygonal swamps (**Table 2**). The vegetation cover has a mosaic character ("spotted tundra"). The Lena River Delta is represented by the dominance of moss-lichen vegetation. Moss groups predominate on loam and lichen predominates on rocks. In addition, often near the thermokarst lakes, moss-lichen vegetation is replaced by sedge-cannon fodder. On the warm southern slopes on well-drained sandy soils and in the river valley there are areas with grassy vegetation (tundra meadows and floodplain meadows) (Boike et al. 2013; Kutzbach et al. 2004; Schneider et al. 2009).

2.2. Ice complex (IC)

The issue of the origin of the Ice Complex (IC) of rocks has not yet been resolved. There are several hypotheses that explain the accumulation of sand-silt sediments and their simultaneous freezing. Some researchers associate this process with aeolian transport and the deposition of a huge amount of mineral material from the atmosphere, and there is also a theory about the formation of IC as a result of alluvial accumulation. Another point of view on the formation of IC is that in front of the ice sheet on the shelf of the Laptev Sea there was a stagnant reservoir in which accumulation of sediments of the IC occurred (Bolshiyanov et al. 2013).

We studied sample № 9, a soil-like body extracted from the IC of the Lena River Delta. The results obtained during its processing differ from the soils investigated in this work. The carbon content in this sample is quite low and more similar in composition to fulvic acid. Apparently, during the long-term storage of organic matter in IC, the carbon content decreases with an increase in the oxygen fraction in HAs macromolecules.

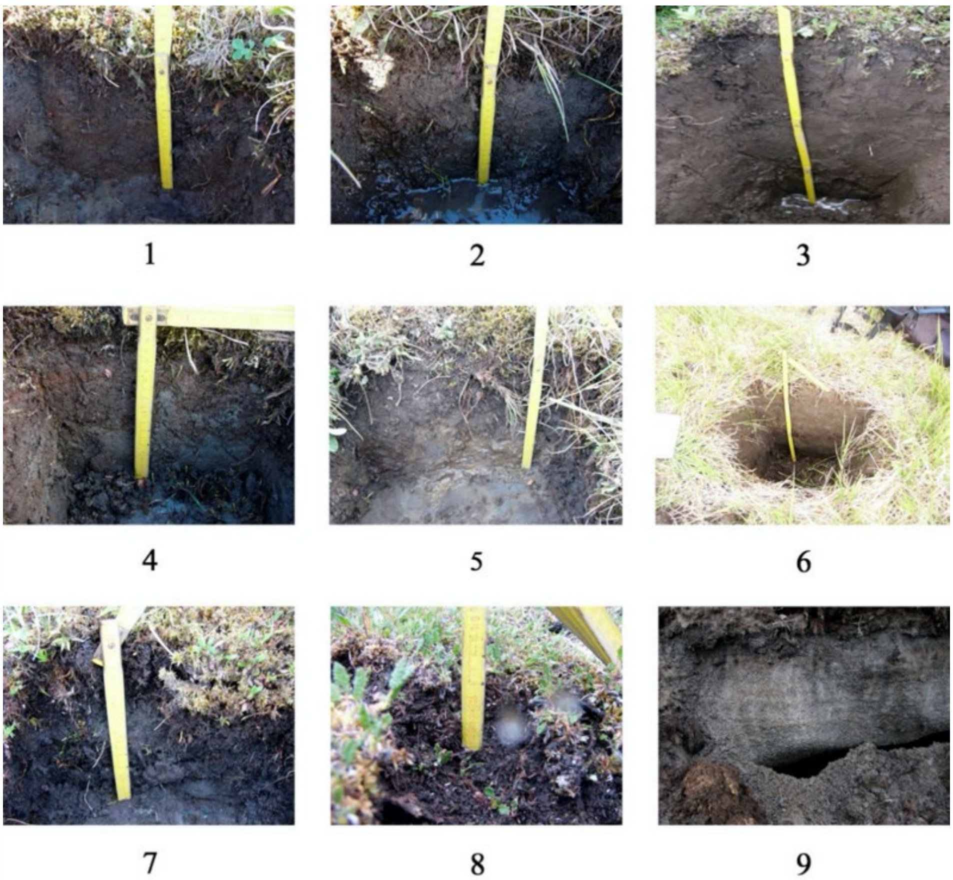

Figure 2. Morphological diversity of study soils. Sample numbers correspond to **Table 2**.

Table 2. Description of the studied soil and soil-like bodies

Site	Sample	Description of the studied soil horizons Coordinates	Color index*	Vegetation	Soil name**
Kurungnah isl.	1	Dark material, roots, iron spots, sandy loam, cryogenic mass exchange, permafrost table from 27 cm. Top of alas. N72.28920 E126.18025.	10 YR 6/1	Cetraria nivalis, Sphagnum, Carex aquatilis.	Turbic Cryosol (Siltic)
	2	Dark material, roots, gleyic processes, sandy loam, water table from 25 cm, permafrost table 29 cm. Bottom of alas. N72.29042 E126.18191.	10 YR 6/1	Cetraria nivalis, Sphagnum sp., Carex aquatilis.	Turbic Cryosol (Loamic)
	3	Roots, sandy loam, water table from 65 cm, permafrost table from 70 cm. Middle of alas. N72.29039 E126.18125.	10 YR 6/1	Sphagnum sp., Carex aquatilis, Salix glauca	Turbic Cryosol (Siltic)
	4	Organic material. Polygon rim. N72.29063 E126.18423.	10 YR 4/3	Cetraria nivalis, Sphagnum sp.,	Turbic Cryosol (Loamic)
	5	Organic material. Inside of alas. N72.29143 E126.18628.	10 YR 4/3	Cetraria nivalis, Sphagnum, Carex aquatilis.	Turbic Cryosol (Loamic)
	6	Buried organic material. Young alas (~100-200 years). N72.32162 E126.25303.	10 YR 3/2	Trisetum, Phragmites.	Folic Cryosol
	9	Organo-mineral material from IC. N72.392402 E125.648261 (~34,299 ± 500 years BP)** *.	10 YR 3/2	-	Soil-like body
Interfluve (Kharaulakh ridge)	7	Organo-mineral material, thixotropy, loamy sand, rocks. Top of ridge. N72.39439 E126.76143.	10 YR 3/2	Cetraria nivalis, Sphagnum, Leptogium lichenoides, Dactylina arctica	Skeletic Cryosol
	8	Organo-mineral material, thixotropy, loamy sand, rocks. Top of ridge. N72.392723 E126.78203.	10 YR 3/2	Cetraria nivalis, Sphagnum, Leptogium lichenoides, Dactylina arctica	Soil-like body

*Munsell Color (Firm) 2010); **Soil name by WRB classification (IUSS Working Group WRB 2015); *** (Schirrmeister et al. 2003).

