1. **GREEN MANURE: ALTERNATIVE TO CARBON SEQUESTRATION IN A TYPIC USTIPSAMMENT UNDER SEMIARID CONDITIONS.** Piraneque GAMBASICA N. V., Aguirre Forero S. E. & Reis Lucheta A.

2. **ASSESSMENT OF COMPOST AND TECHNOSOL AS AMENDMENTS TO INCREASE NUTRIENT CONTENTS IN A MINE SOIL VEGETATED WITH BRASSICA JUNCEA.** Forján R., Rodríguez-Vila A., Cerqueira B., Amano M., Asensio Fandiño V. & F. Covelo E.


4. **EFFECT OF ENMIENDAS ORGÁNICAS Y AZUFRE EN PROPIEDADES QUÍMICAS Y BIOLÓGICAS DE UN SUELO SÓDICO.** Simanca Fontalvo R. M. & Cuervo Andrade J. L.

5. **EFFECT OF INCUBATION TIME ON TRANSFORMATION RATE AND CHEMICAL FORMS OF PHOSPHOROUS IN CALCAREOUS SOILS ALONG A CLIMOTOPOSEQUENCE.** Moazallah M., Baghernejad M. & Naghavi H.
For information regarding how to become a member of the Spanish Society of Soil Science, please go to http://www.secs.com.es or send an e-mail to the Secretary General (secs@upct.es)

For information regarding how to become a member of the Society of Soil Science in your country, please send an e-mail to the Secretary General of the Latin American Society of Soil Science (lbrs@unam.mx)
Letter from the Editor-In-Chief

With great pleasure I present volume 8, issue 3 of the Spanish Journal of Soil Science. On December 5th we will celebrate the World Soil Day, this time dedicated to soil pollution. This year’s theme (Be the Solution to Soil Pollution) is related to two articles published in this issue, specifically on the rehabilitation of technosols through compost amendments; and also on the rehabilitation of sodium soils. The other articles deal with the rehabilitation of soils affected by fires, aspects of phosphorus fertilization and the effect of green fertilization on carbon sequestration. Again, the diversity of the origin of the authors is an added value to the quality of the articles contained in this issue.

As always, on behalf of the editorial team, we thank the authors for sending the manuscripts, the referees for the quality of their reviews, and the team Universia for their professionalism in managing and editing the platform.

Sincerely,

EDITOR-IN-CHIEF
Dr. Rosa M. Poch
Editorial Board

ADVISORY BOARD

Dr. Jaume Pagès. Board member of Universia (Presidency)

Dr. Jaume Porta. Past-President of the Spanish Society of Soil Science (SECS) (Secretary)

Members

Dr. Rosa Menéndez López. President of the Spanish Research Council (CSIC)

Dr. Mario Pérez Bidegain. President of the Latin American Society of Soil Science (SLCS)

Dr. Fatima Maria de Souza Moreira. President of the Brazilian Society of Soil Science (SBCS)

Dr. Jorge Mataix-Solera. President of the Spanish Society of Soil Science (SECS)

Dr. Rosa Calvo de Anta
Departamento de Edafología y Química Agrícola. Universidad de Santiago de Compostela, Spain

Dr. Montserrat Díaz Raviña
Instituto de Investigaciones Agrobiológicas del CSIC. Santiago de Compostela, Spain

Dr. Carlos Ernesto G. R. Schaefer
Universidade Federal de Viçosa, Brasil

Dr. Fernanda Cabral
Departamento de Ciências e Engenharia dos Biossistemas (DCEB), Universidade Tecnica de Lisboa, Portugal

Dr. Irina Kovda
Institute of Geography
Moscow, Russia

Dr. Claire Chenu
INRA AgroParis Tech
Thiverval-Grignon, France

Dr. Margarita Osterrieth
Centro de Geología de Costas, Universidad Nacional Mar de Plata, Argentina

Dr. Irene Ortiz Bernad. Secretary.
Universidad de Granada. Spain

SCIENTIFIC BOARD

Dr. Rosa M. Poch. Presidency
Departament de Medi Ambient i Ciències del Sòl, Universitat de Lleida. Spain

Dr. Gonzalo Almendros
Museo Nacional de Ciencias Naturales, CSIC. Madrid, Spain

Dr. M. Carmen Hermosín
Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC).
Sevilla, Spain

Dr. Carlos García-Izquierdo
Centro de Ecología y Biología Aplicada del Segura (CEBAS, CSIC). Murcia, Spain

Dr. Mario Pérez Bidegain. President of the Latin American Society of Soil Science (SLCS)
EMERITUS SCIENTIFIC COMMITTEE

Dr. Flavio de Oliveira
Past-President of the Brazilian Society of Soil Science (SBCS)

Dr. Gerardo Rubio
Past-President of the Latin American Society of Soil Science (SLCS)

Dr. Julio Alegre Orihuela
Past-President of the Latin American Society of Soil Science (SLCS)

Dr. Stephen Nortcliff
Soil Research Centre, School of Human and Environmental Sciences, University of Reading, United Kingdom.

Dr. Gonçalo Signorelli de Farias
Past-President of the Brazilian Society of Soil Science (SBCS)

Dr. José Aguilar Ruiz
Past-President of the Spanish Society of Soil Science (SECS)

Dr. Ildefons Pla-Sentís
Departament de Medi Ambient i Ciències del Sòl, Universitat de Lleida, Spain

EXECUTIVE BOARD

Editor-in-Chief
Dr. Rosa M. Poch i Claret
Universitat de Lleida (Lleida, Spain)

Executive Editor
Dr. Irene Ortiz Bernad
Universidad de Granada (Spain)

Coordinator Editor
Antonio Castro
Director of Digital Marketing, Channels and Contents
Santander Universidades and Universia

AUDITORS OF ENGLISH LANGUAGE: Malcolm Hayes and Michele L. Francis.
AUDITOR OF PORTUGUESE LANGUAGE: Manuela Abreu.
COVER PHOTO: Fighting a forest wildfire in Galicia (Spain). Author: S. J. González-Prieto.