2.3. Sampling procedure

Samples of soils were collected in various elements of the landforms («Old» alas, «young» alas (buried material), organo-mineral material from IC and interfluve (Kharaulakh ridge)), soil description are presented in **Table 2**. Soil pits are presented in **Figure 2**.

Kurungnah Island, located at the central part of the delta, has a connection with the Olenek Channel from the west and consists of sediment from the IC and the underlying sand from the surface. It is built from the surface by deposits of the IC and the underlying sand. The thickness is composed of two packs of rocks. The lower part is composed of fine-grained, sorted quartz sands. Horizontal layering is inherent in it, and it is rarely wavy. In the lower part there are lenticular layers of plant residues with sand. The entire stratum was formed in the middle of the Late Neopleistocene (Bolshiyanov et al. 2013). Interfluves (Kharaulakh ridge) consist of ultra-fine or fine calcarenite and dolarenite (with minor siliciclastic material) and sandstone. The lithite-quartz-feldspar sandstone contains minor dolomite and limey clasts and is intercalated with calcarenite, mudstone, calcareous mudstone, and silty mudstone, with intraclasts and breccia suggesting occasional landslides (Izokh and Yazikov 2017).

2.4. Soil analysis, ^{13}C NMR spectroscopy and elemental analysis procedure of HAs

Soil samples were air-dried (24 hours, 20 °C), ground, and passed through 2 mm sieve. Routine chemical analyses were performed using classical methods: C and N content were determined using an element analyzer (EA3028-HT EuroVector, Pravia PV, Italy) and pH in water and in salt suspension (soil-dissolvent ratios 1:2.5 in case of mineral horizons and 1:25 in case of organo-mineral horizons) suspensions using a pH meter (pH-150M Teplopribor, Moscow, Russia).

Humic acids were extracted from each sample according to a published IHSS protocol (Swift 1996). HAs extraction yields were calculated as the percentage of carbon recovered from the original soil sample (Vasilevich et al. 2018). Solid-state CP/MAS ^{13}C–NMR spectra of HAs were measured with a Bruker Avance 500 NMR spectrometer in a 3.2-mm ZrO_2 rotor. The magic angle spinning frequency was 20 kHz in all cases and the nutation frequency for cross polarization was u1/2p 1/4 62.5 kHz. Repetition delay were 3 seconds. The number of scans was 6500-32000. Contact time is 0.2 us.

The elemental composition of HAs represents the percentage of C, H, N, O elements in them. The high variability of the elemental composition of HAs among different soils is explained by the varying degree of accumulation of elements in the HAs. The highest C content is normally typical of Chernozems with a well-developed mollic layer and high degree of organic matter humification. In the soils of the Arctic zone, the carbon content is much lower. This peculiarity is explained by the effect of increased acidity and humidity. A reduced nitrogen content in soils of the arctic environment is also observed, which is associated with its low content in peat and an increase in hydrogen content. Information on the elemental composition of organic substance provides significant information on the general principles of molecular construction and some of their properties (Orlov 1990). To conduct a graphical analysis of the elemental composition, we used the van Krevelen diagram (van Krevelen 1950), using the H/C-O/C ratios to identify the direction of the transformation processes of various organic compounds in natural conditions. Thus, we can evaluate the processes of oxidation/reduction and hydration/dehydration in HA macromolecules. Elemental compositions were corrected for gravimetric water and ash content. Oxygen content was calculated by difference of whole samples mass and gravimetric concentration of C, N, H and ash.

3. Results and Discussion

3.1. Elemental composition of humic acids isolated from study soils

The elemental composition of HAs is the most important indicator determining the progress of humification, oxidation and degree of condensation of HAs (Abakumov et al. 2015; Beznosikov and Lodygin 2010). Characteristic features of HAs formed in cold conditions, and especially in permafrost-affected soils, are a relatively high H content and a reduced O content compared to boreal and sub-boreal soils (Lupachev et al. 2017).

The obtained data on HAs elemental composition, atomic rations and degree of oxidation (W) are presented in **Table 3**.

From the obtained data, the carbon content in the study samples confined to modern soil formation is quite small and in a narrow range (41-45%), while the carbon content in the sample

Table 3. Elemental composition of the studied HAs from soils. Gravimetric concentration is given for C, H, O and N content. C/N, H/C, O/C, H/Cmod and W were calculated from mole fraction of C, H, O and N content. H/Cmod is the number of substituted hydrogen atoms in HAs; H/Cmod = H/C + 2 (O/C)*0.67; H/C and W indexes were calculated according to (Orlov 1985). Sample numbers correspond to **Table 2**. SD ± 0.05 for N, H and C content

Site	Sample	N, %	C, %	H, %	O, %	C/N	H/C	O/C	H/C mod	W
Kurungnah isl.	1	3.0±0.1	41±2	5.0±0.2	45	13.81	1.50	0.82	2.60	0.15
	2	4.0±0.2	44±2	5.0±0.2	42	13.91	1.43	0.71	2.38	–0.01
	3	3.0±0.1	44±2	5.0±0.2	42	15.16	1.43	0.73	2.40	0.03
	4	3.0±0.1	43±2	5.0±0.2	43	15.18	1.44	0.76	2.45	0.07
	5	3.0±0.1	44±2	5.0±0.2	42	16.82	1.38	0.72	2.35	0.06
	6	3.0±0.1	45±2	5.0±0.2	42	15.78	1.37	0.70	2.32	0.03
	9	3.0±0.1	36±1	5.0±0.2	51	13.93	1.59	1.05	3.00	0.52
Interfluve (Kharau-lakh ridge)	7	3.0±0.1	44±2	5.0±0.2	42	15.16	1.42	0.71	2.37	0.01
	8	4.0±0.2	42±2	5.0±0.2	44	12.65	1.46	0.80	2.53	0.13

from the frozen soil of the IC is noticeably lower (36%). This may indicate that less organic residue reached the IC during its formation. The C/N ratio various from 12 to 16, indicating a low enrichment of carbon by nitrogen. It is associated with a nitrogen reservoir in the Arctic systems where the processes of nitrogen fixation and ammonification are low due to the low microbiological activity. Moreover, the oxygen content in the studied samples is comparable with the carbon content; there is a high oxygen content in the sample from the IC (51%). The high oxygen content is due to the better solubility of oxygen-enriched hydrophilic HAs molecules and their migration ability (Lodygin et al. 2014). The H/C ratio is an indicator of the stability of HAs in soils. The lower this indicator, the higher the process of condensation of monomers in high-molecular substances. From the obtained data, it can be seen that the sample from the IC has the highest H/C ratio, which is the result of a low level of molecular condensation in the absence of a relationship with the upper horizons of the soils. In samples from «old» alas (№ 1-5), depending on the landscape position, the accumulation of high-molecular compounds from the top of the alas to its bottom also occurs. In buried soils of «young» alas (№ 6), the lowest H/C ratio was observed, which indicates that selective degradation of condensed structures occurs with depth inside the profile and an increase in the proportion of herbaceous vegetation, which is the reason for the condensation of high molecular weight compounds (Hatcher et al. 1981; Vasilevich et al. 2019).