UNIVERSIA HOLDING, S.L. has adopted Creative Commons licensing: Attribution-NonCommercial 3.0 Spain (CC BY-NC 3.0). You are free to share (copy, distribute and transmit) and to remix (adapt) the work. You must attribute the material you use to the author(s) or the licensor (but not in any way that suggests they endorse you or your use of the work). You may not use this work for commercial purposes. In no way are any of the following rights affected by the license: (i) your fair dealing or fair use rights, or other applicable copyright exceptions and limitations, (ii) the author’s moral rights; (iii) rights other persons may have either in the work itself or in how the work is used, such as publicity or privacy rights. For full details see http://creativecommons.org/licenses/by-nc/3.0/es/legalcode.es.
List of Reviewers

We would like to acknowledge the support of the following people who contributed to peer review of articles from March 2018 to November 2018:

Manuela Abreu
Gonzalo Almendros
Silvia Amicone
Daniel Arenas Lago
Ester Álvarez Ayuso
Omar Bachmeier
Marta Benito Capa
Aleksander J. Borejsza
Héctor Cabadas Báez
Rosa M. Calvo de Anta
Pilar Carral González
Juan Castro Rodríguez
Nathalie Cools
Nuno Cortez
Heajoo Chung
Necdet Dagdelen
Eduardo de Sá Pereira
Fernando del Moral Torres
Emilia Fernández Ondoño
Desiderio A. Francisco Bethencourt
José María García-Ruiz
José A. Gómez
Mª Carmen Gutiérrez Castorena
Munima Haque
Peter Jacobs
Mª Noelia Jiménez Morales
Irina Kovda
Peter Kühn
Juan Carlos Loaiza
Mª Elvira López-Mosquera
Francisco Martín Peinado
Christopher Miller
Ana Moliner Aramendia
F. G. Monteiro
Juan Luis Mora Hernández
Przemyslaw Mroczek
Jorge Mataix-Solera
Christopher M. Menzel
José Navarro Pedreño
Stephen Nortcliff
Juan Carlos Nóvoa Muñoz
Irene Ortiz Bernad
Josep Oriol Ortiz Perpiñá
Xose Luis Otero Pérez
Hamidreza Owliaie
Matthew C. Pailes
Ildefons Pla Sentís
Rosa M. Poch
Jaume Porta Casanellas
Blanca Prado
José Luis Punzo Díaz
Patrick Quinn
Yann Ramos Arroyo
Alfonso Rodríguez-Vila
Adriana A. Rollán
Ali Akbar Safari Sinegani
Gabriela Sánchez Viveros
Cristina Santín
Sergei Sedov
Fabio Scarciglia
Carlos Schaefer
Raimund Schneider
Manuel Sierra Aragón
Rafael Silva Guedes
Martín M. Silva Rossi
Tobias Sprafke
Karl Stahr
Guillermo A. Studdert
Lukasz Usarowicz
Asunción Usón Murillo
Ricardo D. Valdez Cepeda
César Villalobos Acosta
Françoise Watteau
Raúl Zornoza Belmonte
Summary

VOLUME 8 • ISSUE 3

1. Green manure: Alternative to carbon sequestration in a Typic Ustipsamment under semiarid conditions.
   Abono verde: una alternativa al secuestro de carbono en un Typic Ustipsamment bajo condiciones semiáridas
   Piraneque Gambasica N. V., Aguirre Forero S. E. & Reis Lucheta A.

2. Assessment of compost and Technosol as amendments to increase nutrient contents in a mine soil vegetated with Brassica juncea.
   Evaluación de enmiendas elaboradas con compost y un Tecnosol sobre el incremento del contenido de nutrientes en un suelo de mina vegetado con Brassica juncea
   Forján R., Rodríguez-Vila A., Cerqueira B., Amano M., Asensio Fandiño V. & F. Covelo E.

3. Medium-term impact of post-fire emergency rehabilitation techniques on a shrubland ecosystem in Galicia (NW Spain).
   Impacto a medio plazo de las técnicas de rehabilitación de emergencia post-incendio sobre un ecosistema de matorral en Galicia (NO de España)
   Díaz-Raviña M., Lombao Vázquez A., Barreiro Buján A. I., Martín Jiménez A. & Carballas Fernández T.

4. Effect of organic amendments and sulfur on chemical and biological properties of a sodic soil.
   Efecto de enmiendas orgánicas y azufre en propiedades químicas y biológicas de un suelo sódico
   Simanca Fontalvo R. M. & Cuervo Andrade J. L.

5. Effect of incubation time on transformation rate and chemical forms of phosphorous in calcareous soils along a climotoposequence.
   Efecto del tiempo de incubación sobre el grado de transformación y las formas químicas de fósforo en suelos calcáreos a lo largo de una climotopossecuencia
   Moazallah M., Baghernajad M. & Naghavi H.
Green manure: Alternative to carbon sequestration in a Typic Ustipsamment under semiarid conditions

ABSTRACT

Vegetative soil cover mitigates climatic variability and enhances the balance between mineralization and humification processes. Under aerobic conditions, most of the carbon that enters the soil is labile, but a small fraction (1%) is humified and stable, contributing to the soil carbon reserve; therefore, it is important to assess the carbon content captured after green manure cultivation and decomposition. During two consecutive semesters, July to December 2016 and January to June 2017, green manure plots (Zea mays L., Andropogon sorghum subsp. sudanensis and Crotalaria longirostrata) were cultivated individually, in a consortium or amended with palm oil agro-industrial biosolids in a randomized complete block design with 12 treatments. Once decomposed, the different carbon fractions (organic, oxidizable, non-oxidizable, removable and total) were determined. The results showed high total and organic carbon contents under the sorghum treatment, at 30 and 28 Mg ha⁻¹, respectively, followed by those under the fallow + biosolid treatment, at 29.8 Mg ha⁻¹ and 27.5 Mg ha⁻¹, respectively. Despite the short experiment duration and the possible contributions of previous management on recalcitrant carbon soil stocks, these findings suggest the importance of maintaining plant cover and utilizing green manure in the Colombian Caribbean region. Long-term experiments may be conducted to confirm the full potential of cover crops on carbon sequestration under tropical semiarid conditions.

RESUMEN

La cobertura vegetal mitigá la variación climática y favorece el balance entre los procesos de mineralización y humificación del suelo. En condiciones aeróbicas, parte del carbono que entra en el suelo es labil, pero una pequeña fracción (1%) es humificada, estable y constituye la reserva de carbono del suelo; por ello, es importante conocer la cantidad de carbono capturada después del cultivo y descomposición de abonos verdes. Durante dos semestres consecutivos, julio a diciembre de 2016 y enero a junio de 2017, parcelas fueron cultivadas con abonos verdes (Zea mays L., Andropogon sorghum subsp. sudanensis y Crotalaria longirostrata) individuales, asociadas o con aplicación de biosólidos de la agroindustria de palma de aceite, totalizando 12 tratamientos en un diseño en bloques...
al azar. Una vez descompuestos se determinaron las diferentes fracciones de carbono (orgánico, oxidable, no oxidable, removible y total). Los resultados indicaron altas concentraciones de carbono total y orgánico en el tratamiento con sorgo con 30 y 28 Mg ha\(^{-1}\), respectivamente, seguido por barbecho + biosólido con 29.8 Mg ha\(^{-1}\) de carbono total y 27.5 Mg ha\(^{-1}\) de carbono orgánico. Pese a la corta duración del experimento y la posible contribución de cultivos previos en el almacenamiento de carbono recalcitrante en el suelo, los resultados sugieren la importancia de mantener la cobertura vegetal y la utilización de abonos verdes en la región del Caribe Colombiano. Más estudios de larga duración deben ser conducidos para la determinación del potencial máximo de los abonos verdes en el secuestro de carbono bajo condiciones tropicales semiáridas.