From the W index, it follows that most of the study samples are in an oxidized state, only sample 2, located in the bottom of the «old» alas, has reducing conditions associated with a relatively high level of hydromorphism in the soil. Oxidative environmental conditions are associated with the ability of elements to migrate to the bottom of the profile, in particular, iron and aluminum ions, which actively migrate in weakly-acidic and acidic soil solutions. Weak reducing conditions are due to the production of fresh organic residues and the process of humification in the specific bioclimatic conditions of the river delta (Vasilevich et al. 2018).

One of the methods for graphical representation of the elemental composition of HAs from soils in order to identify patterns of their formation is based on the method outlined by Kleinhempel (1970) and van Krevelen (1950). The method is based on constructing H/Cmod and O/C diagrams and serves as a technique to demonstrate the contribution of oxidation and condensation to changes in the elemental composition of HAs (Lodygin et al. 2014; Polyakov et al. 2019a). Based on the obtained diagram (**Figure 3**), the H/Cmod and O/C integral indicator is relatively low in most of the studied HAs, which indicates a low content of oxygen-containing fragments in the HAs and a relatively low migration ability. As already mentioned above, a high value of H/Cmod and O/C is noted in the sample from the IC and indicates that the sample was formed in a

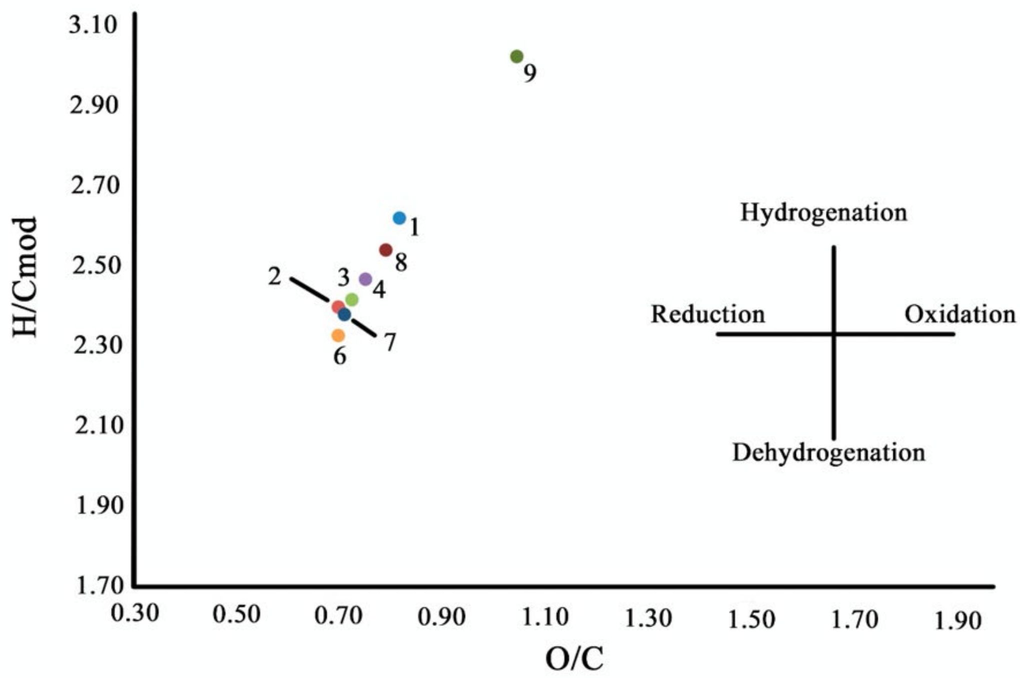

Figure 3. Elemental composition of the studied HAs isolated from study soil. H/Cmod – the number of substituted hydrogen atoms in the HA. Sample numbers correspond to **Table 2**.

environment with a high level of hydromorphism with a low level of microbiological activity, thereby contributing to better conservation of carbohydrate and amino acid HAs fragments. A decrease in H/Cmod indicates the accumulation of aromatic fragments in the composition of soil soils (Pengerud et al. 2017; Strebel et al. 2010).

The obtained data correspond to the previously published work by a numerous of scientists. The Arctic environment is characterized by low microbiological activity and the composition of the precursors of humification. The most characteristic distribution is from west to east of the country as the H/C ratio increases and the proportion of aliphatic compounds grows. Freezing/thawing processes lead to the evolutionary selection of macromolecules of HAs, which is characteristic of the tundra and boreal zones (Abakumov et al. 2015; Beznosikov and Lodygin 2010; Lupachev et al. 2017; Polyakov et al. 2019b).

3.2. Characterization of HAs by ^{13}C–NMR spectroscopy

Numerous molecular fragments were identified by CP/MAS ^{13}C–NMR spectroscopy (**Table 4**): carboxyl (–COOR), carbonyl (–C=O); CH_3–,

CH_2–, CH–aliphatic, –C–OR alcohols, esters and carbohydrates, phenolic (Ar–OH), quinone (Ar = O) and aromatic (Ar–) groups, which indicates the great complexity of the structure of HAs and the polyfunctional properties that cause their active participation in soil processes (Lodygin et al. 2014; Yao et al. 2019).

Table 4. Chemical shifts of atoms of the ^{13}C molecular fragments of HAs

Chemical shift. ppm	*The type of molecular fragments*
0-46	C, H-substituted aliphatic fragments
46-60	Methoxy and O, N-substituted aliphatic fragments
60-110	Aliphatic fragments doubly substituted by heteroatoms (including carbohydrate) and methine carbon of ethers and esters
110-160	C, H-substituted aromatic fragments; O, N-substituted aromatic fragments
160-185	Carboxyl groups, esters, amides and their derivatives
185-200	Quinone groups; Groups of aldehydes and ketones

Six chemical groups in HAs were identified according to the ^{13}C–NMR spectroscopy method. Signals from non-polar alkyls (0-46 ppm), *N*–alkyl/methoxyl (46-60 ppm), *O*-alkyl and anomerics (60-110 ppm), aromatics (110-160 ppm), carboxyl, esters, amides (160-185 ppm) and quinone (185-200 ppm).