RESUMO

A cobertura vegetal atua na mitigação das alterações climáticas e favorece o balanço entre os processos de mineralização e humificação da matéria orgânica no solo. Em condições aeróbicas, a maior parte do carbono que entra no solo é lábil, porém uma pequena fração (1%) é húmica, estável e contribui para a reserva de carbono no solo, assim sendo, é importante conhecer a quantidade de carbono sequestrada após o cultivo e decomposição dos adubos verdes. Durante dois semestres consecutivos, Julho a Dezembro de 2016 e Janeiro a Junho de 2017, foram cultivadas parcelas com adubos verdes (Zea mayz L., Andropogon sorghum subsp. sudanensis and Crotalaria longirostrata) individuais, consociadas ou com aplicação de biosólidos da agroindustria do óleo de palma, totalizando 12 tratamentos delineados em blocos totalmente casualizados. Após a decomposição, foram determinadas as diferentes frações de carbono (orgânica, oxidável, não oxidável, lábil e total). Os resultados indicaram altas concentrações de carbono total e orgânico no tratamento com sorgo, 30 e 28 Mg ha\(^{-1}\), respectivamente, seguido por pousio + biossólido com 29,8 de carbono total e 27,5 Mg ha\(^{-1}\) de carbono orgânico. Apesar da curta duração dos ensaios e da possível contribuição de culturas prévias no armazenamento de carbono recalcitrante no solo, os resultados obtidos sugerem a importância da manutenção da cobertura vegetal e utilização da adubação verde na região do Caribe Colombiano. Ensaios de longa duração deverão ser conduzidos para a determinação do potencial máximo dos adubos verdes no sequestro de carbono em condições tropicais semiáridas.

1. Introduction

Agricultural practices can be a source of carbon dioxide (CO\(_2\)) to the atmosphere (Lal 2007, 2014), contributing to climate variability when it surpasses plant carbon fixation by photosynthesis. Reicosky (2002) estimated that soil tillage promotes carbon losses between 30% and 50% and results in moisture and biodiversity losses in edaphic systems. However, biomass incorporation into soil has the potential to capture CO\(_2\) through the humification of organic matter (OM) fractions after the mineralization process (Escalante 2015) and increase the soil carbon (SC) content.

The adoption of more sustainable agricultural practices, such as cover crops, can increase SC and the retention of soil moisture (Carvajal et al. 2014). In humid tropical climates, biomass added to soil is readily mineralized to CO\(_2\) and quickly released into the atmosphere due to the higher turnover rates (Davidson and Janssens 2006). However, under semiarid conditions, soils are more resistant to carbon losses (Donohue et al. 2013; Romanyá et al. 2000) and can be considered an important sink for atmospheric CO\(_2\) (Evans et al. 2014). It was also demonstrated that the type of cover crop directly affects the soil organic carbon (SOC) mineralization rates under semiarid conditions (Ghimire et al. 2017). Exposing semiarid soils to elevated concentrations of CO\(_2\) resulted in increases in net ecosystem productivity and carbon storage primarily as a result of direct effects on photosynthesis.
GREEN MANURE: ALTERNATIVE TO CARBON SEQUESTRATION IN A TYPIC USTIPSAMMENT UNDER SEMIARID CONDITIONS

(Dijkstra et al. 2010; Evans et al. 2014). The indirect effect of SOC on soil physical properties and water retention, together with total N and K, was also identified as a key ecosystem function driving plant biodiversity and contributing to the rehabilitation of deserted areas (Qiu et al. 2018).

Another alternative to semiarid SC stocks is amendment with agro-industrial wastes. During oil palm production, a large quantity of solid and liquid wastes in the form of empty fruit bunches, pericarp fibers, and palm biosolids is generated. It is estimated that 0.3-0.6 t of biosolids are produced for every t of oil palm fresh fruit bunches processed (Rashid et al. 2009). The biosolid produced is a colloidal suspension with a sufficient amount of nutrients for N, P and K as well as cellulose, which could be favorable for microbial growth and soil chemical and physical characteristics. Many attempts have been reported in the bioconversion of these oil palm wastes (Alam et al. 2008), but the high production of these wastes forces the search for alternative uses.

Present and future greenhouse gas (GHG) emissions from agriculture are highly uncertain in Latin America due to the real adoption or absence of climate mitigation policies (Calvin et al. 2016). FAO (2014) estimates that GHGs derived from agricultural activities in Latin America account for 17% of agricultural emissions and have doubled in the last 50 years. Alternatives are necessary to mitigate GHG emissions and reduce the impacts of agricultural practices on global warming. Because semiarid and arid ecosystems cover 47% of the terrestrial surface (Evans et al. 2014), it is very important to accurately determine carbon capture under these environmental conditions and their potential as atmospheric carbon sinks under the current atmospheric CO₂ concentration.

Here, we evaluated the carbon fixation rates of a Typic Ustipsamment planted with green manures (Zea mays L., Andropogon sorghum subsp. sudanensis, and Crotalaria longirostrata) to which biosolids were added (residue from palm oil processing), in a short-term experiment in Santa Marta, Colombia, in a semiarid ecosystem with tropical dry forests converted to intensive agricultural fields after deforestation.

2. Materials and Methods

2.1. Site description and experimental design

The study site is characterized by a tropical dry climate, with an average annual precipitation of 674 mm and bimodal rainfall. The seasons are divided into rainy (March to May and September to November) and dry (December to February and June to August) periods. The mean temperature and relative humidity are 29.5 °C and 70%, respectively. The natural plant community is composed of a dry tropical forest with xerophytic vegetation (IDEAM et al. 2015). Soils in the area are derived from strata of sedimentary parent rock from the Tertiary period of the Cenozoic Era with successive strata of sandstones and schistose clays, colored yellow, brown, greenish-gray and bluish, from the Oligocene and Miocene eras. There are also calcareous limestones, calcareous sandstones, gravel, coal layers and marly limestone from the Pliocene. This formation has considerable thickness and is characterized by its Miocene fauna, plant remains, and strongly bituminous lignite layers. The soil mineralogy is dominated by kaolinite (50%), quartz (5-15%), feldspar (5-15%), interstratified trace minerals, micas (5-30%), montmorillonite (30-50%) and vermiculite (5-30%) (IGAC 2009; Aguirre et al. 2015).