The obtained spectra are presented in **Figure 4**. According to the obtained data, we can identify three main groups of fragments that accumulate in the delta soils, these are C,H–alkyl ($(CH_2)_n$/CH/C and CH_3), aromatic compounds (C–C/C–H, C–O) and OCH group (OCH/OCq). The aromatic group is calculated from the sum of the shifts of 110-185 ppm. Aliphatic fragments are calculated from the sum of the shifts of 0-110 ppm, 180-200 ppm, AL h,r + AR h,r (total number of unoxidized carbon atoms) – the signals were summed over the regions 0-46 and 110-160 ppm, C,H–AL/O,N–AL. Signals from C, H–alkyls were summed in the range 0-47 ppm. O, *N*-alkyl at regions 46-60 and 60-110 ppm. The presence of all peaks of the carbon species which are required for identification of the studied substances as HAs has been revealed (Yao et al. 2019). Data of chemical shifts in the studied soil are presented in **Table 5**.

Aliphatic fragments of HAs (53-59%) dominate in the studied soils, which indicates the dominant mineralization process of organic matter in the soils of the Lena River Delta. The predominance of aliphatic fragments indicates the scarcity of vascular plant remnants or the low maturation of humic substances in the terrestrial environment. The predominance of aliphatic structures is typical of humic substances formed under reducing conditions, including aquatic humic substances. The microbial and algal biomass consists of protein and membrane lipids, and sometimes carbohydrate. An aliphatic enhancement in humic substances often occurs when there is contribution of microbial biomass. At the same time, relative to other polar sectors of the Arctic (the Yamal Peninsula, a number of Russian northern islands in the Barents, Kara Seas and the Svalbard archipelago), a significant amount of aromatic fragments accumulate in soils (41-45%). This ratio is closer to the soils of the taiga zone (Abakumov et al. 2019; Lodygin et al. 2014; Polyakov et al. 2019b; Strebel et al. 2010). We previously studied a number of samples from the Lena Delta in recent works, where we noted that a significant amount of aromatic fragments accumulate in the delta in the alluvial soils of the first terrace of the river (annually flooded), as well as samples from the island of Kurungnah (typical permafrost soils). From the data obtained, it was seen that alluvial soils are more enriched in aromatic fragments, and the ratio of aliphatic to aromatic in some samples was more than one (Polyakov et al. 2018; Polyakov et al. 2019b).

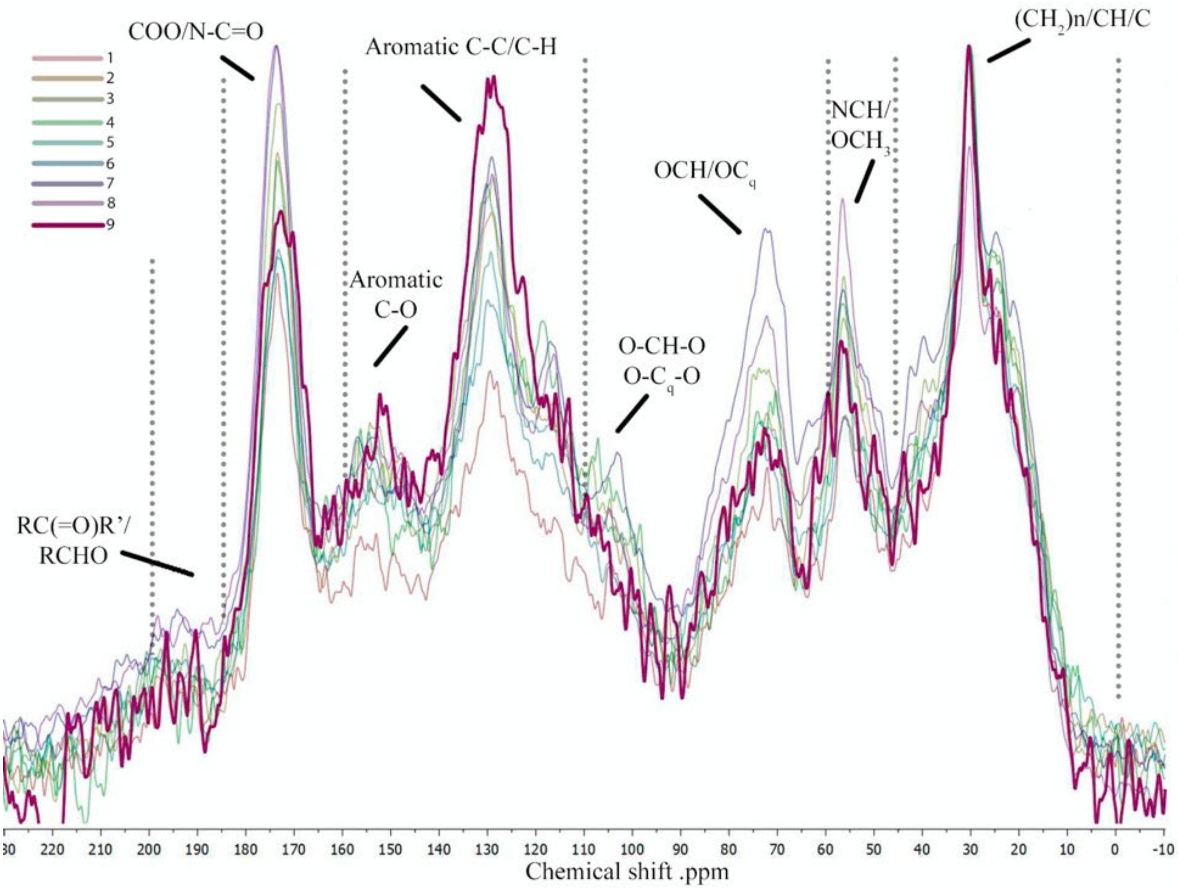

Figure 4. CP/MAS ^{13}C–NMR spectra of HAs from study soils. Number corresponds to**Table 2**.