Field experiments were carried out in 2016 at the Center for Agricultural and Forestry Development of the Universidad del Magdalena, Santa Marta, Colombia (11°13'24" N; 74°10'56" W) (Figure 1), with mean monthly temperatures that varied within the annual cycle from 27.9-29.9 °C and with an average annual rainfall of 565.5 mm. Approximately 60% of the annual rainfall occurred during the rainy season.

Soils were classified as Typic Ustipsamments of alluvial origin. According to Idárraga et al. (2011), these are recent unconsolidated alluviums composed of sands, gravels, and pebbles that are poorly selected and have fragments of plagioclase, mica biotite, quartz and rocks from the Batolito de Santa Marta rocks. They are located in the western part of the study area and come from the mountainous part of the
Sierra Nevada de Santa Marta - SNSM, forming small fans and fluvial terraces and showing a pH (determined in a 1:1 soil and distilled deionized water mixture) of 8.06, bulk density (\(\rho_a\)) of 1.35 g cm\(^{-3}\), organic carbon (OC) of 1.17% (15.8 Mg ha\(^{-1}\)), and electric conductivity (EC\(_{1:1}\)) - laboratory measured electrical conductivity of a 1:1 soil to water extract - of 0.80 dS m\(^{-1}\) at 25 °C. Seeds of maize (\textit{Zea mays}), sorghum (\textit{Andropogon sorghum} subsp. \textit{sudanensis}) and crotalaria (\textit{Crotalaria longirostrata}) were sown as green manure in 5 x 5 m plots in addition to a control plot in which weeds grew freely. The experimental design was a randomized complete block with twelve treatments and three replications (Table 1), totaling 36 experimental units distributed across 900 m\(^2\).

Fertilizer rates were calculated according to the species’ nutrient requirements; fertilizer applications consisted of 0.5 kg of 17-6-18-2 (N-P-K-Mg) fertilizer and 5 kg plot\(^{-1}\) of biosolids (4.3% N, 1.2% P, 1.5% K, 1.7% Ca, and 1.2% Mg, with 23.4% ash and a pH of 6.8) from the palm agro-industry in T6, T7, T8, T9, T10, and T12. SC fractions were determined during two harvesting cycles. Treatments were randomly distributed, and plants were cut and laid out on the ground after they reached 75% flowering. Four soil samples were collected from a 0-10 cm depth in three stages: before planting, 45 days after planting, and 30 days after cutting or being laid out for organic material on the ground, for a total of 24 samples per plot. Fifty grams of each undisturbed soil sample was taken for bulk density (\(\rho_a\)) measurement and stored carbon.

Figure 1. Map showing the location of the Universidad del Magdalena, Santa Marta - Department of Magdalena, Colombia, where the carbon capture rates of a Typic Ustipsamment planted with green manures were determined.
2.2. Biomass and dry mass production by green fertilizers

Plant samples were taken from each plot and replicated when the plants showed 75% flowering after cutting all the plants at their maximum flowering stage (75 days after planting). Random samples using an iron square of 0.25 m² were taken, with three replications per plot. All plants present in the area were cut at the root's neck and weighed for biomass determination. Then, 500 g of plant material was dried at 65 °C for 72 hours and weighed to determine the dry matter.

2.3. Determination of SC fractions

Total SC (TSC) and SC fractions of different labilities were determined based on the methodology developed by Macías et al. (2004) at the Soil Laboratories of the University of Magdalena and the University of Santiago de Compostela, Spain. The soil samples from the three sampling stages were mixed to create a composite sample before SC fraction analysis. A brief description of each method is provided in Table 2.

SOM was estimated from the SOC multiplied by empirical factors such as van Benmelen’s (Pribyl 2010), with a value of 1.724 in surface horizons.

SOC differs from labile carbon (LC) or available carbon as an energy source in that it maintains the chemical characteristics of the source material (carbohydrates, lignin, proteins, tannins, and fatty acids) and a stable humic fraction, constituted by fulvic acids, humic acids and humins (Martínez et al. 2008). Removable carbon with pyrophosphate (Cp) is related to the humic fractions of OM (Islas et al. 2014) and represents the “humified C” or “active C,” which forms different C compounds bound to metallic elements and even to soil mineral components. Additionally, oxidizable carbon with dichromate

Table 1. Treatment descriptions and crop density during two cycles of green manures

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Crop Density (kg of seeds ha⁻¹)</th>
<th>Fertilizer (N-P-K-Mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Maize</td>
<td>30</td>
<td>17-6-18-2</td>
</tr>
<tr>
<td>T2 Sorghum</td>
<td>25</td>
<td>17-6-18-2</td>
</tr>
<tr>
<td>T3 Crotalaria</td>
<td>15</td>
<td>17-6-18-2</td>
</tr>
<tr>
<td>T4 Maize + Crotalaria</td>
<td>21-4.5</td>
<td>17-6-18-2</td>
</tr>
<tr>
<td>T5 Sorghum + Crotalaria</td>
<td>17.5-4.5</td>
<td>17-6-18-2</td>
</tr>
<tr>
<td>T6 Maize + Biosolids</td>
<td>30-2</td>
<td>17-6-18-2+biosolids</td>
</tr>
<tr>
<td>T7 Sorghum + Biosolids</td>
<td>25-2</td>
<td>17-6-18-2+biosolids</td>
</tr>
<tr>
<td>T8 Crotalaria + Biosolids</td>
<td>15-2</td>
<td>17-6-18-2+biosolids</td>
</tr>
<tr>
<td>T9 Maize + Crotalaria + Biosolids</td>
<td>21-4.5-2</td>
<td>17-6-18-2+biosolids</td>
</tr>
<tr>
<td>T10 Sorghum + Crotalaria + Biosolids</td>
<td>17.5-4.5-2</td>
<td>17-6-18-2+biosolids</td>
</tr>
<tr>
<td>*T11 Control plot</td>
<td>Fallow</td>
<td>Without</td>
</tr>
<tr>
<td>*T12 Control plot + Biosolids</td>
<td>Fallow</td>
<td>Biosolids</td>
</tr>
</tbody>
</table>

*The control plots were established by the spontaneous vegetation (fallow) of natural weed species.
(C_{od}), but not C_p, refers to the fractions that escaped biological activity and oxidative processes (hot oxidation with dichromate in sulfuric medium) (Macias et al. 2005). Likewise, oxidizable carbon (C_{od}), resulting from the difference between C_{od} and C_p, is the readily available carbon. Finally, the residue between TSC and C_{od} represents nonoxidizable carbon (C_{nox}), which is recalcitrant C and is not oxidized under extreme or forced conditions (Macias et al. 2005, quoted by Islas et al. 2014).