Table 5. Percentage of carbon in the main structural fragments of HAs from the studied surface soil horizons (according to CP/MAS ^{13}C–NMR data). Sample numbers correspond to **Table 1**; AR – aromatic fraction; AL – aliphatic fraction; AL h,r + AR h,r % – hydrophobicity degree; C,H–AL/O,N–AL – the degree of decomposition of organic matter

Sample	Chemical shifts, % of total C-13 signal						AR	AL	AR/AL	AL h,r + AR h,r, %	C,H –AL/ O,N – AL
	0-46	46-60	60-110	110-160	160-185	185-200					
1	28	8	21	27	14	2	41	59	0.69	76	0.97
2	26	8	20	30	13	3	43	57	0.75	76	0.93
3	24	8	21	30	14	3	44	56	0.79	75	0.83
4	25	7	22	30	13	3	43	57	0.75	77	0.86
5	25	7	22	29	13	4	42	58	0.72	76	0.86
6	25	7	20	31	14	3	45	55	0.80	77	0.96
7	24	8	24	28	14	2	42	58	0.72	76	0.75
8	21	9	22	30	15	3	45	55	0.82	73	0.68
9	22	7	21	33	14	3	47	53	0.89	76	0.79

3.3. Characterization of HAs from «old» alas (Kurungnach isl.)

In the «old» alas, depending on the position in the landscape, 5 soil pits were made (**Figure 5**). Depending on the height, different amounts of aromatic and aliphatic HAs fragments accumulate in soils. First of all, this is due to the predominance of various types of vegetation; mosses and lichens, as precursors of humification, dominate on the windy top of alas (№ 1); mosses and lichens are characterized by the presence of aliphatic constituents and lead to accumulation of aliphatic compounds in the composition of HAs. Sample № 3 is characterized by a higher content of aromatic fragments due to more favorable climatic parameters and the absence of stagnant moisture, the thickness of the active layer reaches 70 cm. This allows a greater number of shrubs to develop on the slope surface, and the southern exposure of the slope is more warmed up during the summer season, which allows the soil microbiota to transform the soil organic matter for a longer season. Sample № 2 (the bottom of the slope) is characterized by a slightly lower content of aromatic fragments. Permafrost processes are more active here, and together with a high permafrost table, stagnic conditions and the mosses and lichens prevailing in the vegetation cover, this leads to accumulation of C,H–alkyl fragments in HAs. According to the composition of HAs, it is most similar to sample 1 and aliphatic fragments predominate the humic substances. Samples № 4 and 5, developed under conditions of excessive moisture and confined to the polygonal tundra, with increasing stagnification in the soil, and an increasing proportion of oxygen-containing fragments according to spectroscopy.

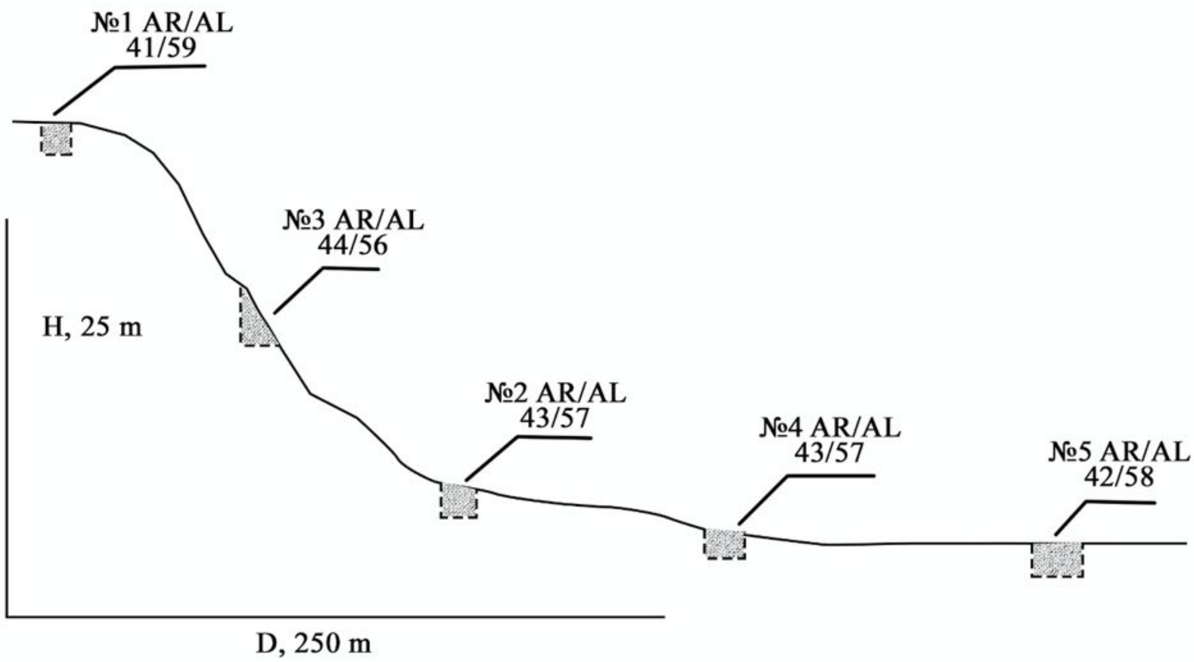

Figure 5. Soil catena of “old” alas in Kurungnah isl. AR/AL – aromatic to aliphatic compounds ratio; H –height of alas; D – distance from point 1 to point 5.

In general, the obtained spectra for this locality are characterized by a rather close position of the chemical shifts and the composition of the HAs, which indicates the homogeneity of the precursors of humification.

3.4. Characterization of HAs from «young» alas

«Young» alas landscapes and soils are about 200 years old and zonal cryoturbation processes here are not as clearly traced as in previous samples. About 200 years ago, there was a lake that began to dry out due to soil and coastal degradation. Today it represents a lowering with a several sporadic pingos, on the tops of which vegetation different from the typical tundra vegetation. The vegetation cover plays a rather important role in the formation of soil and its reserves of organic matter; in «young» alas, it is represented by perennial herbs. At that time, when there was a lake at the place of alas, a significant amount of organic residues accumulated in its bottom sediments, which, after drainage, began to actively interact with the atmosphere and already modern soils began to form in their place.

The SOM buried here turned out the most humified (45% AR) among the studied samples, as well as a high content of organic carbon. Relative to the studied soils, the highest indicator of the content of aromatic fragments (C–C/C–H, C–O) is here, this also confirms the thesis that selective degradation of condensed structures and condensation of high molecular compounds occur with depth. With an increase in the proportion of aromatic compounds, soil organic matter stabilizes, and so the soils buried here will not be subjected to active microbiological effects due to the complex structure of high molecular compounds. The vegetation cover of this site is characterized by a predominance of vascular plants with a developed root system. This results in an intensive input of lignin-derived compounds into soils. Herbaceous plants and their root system show accumulation rates of approximately 30% lignin compounds. These substances play an important role in the formation of HAs. Lignin is crucial component for formation of HAs macromolecules. Chemically, it is a three-dimensional polymer with highly branched molecule composed of phenol units with strong intramolecular bonding (Mišurcová et al. 2012). Lignin transformation ways in soils are presented both by decaying of monomers and by partial changes in the macromolecule. During lignin transformation, a decrease in the group of $–OCH_3$ fragments occurs. Demethylation is a characteristic elementary process of humification, during the decomposition of lignin the accumulation of the COOH group occurs. Thus, an increase in the proportion of vascular plants and lignin may indicate the formation of macromolecules that are more resistant to microbial decomposition indicates the maturity of HAs. The vegetation cover of young alas is a mixture of perennial grasses with a predominance of cereals; annually, as a result of the growing season, straw forms here, which is enriched with nitrogen, carbohydrates, easily and hardly hydrolysable polysaccharides and lignin, during the transformation of which high-molecular compounds are condensed (Verkhoturova and Evstaf'ev 2016).

3.5. Characterization of HAs from Interfluve (Kharaulakh ridge)

The soils formed here are zonal variant of soil formation and can be considered the baseline for this region, this site is not under the active influence of the river. Soils are formed here under continuously windy conditions, therefore, they are represented by low-developed profiles and soil formation takes place on stony rock including carbonates. Nevertheless, the organic matter that is formed here has relatively high levels of humification and up to 45% of aromatic fragments accumulate here; the content of aliphatic fragments is mainly in the C, H–alkyl and OCH group. The high content of aromatic fragments may be due to the lack of permafrost table in the soil, since the soil cover is formed directly on the rock, while the presence of many cracks in the stones prevents the accumulation of excess moisture here and the carbonates available (from the parent rock). The precursors component composition of humic substances depends fundamentally on the type of vegetation and the microbial biomass.

3.6. Characterization of HAs from soil-like body of IC

Annually, due to the action of the river coastal erosion and abrasion, the IC of the Lena River Delta is destroyed and a huge amount of organo-mineral substance enters the atmosphere from the frozen state (**Figure 6**). During the interaction of organomineral components with the atmosphere, SOM is mineralized, and carbon dioxide, water and mineral salts are released. Due to the activation of microorganisms, an enhanced cleavage (mineralization) of the aliphatic structures occurs with an increase in the degree of benzoidicity (Orlov 1990). We have conducted an analysis of this organic matter. According to radiocarbon analysis, the age of these deposits is about 34,299 ± 500 years BP (Schirrmeister et al. 2003). During the analysis, we found that this material was the most humified among the samples we studied. If we consider theories of the origins of the IC, our data show that a reservoir could exist in place of the IC in which the accumulation and transformation of organic matter took place. Frozen organic matter from the IC is the most stable of all the samples we studied. It seems that as a result of the accumulation of various organic residues here and their long-term transformation during the selective degradation of condensed structures, condensation of high-molecular compounds occurred, which led to an increase in the aromaticity of HAs in the soil-like bodies of the IC.

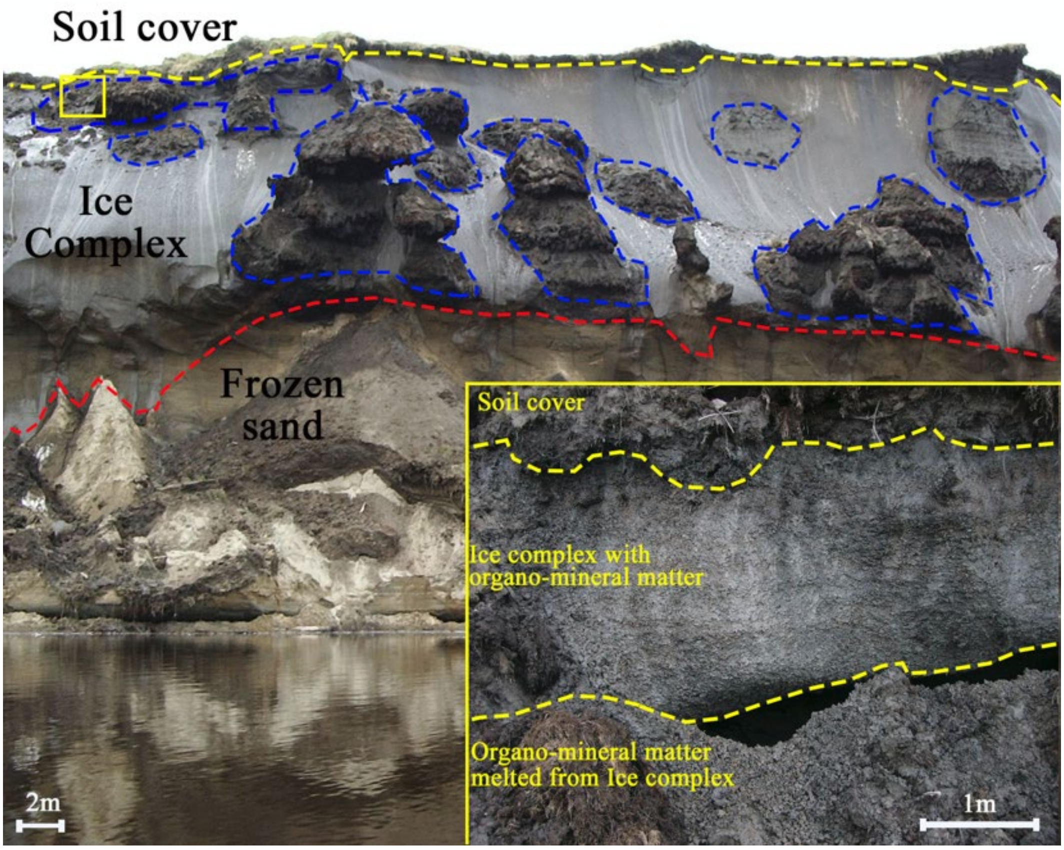

Figure 6. Structure of IC in the Lena River Delta.