Table 2. Methods used in the determination of carbon soil fractions

<table>
<thead>
<tr>
<th>Carbon fraction</th>
<th>Principles</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Soil Carbon (TSC)</td>
<td>High-temperature combustion of the soil sample with an elemental analyzer (Leco Truspec cn.)</td>
<td>Macias et al. 2005</td>
</tr>
<tr>
<td>Labile Soil Carbon (LC)</td>
<td>TSC* 0.02</td>
<td>Macias et al. 2005</td>
</tr>
<tr>
<td>Stored Carbon</td>
<td>TSC x MCA - MCA = Soil volume*ρa</td>
<td>Guo and Gifford 2002</td>
</tr>
<tr>
<td>Soil Organic Carbon (SOC)</td>
<td>Oxidation of organic matter by dichromate in sulfuric medium</td>
<td>Macias et al. 2004</td>
</tr>
<tr>
<td>Oxidizable Carbon in Dichromate (C_{od})</td>
<td>Oxidizable organic carbon with potassium dichromate in acid medium, cold and hot</td>
<td>Walkley and Black 1934, modified by Macias et al. 2004</td>
</tr>
<tr>
<td>Removable Carbon (C_r)</td>
<td>Removable carbon with sodium pyrophosphate C. Humified – Active</td>
<td>Bascomb 1968</td>
</tr>
<tr>
<td>Oxidizable Soil Carbon (C_{ox})</td>
<td>C_{ox} = (C_{od} - C_p)</td>
<td>Macias et al. 2005 in Islas et al. 2014</td>
</tr>
<tr>
<td>Nonoxidizable Carbon (C_{nox})</td>
<td>TSC - C_{ox} - C_{ox} Fractions that have escaped biological activity and even oxidative processes; among them is removable C</td>
<td>Macias et al. 2004</td>
</tr>
</tbody>
</table>

2.4. Data analysis

The data were subjected to ANOVA (P < 0.05) and Tukey’s test (P < 0.01) for means comparison. Subsequently, the degree of association of the variables was verified by Pearson’s correlation test to establish the correlation between the different forms of carbon. Statistical analyses were performed using R software v. 3.02 (R Development Core Team 2015, available at www.r-project.org).

3. Results and Discussion

Once the green manures were degraded, low average values of EC_{1:1} (0.73 dS m^{-1} at 25 °C) and pH (7.8) were observed in the soil, probably due to the production of organic acids resulting from the OM cycling. SOC concentration decreases with depth and is linked to organic residues (carbohydrates, lignins, proteins, and humus), promoting the aggregation and distribution of the porous space and the chemical characteristics of soil. Under natural conditions, SOC results from the balance between the incorporation of fresh organic material and the C output as CO_2 into the atmosphere (Duval et al. 2014).
Vegetative development depends in large part on the morphology of the species, but climate limits the capacity for CO₂ fixation by the plant (Azcón-Bieto et al. 2008). In the present study (Table 3), T2, T3, and T4 showed significant differences in the accumulation of dry weight (DW) when compared to the mixed treatments (green manure + biosolids). However, sorghum was more efficient than corn and crotalaria in the environmental conditions of the studied area when comparing with the controls treatments (T11 and T12).

All the isolated green manure cultures, as well as combined treatments (T4, T5 and T6), showed significantly (P < 0.05) higher fresh biomass production compared to that of the control plots (T11 and T12) (Table 3).

### Table 3. Means of plant biomass, fresh and dry weights in each treatment. Means calculated from three replicates per treatment

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>FW Mg ha⁻¹</th>
<th>SD</th>
<th>DW Mg ha⁻¹</th>
<th>SD</th>
<th>FW/DW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Maize</td>
<td>55.09⁷</td>
<td>± 14.44</td>
<td>11.02⁷</td>
<td>± 4.99</td>
<td>20</td>
</tr>
<tr>
<td>T2 Sorghum</td>
<td>67.29⁴</td>
<td>± 4.40</td>
<td>13.46⁴</td>
<td>± 6.10</td>
<td>20</td>
</tr>
<tr>
<td>T3 Crotalaria</td>
<td>51.90⁸</td>
<td>± 20.26</td>
<td>10.38⁸</td>
<td>± 4.70</td>
<td>20</td>
</tr>
<tr>
<td>T4 Maize + Crotalaria</td>
<td>51.56⁸</td>
<td>± 21.28</td>
<td>10.31⁸</td>
<td>± 4.67</td>
<td>20</td>
</tr>
<tr>
<td>T5 Sorghum + Crotalaria</td>
<td>42.85⁸</td>
<td>± 17.49</td>
<td>8.57⁸</td>
<td>± 3.88</td>
<td>20</td>
</tr>
<tr>
<td>T6 Maize + Biosolids</td>
<td>41.12⁸</td>
<td>± 14.82</td>
<td>8.23⁸</td>
<td>± 3.73</td>
<td>20</td>
</tr>
<tr>
<td>T7 Sorghum + Biosolids</td>
<td>33.91⁹</td>
<td>± 10.95</td>
<td>6.78⁹</td>
<td>± 3.07</td>
<td>20</td>
</tr>
<tr>
<td>T8 Crotalaria + Biosolids</td>
<td>33.75⁹</td>
<td>± 10.32</td>
<td>6.75⁹</td>
<td>± 3.06</td>
<td>20</td>
</tr>
<tr>
<td>T9 Maize + Crotalaria + Biosolids</td>
<td>32.43⁹</td>
<td>± 12.25</td>
<td>6.49⁹</td>
<td>± 2.94</td>
<td>20</td>
</tr>
<tr>
<td>T10 Sorghum + Crotalaria + Biosolids</td>
<td>31.65⁹</td>
<td>± 10.29</td>
<td>6.33⁹</td>
<td>± 2.87</td>
<td>20</td>
</tr>
<tr>
<td>T11 Control plot (fallow)</td>
<td>23.49⁴</td>
<td>± 6.51</td>
<td>9.96⁴</td>
<td>± 4.51</td>
<td>42.4</td>
</tr>
<tr>
<td>T12 Control plot + Biosolids</td>
<td>20.49⁴</td>
<td>± 11.85</td>
<td>9.58⁴</td>
<td>± 4.34</td>
<td>46.76</td>
</tr>
</tbody>
</table>

FW: Fresh weight. DW: Dry weight. Means in the columns followed by the same letter were not significantly different (P > 0.01) according to Tukey’s test. SD: Standard deviation.