3.7. Stabilization rate of organic matter from study soils

In the study samples up to 47% of aromatic compounds accumulate, which indicates the stabilization of organic matter in the soils of the Lena delta. However, aliphatic fragments remain dominant and their accumulation is associated with the predominant mineralization process in soils. The decrease in the portion of aromatic fragments is primarily associated with low microbiological activity and precursors of humification. This ratio AR/AL in study samples leads to the accumulation of organic matter in the soil. As a result of studying the composition of structural fragments of the studied soils, we can conclude the contribution of plant communities to the composition of HA. Thus, in sample № 6, which is formed under vascular plants with about 30% lignin, an increase in signals is observed in the interval of 110-160 ppm. Aromatic and carboxyl fragments in the structure of HA are formed around lignin transformations, which leads to increasing of the stability of HAs to biodegradation. The highest AR/AL ratio (0.89) was observed in the sample from the IC (№ 9). The aromatic fragments content is higher than in the sample formed under vascular plants (№ 6) AR/AL (0.8), this trend may be associated with cryogenic and thawing/freezing processes. The temperature amplitude in the Arctic environments can reach 90 ºC. It was suggested that the humid season allows the formation of soluble precursors, and the dry season favors molecular condensation. In areas that are formed under conditions where the lignin content is minimal, under a moss-lichen cover, a decrease in aromatic fragments in the HA is noticeable due to the low content of aromatic precursors in the precursors of humification. Moreover, the condensation of macromolecules is apparently associated with climatic features in this region. The Lena delta region is quite different from the continental part of Siberia; it has a much milder climate due to the proximity of the sea, and in the summer, the warm Lena waters with temperatures up to 18 °C also contribute to the heating of air, and, accordingly, soils. Thus, the influence of the river on this region is quite high, which favors the development of soils and the formation of HAs with a relatively high proportion of aromatic and carboxyl groups, which are more resistant to biodegradation, compared to other Arctic regions. At the same time, evolutionary selection of organic compounds takes place and high-molecular organic compounds condense at the permafrost table.

The following parameters were used to standardize the quantitative characteristics of HAs macromolecules: the ratio of carbon of aromatic structures to aliphatic, degree of decomposition of organic matter (C-alkyl/O-alkyl) and integral indicator of hydrophobicity of HAs (AL h, r + AR h, r) (**Figure 7**).

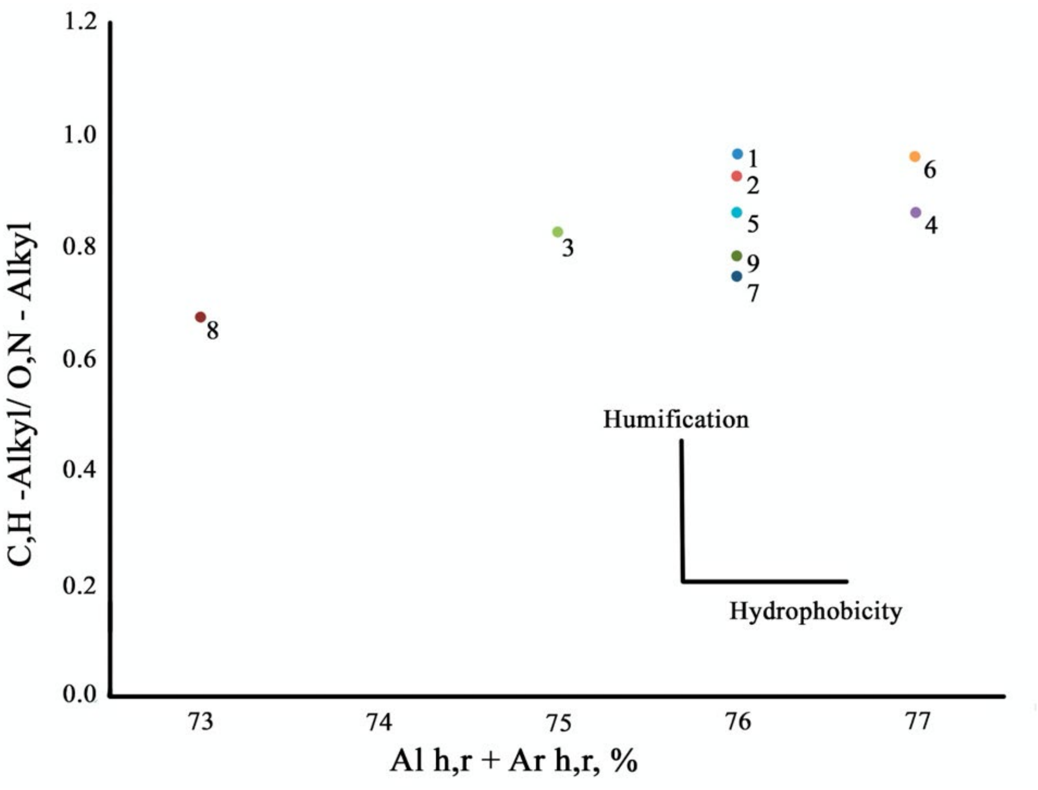

Figure 7. The diagram of integrated indicators of the molecular composition of HAs. Samples correspond to Table 2; AL h,r + AR h,r indicates the total number of unoxidized carbon atoms.

Based on the data obtained, we can conclude that aliphatic compounds ($(CH_2)_n$/CH/C and CH_3) and aromatic compounds (C–C/C–H, C–O) accumulate in the soils investigated. The ratio of aromatic to aliphatic fragments ranges from 0.69 for the sample on top of the «old» alas up to 0.89 for the frozen organics from the IC. The increased portions of aromatic is associated with the local position of the soils in the relief, the exposure of the slope, wind conditions, altitude, hydromorphism of the territory and plant composition. It should be noted the cryogenic activities in soil under the conditions of the Lena Delta, the parent material of which are alluvial sands, lead to a lesser development of cryogenic processes in soils. Under conditions of good drainage and soil aeration, there is a rapid heat exchange with the atmosphere, which affects the level of soil microbiological activity and thereby increases the rate of humification. Thus, during relatively active microbiological processes, a significant proportion of aromatic fragments accumulate in the delta soils. **Figure 7** shows that the most humified and hydrophobic constituents/structures are those obtained from the buried organic matter of «young» alas. This is primarily due to the precursors of humification, and thermodynamic evolutionary selection of high-molecular compounds that accumulate at the permafrost table. In general, from the obtained diagram we can conclude that the accumulation oxygen-containing –OCH and –COOH fragments influences the redistribution of organic acids along the profile, which occurs together with the destruction of the mineral part of the soil. An increase in the proportion of aromatic fragments of HAs leads to stabilization of the organic matter in the soils of the Lena River Delta. The condensation of macromolecular compounds, which includes aromatic/unsaturated structures between 110–185 ppm, indicates an increase in the degree of hydrophobicity of soil organic matter and its low availability to soil microbiome (Semenov et al. 2009).

We carried out a statistical analysis of the principal components in the HAs of the studied soils (**Figure 8**).

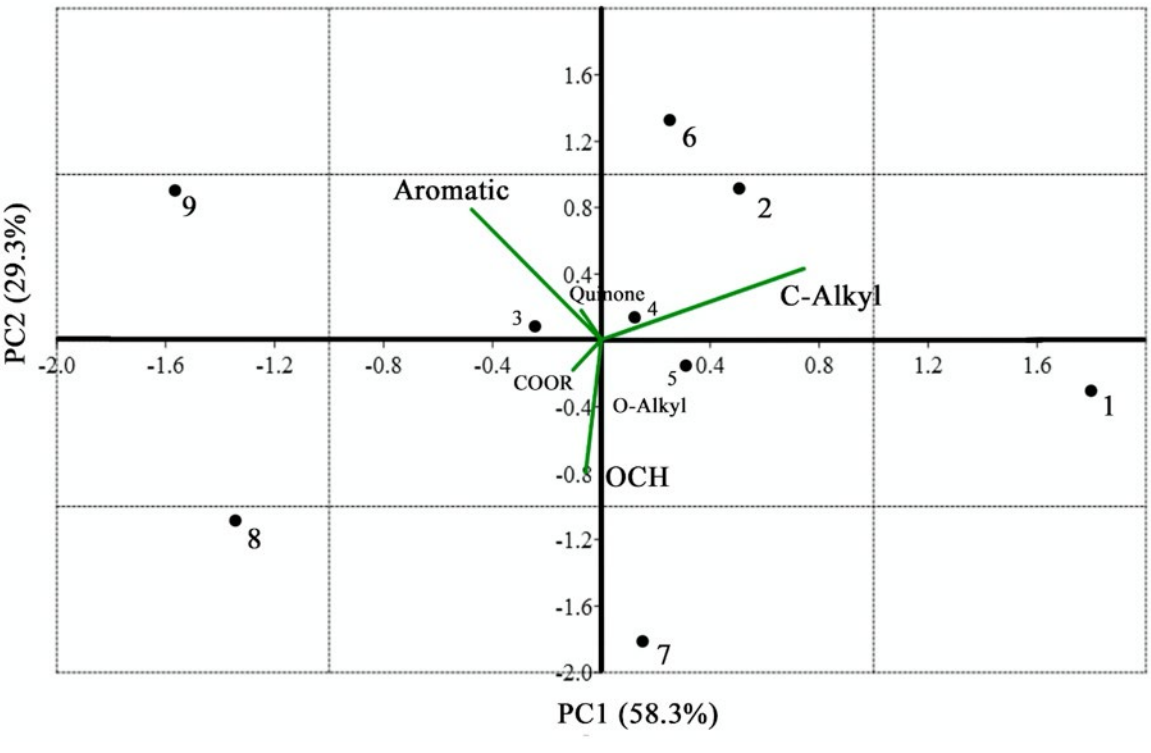

Figure 8. Representation of the samples of humic acids in the plane defined by the first two axes obtained by Principal Component Analysis (88% of the total variance explained).

Based on statistical analysis, we can conclude that there is a high correlation between the studied samples associated with the aliphatic fragments (C-alkyl PC1 (58.3%) and the aromatic fragments PC2 (29.3%). Thus, we can say that the formation processes are primarily responsible for the accumulation of long aliphatic chains present in lipids (fatty acids, paraffins), which are the result of decomposition of moss-lichen plant residues (Karmanov et al. 2015). The aromatic C component, including the aromatic group of chemical compounds, is associated with the transformation of lignin from vascular plants. In general, n-alkyl and carboxylic acids predominate among mosses, which corresponds to the composition of HAs isolated from the studied soils.

Our data are confirmed by previously published materials from scientists working in the Arctic sector and for permafrost-affected soils (Abakumov et al. 2015; Beznosikov and Lodygin 2010; Ejarque and Abakumov 2016). The dominance of aliphatic HAs' fragments is associated with the specific composition of the vegetation cover, soil microbiological composition, and climatic conditions (Lupachev et al. 2017; Polyakov and Abakumov 2020; Szymański 2017). The taiga zones are more similar in terms of HAs composition; here, the aromaticity of the studied HAs of podzols increases to 44% and the European Arctic of Russia to 50% (Lodygin et al. 2014; Pengerud et al. 2017). These regions combine the features of the vegetation cover; the studied tundra and taiga zone is characterized by the predominance of moss-lichen vegetation, which is a source of carbohydrates and various lipids. Thus, the annual change in climatic parameters, cryogenic processes, and the precursors of humification determine the composition of HAs in the study area. The predominance of moss-lichen communities contributes to the formation of long aliphatic chains in the HAs macromolecules. The change of plant communities to vascular plants and the alternation of humid and dry seasons promotes the condensation of aromatic and carboxylic fragments of HAs, which are associated with the resistance of organic material to biodegradation (Abakumov et al. 2015; Beznosikov and Lodygin 2010; Lodygin et al. 2014).

4. Conclusions

The key role in the formation of HAs in the soil is related to the processes of condensation and the polymerization of compounds formed from precursors of plant and microbial origin. Humic acids are structurally dynamic macromolecules, the condensation of which depends on external and internal environmental factors that determine their resistance to biodegradation. During the temporal development of soils, the H/C ratio decreases, which leads to the accumulation of aromatic fragments in the composition of HAs. An increase in the aromaticity of soil HAs lead to a decrease in the rate of soil mineralization and as a result, its destabilization (protection) from microbiological decomposition.

Analysis of the molecular composition of HAs by ^{13}C–NMR spectroscopy also shows that aromatic fragments (up to 47%) are accumulated in HAs of the Lena River Delta soils. The ratio AR/AL varies in soils from 0.69 to 0.89, which indicates the leading processes of mineralization in the soil. The predominance of moss-lichen communities consisting of lignin-lacking plants leads to the accumulation of aliphatic fragments of HAs, which are dominant in the Lena River Delta. In comparison with the HAs that were formed under the current processes of humification we can conclude that the organic residues from the IC are the most humified among the studied samples, which may be the result of long-term climatic changes and condensation of HAs macromolecules in the IC. Plant communities with a predominance of lignified vascular plants are characterized by a higher content of aromatic and carboxylic fragments in the composition of HAs, which indicates the maturity of organic matter in the Lena Delta region.

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