The treatment T2 (sorghum) exhibited the highest TSC content, with 30.036 Mg ha⁻¹ (Table 4), followed by T12 (fallow + biosolids) with 29.876 Mg ha⁻¹. In contrast, T7 (sorghum + biosolids) showed the lowest values of TSC, at 22.571 Mg ha⁻¹. No significant differences were found (P > 0.05) in the carbon content between cycles and sampling periods.

These results agree with those reported by Prager et al. (2012), who evaluated Sorghum bicolor, Crotalaria juncea and Canavalia ensiformis as green manure in the tropics and found that the soils treated with Sorghum bicolor associated with mycorrhizae showed the highest TSC content after being degraded in the soil, with a value of 5.94 Mg ha⁻¹. However, these values were lower than those found in the present study.

Salazar et al. (2004) evaluated the effect of cut and distribution of Zea mays, S. bicolor, Phaseolus vulgaris and Panicum miliaceum species on the TSC content of an inceptisol during a growing season and reported that sorghum provided 19.78 Mg ha⁻¹, surpassing the others, supporting the findings of the present research, where T2 (sorghum) surpassed the other treatments.
The FAO (2002) studied grasslands, savannas and fallow fields with spontaneous vegetation and showed that these species had a high capacity for CO₂ storage. Likewise, González et al. (2014) proposed improved grasslands, fallow covers, and resting areas as zones with high potential for SC fixation. This hypothesis agreed with the results obtained here, which showed that T12 (fallow + biosolids) exhibited a high TSC fixation capacity.

Porlles (2011) argued the importance of C fixation in arid lands for contributing to the global C balance and reducing the rate of desertification with native species in each zone. According to the FAO (2002), a great proportion of arid lands are in the tropics, which represent 37.2% of the terrestrial surface (4900 million hectares) and could, through appropriate management, provide benefits of carbon sequestration in addition to their contribution to ecosystem restoration. T12, a fallow field composed of spontaneous vegetation of *Lolium perenne*, *Hordeum jubatum* L., *Cynodon dactylon*, *Panicum maximum*, *Cucumis melo*, *Boerhavia erecta*, *Amaranthus retroflexus*, *A. spinosus* and *Momordica balsamina*, showed acceptable biomass production and ability to fix TSC, probably due to the adaptation and aggressiveness of the species in the area.

In Table 4, the SOC content is reported in its C_{col} and LC fractions. T2 and T12 were the treatments that fixed the greatest amounts of stable carbon, which is an important trait in the selection of species (*Sorghum*) for TSC sequestration in dry tropics. Ghimire et al. (2017) evaluated the amount and quality of cover crop biomass on carbon mineralization in semiarid soils, suggesting that large pools of canola and pea biomass can stimulate turnover rates. Likewise, the aggressiveness and adaptation of spontaneous species (T12) are remarkable for the revegetation of the area, suggesting the potential value of protecting fallow land in the area.

C_{col} is the fraction of C that has already escaped biological activity and oxidative processes and represents a dynamic balance between the absorption of dead plant material and loss by decomposition (Macías et al. 2004). Treatment

**Table 4.** Carbon content fractions (Mg ha⁻¹) in *Typic Ustipsamments* cultivated with green manures

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TSC</th>
<th>LC</th>
<th>C_{col}</th>
<th>C_{p}</th>
<th>C_{ex}</th>
<th>C_{nox}</th>
<th>SOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Maize</td>
<td>24.76</td>
<td>0.49</td>
<td>4.69</td>
<td>2.33</td>
<td>3.35</td>
<td>22.40</td>
<td>22.90</td>
</tr>
<tr>
<td>T2 Sorghum</td>
<td>30.04</td>
<td>0.60</td>
<td>4.64</td>
<td>2.95</td>
<td>1.69</td>
<td>28.34</td>
<td>28.94</td>
</tr>
<tr>
<td>T3 Crotalaria</td>
<td>25.95</td>
<td>0.52</td>
<td>5.32</td>
<td>2.31</td>
<td>3.01</td>
<td>22.94</td>
<td>23.46</td>
</tr>
<tr>
<td>T4 Maize + Crotalaria</td>
<td>23.21</td>
<td>0.46</td>
<td>4.63</td>
<td>2.18</td>
<td>2.45</td>
<td>20.76</td>
<td>21.22</td>
</tr>
<tr>
<td>T5 Sorghum + Crotalaria</td>
<td>24.29</td>
<td>0.48</td>
<td>4.33</td>
<td>2.41</td>
<td>1.92</td>
<td>22.37</td>
<td>22.86</td>
</tr>
<tr>
<td>T6 Maize + Biosolids</td>
<td>27.37</td>
<td>0.55</td>
<td>6.15</td>
<td>2.00</td>
<td>4.15</td>
<td>23.22</td>
<td>23.77</td>
</tr>
<tr>
<td>T7 Sorghum + Biosolids</td>
<td>22.57</td>
<td>0.45</td>
<td>4.15</td>
<td>2.44</td>
<td>1.71</td>
<td>20.86</td>
<td>21.31</td>
</tr>
<tr>
<td>T8 Crotalaria + Biosolids</td>
<td>25.11</td>
<td>0.50</td>
<td>5.74</td>
<td>2.64</td>
<td>3.10</td>
<td>22.01</td>
<td>22.51</td>
</tr>
<tr>
<td>T9 Maize + Crotalaria + Biosolids</td>
<td>24.55</td>
<td>0.49</td>
<td>5.94</td>
<td>2.90</td>
<td>3.06</td>
<td>21.21</td>
<td>21.70</td>
</tr>
<tr>
<td>T10 Sorghum + Crotalaria + Biosolids</td>
<td>26.04</td>
<td>0.52</td>
<td>5.04</td>
<td>1.97</td>
<td>3.06</td>
<td>22.97</td>
<td>23.49</td>
</tr>
<tr>
<td>T11 Control plot (fallow)</td>
<td>24.43</td>
<td>0.48</td>
<td>4.48</td>
<td>1.81</td>
<td>2.66</td>
<td>21.77</td>
<td>22.26</td>
</tr>
<tr>
<td>T12 Control plot + Biosolids</td>
<td>29.88</td>
<td>0.59</td>
<td>6.18</td>
<td>3.19</td>
<td>2.97</td>
<td>26.90</td>
<td>27.50</td>
</tr>
</tbody>
</table>

Means in the columns followed by the same letter were not significantly different (P > 0.05) according to Tukey’s test. TSC: Total Soil Carbon; LC: Labile Soil Carbon; C_{col}: Oxidizable Carbon in Dichromate; C_{p}: Removable Carbon; C_{ex}: Oxidizable Soil Carbon; C_{nox}: Nonoxidizable Carbon; SOC: Soil Organic Carbon.

The FAO (2002) studied grasslands, savannas and fallow fields with spontaneous vegetation and showed that these species had a high capacity for CO₂ storage. Likewise, González et al. (2014) proposed improved grasslands, fallow covers, and resting areas as zones with high potential for SC fixation. This hypothesis agreed with the results obtained here, which showed that T12 (fallow + biosolids) exhibited a high TSC fixation capacity.

Porlles (2011) argued the importance of C fixation in arid lands for contributing to the global C balance and reducing the rate of desertification with native species in each zone. According to the FAO (2002), a great proportion of arid lands are in the tropics, which represent 37.2% of the terrestrial surface (4900 million hectares) and could, through appropriate management, provide benefits of carbon sequestration in addition to their contribution to ecosystem restoration. T12, a fallow field composed of spontaneous vegetation of *Lolium perenne*, *Hordeum jubatum* L., *Cynodon dactylon*, *Panicum maximum*, *Cucumis melo*, *Boerhavia erecta*, *Amaranthus retroflexus*, *A. spinosus* and *Momordica balsamina*, showed acceptable biomass production and ability to fix TSC, probably due to the adaptation and aggressiveness of the species in the area.

In Table 4, the SOC content is reported in its C_{col} and LC fractions. T2 and T12 were the treatments that fixed the greatest amounts of stable carbon, which is an important trait in the selection of species (*Sorghum*) for TSC sequestration in dry tropics. Ghimire et al. (2017) evaluated the amount and quality of cover crop biomass on carbon mineralization in semiarid soils, suggesting that large pools of canola and pea biomass can stimulate turnover rates. Likewise, the aggressiveness and adaptation of spontaneous species (T12) are remarkable for the revegetation of the area, suggesting the potential value of protecting fallow land in the area.

C_{col} is the fraction of C that has already escaped biological activity and oxidative processes and represents a dynamic balance between the absorption of dead plant material and loss by decomposition (Macías et al. 2004). Treatment
T2 showed the highest C_{nox} content, followed by T12 and T6, with possible stable forms characteristic of SC stabilization mechanisms (Vásquez et al. 2011); however, no differences at the statistical significance level (\(P > 0.05\)) applied were observed. It is possible that soil C_{nox} pool was affected by previous crop managements in the studied area. The added organic matter may not be completed stabilized in the soil due to the short-time experiment and long-term studies would be needed for a proper evaluation.

Under aerobic soil conditions, microorganisms readily attack C. According to García (2013), only a small fraction enters and accumulates in the stable humic fraction; however, under semi-arid conditions, lower respiration rates result in less OM oxidation favoring the humification process and OM stabilization in the form of complex compounds (Sierra et al. 2016). The ability of microorganisms to use C_{nox} as a substrate and as a source of carbon assimilation in their metabolic processes is related to the amount of carbon flux, as CO₂ can disperse into the atmosphere. In this respect, soils treated with Poaceae, Fabaceae and herbaceous species (T11 and T12) increase the stable forms of SC. This condition coincides with Phillips et al. (2011), who state that rest systems (fallow fields) store large amounts of atmospheric carbon in the soil, possibly because of the ecological dynamics of the system and the higher diversity of species.

Analyses showed that C_{ex} contents did not differ among treatments (\(P < 0.05\)). The highest contents were reported in T6, followed by T9, coinciding with Robledo et al. (2011), who indicated that maize plant residues contain cellulose (45%), hemicellulose (35%), and lignin (15%), which are broken down by microorganisms at a low or medium rate of decomposition. The results are consistent with findings on the combination of maize-biosolids and maize-crotalaria-biosolids, which showed the highest content for the oxidized form. Redin et al. (2014) have shown that the contribution of roots to soil organic C is 2.4 to 13 times higher than the contribution of shoots. On the other hand, the roots of the Fabaceae (legumes) are characterized by their higher cellulose and lignin fractions compared to those of Poaceae roots (Redin et al. 2014). Conversely, the proportion of root hemicellulose of the Poaceae family is higher than that of Fabaceae species, which are characterized by higher total N than that of Poaceae species. This may explain the behavior of Poaceae in terms of higher stable C than that of the Fabaceae or mixture treatments; thus, the decomposition is slower, and there is greater probability of humification.

Macias et al. (2005) claim that the OM derived from cellulose is more labile or has a lower cycle of resilience than the existing lignified OM present in many agroforestry systems. However, C appears in soil in recalcitrant fractions (C_{nox}), with possible stable forms inherent to the stabilization mechanisms of soil C (Vásquez et al. 2011). Those C portions are considered highly stable fractions that do not participate in the biotic processes of the soil, and for this reason, they can maintain stability over long periods of time, representing values that were observed in the field.

For removable carbon (C_{ex}; humified or active carbon, which results in the most recalcitrant forms of carbon present in the soil), there were no differences among treatments; however, the treatments with the best performance were T12, followed by T2, T8 and T9. These results suggest that the presence of Poaceae enhanced the humification of the material.

Plant cover enhances SC sequestration, which is one way to reduce the GHG effect (GEI) in the atmosphere. In this research, green manures were deposited on the soil, allowing the biological incorporation of the organic material, and the biomass produced by the root system was not quantified. According to Carrera et al. (2008), roots typically contain more lignified cells than do aerial plant parts and, in this form, may contribute differently to soil C inputs.

SOC showed significant differences, and the highest SOC was in T2, followed by T12, showing the potential for Poaceae to contribute to TSC. As shown in Table 4, approximately 90% of TSC corresponds to organic fraction forms with different degrees of oxidization.

The FAO (2002) argues that rangelands and rest areas, through extension and diversity, have a significant impact on carbon sequestration,
and fallow areas can be an economic tool to be included in agricultural practices with the purpose of making production more sustainable and mitigating soil degradation. In this work, soil treated with fallow + biosolids (T12) reported high SOC and TSC contents, contributing to the formation of biomass (from weeds) and thus the accumulation of carbon in the soil.

Jiménez et al. (2012) concluded that the carbon content of a soil under pasture and fallow land is higher than that in conventional production systems; this conclusion agrees with the results obtained by Bayer et al. (2006), who found that plowed and cultivated soils present a 36% reduction in carbon fraction contents. According to the FAO (2002), the values for different reservoirs of organic carbon fluctuate from 8-10 Mg ha⁻¹ in cultivated lands, 20 Mg ha⁻¹ in fallow lands and vineyards, 50 Mg ha⁻¹ in pastures and 60 Mg ha⁻¹ in forests without intervention. These values greatly exceed those of green manures incorporated into the production system and thus provide an easy and economical way to increase the SOC.

Diekow et al. (2005) showed that Fabaceae species with high biomass production, such as Canavalia, Mucuna and Crotalaria, provide an easy way to increase carbon and nitrogen reserves in the soil due to the high contribution of residues and to the biological nitrogen-fixing capacity of these species. In the present study, treatments T3, T4, T5, T9, and T10 in which Crotalaria was included showed high SOC, with additions higher than 20 Mg ha⁻¹ (Table 4). Increasing OC in terrestrial ecosystems is a means of removing considerable amounts of CO₂ from the atmosphere and decreasing the effects of global warming (Jiménez et al. 2012), and the increase in SOC depends on the quantity and quality of the residuals as well as on the climatic conditions.

In tropical regions, the rate of carbon mineralization is higher than that in temperate regions; therefore, the conservation of carbon must be more efficient. According to Méndez (2016), soils with the highest humified carbon content are associated with high total carbon content. This relationship is confirmed by the fact that in the present study, treatment T2 provided the highest total carbon content (TSC = 30.04 Mg ha⁻¹) and also had the highest stabilized carbon content (Cnox = 28.3 Mg ha⁻¹). The same author reports that one-quarter of the total carbon that is found in the surface horizons exhibits recalcitrance due to the processes of OM humification from the necromass contributed by vegetative residues.

The main characteristic of arid lands is the scarcity of water, which, together with high pH, severely limits the productivity of plants and, therefore, the accumulation of carbon in soils. However, certain aspects of dryland soils favor carbon sequestration because they are less likely to lose carbon than are wet soils. Robert (2002) argued that the lack of water limits the mineralization of the soil; therefore, the flow of carbon into the atmosphere is lower, which promotes carbon fixation in these environments.

The SOC associated with soil colloids (OM and clay) increases the cation exchange capacity and has a direct effect on the physical properties. Over a long time period, the SOC could modify the structure and distribution of the porous space, which is directly related to the water flow and the water storage capacity.

From an environmental point of view, SOC is related to system sustainability and is linked to the availability of soil nutrients by providing nutrients, such as N, which is normally deficient in arid ecosystems; thus, SOC regulates the dynamics of plant biomass production and biodiversity. Inadequate management practices in vulnerable ecosystems, including the Colombian Caribbean coast, could lead to SOC degradation and increase the CO₂ content in the atmosphere. Therefore, the results obtained here should be considered for sustainable environmental policies in the area and to improve agricultural processes.

The SOM contents in arid and semiarid zones are very low, the biomass in these zones is limited, and the N by mineralization processes is transformed into inorganic compounds. The nitrogenase enzyme used by the bacteria to fix N decreases its activity according to the ecosystem temperature and humidity (Celaya-Michel and Castellanos-Villegas 2011; Moreno et al. 2014). In the present research, we found a low association (r ≤ 0.5) between the
accumulation of TSC and biomass and dry mass yield, a situation that may be related to the fact that the fraction represented by the plant root system was not measured, which should be the object of a future study.

Due to the climate zone, the mineralization of SOC is intense, and the C fixation in stable forms is reduced. If combined with inadequate edaphic management of the system, this situation could cause rapid degradation and desertification. Therefore, we recommend prioritizing plant cover with the species that showed better results, a simple and rapid technique that promotes an increase in TSC and mitigates the effects of climate change.

4. Conclusions

The results of this study lead to the following conclusions regarding the use of green manures as an alternative to SC sequestration under semiarid conditions: 1) Under semiarid conditions, green manures allow carbon sequestration in soils, which can help to mitigate climate change effects. 2) With the methodology used, the carbon capture ability of sorghum (*Andropogon sorghum sudanensis*) was verified. 3) Fallow with weeds showed a high capacity for SC storage. Therefore, proper management is important to mitigate climate change effects. 4) The results presented here were obtained over a short time, and suggest that probably the previous management might have had some effect on the more recalcitrant carbon fraction contents. Despite that, differences during the experimentation year were observed on SOC. Further studies are necessary to investigate the long-time treatments’ effects. 5) The results of this study demonstrate the importance of maintaining plant cover and implementing green manures in the Colombian Caribbean region.

REFERENCES


• Moreno Conn LM, Pérez A, Ramírez M, Franco M. 2014. Efecto de la temperatura de almacenamiento sobre la viabilidad de bacterias simbióticas fijadoras de nitrógeno utilizadas en la elaboración de inoculantes biológicos para arveja (Pisum sativum) y soya (Glycine max). Revista Colombiana de Biotecnología 16(2):45-56.


• Qiu K, Xie Y, Xu D, Pott R. 2018. Ecosystem functions including soil organic carbon, total nitrogen and available potassium are crucial for vegetation recovery. Scientific Reports 8:7607.


Assessment of compost and Technosol as amendments to increase nutrient contents in a mine soil vegetated with *Brassica juncea*

Evaluación de enmiendas elaboradas con compost y un Tecnosol sobre el incremento del contenido de nutrientes en un suelo de mina vegetado con *Brassica juncea*

Avaliação de um composto e de um Tecnossolo como corretivos para aumentar o teor de nutrientes em um solo de mina vegetalizado com *Brassica juncea*

**ABSTRACT**

Abandoned mines pose potential risks to the environment and human health, and the reclamation of these areas is difficult. Soils from mining areas are usually characterised by degraded structure, high concentration of potentially toxic elements and deficiencies in nutrients. A greenhouse experiment was carried out in cylinders with the mine soil from the settling pond of the depleted copper mine of Touro (Galicia, Northwest Spain) amended with compost or technosol made from organic and inorganic wastes, and planted with *Brassica juncea* for 11 months. The aim of the study was to evaluate the effect of compost and technosol amendments on nutrient concentrations at different depths in a mine soil planted with *Brassica juncea*. The results revealed that at depths 0-15 and 15-30 cm, soil+technosol+*Brassica juncea* (STP) and soil+compost+*Brassica juncea* (SCP) treatments had higher pH than untreated mine soil S at the end of experimental. At depths 0-15 and 15-30 cm, SCP had the highest carbon total content. The nitrogen was only detected at depth 0–15 cm and only in the treated settling pond soil. STP and SCP had higher percentage of base saturation (V%) and lower percentage of aluminum saturation (A%) than S and SS (sand). At depth 0-15 cm, soil+technosol+*Brassica juncea* (STP) and soil+compost+*Brassica juncea* (SCP) did not show generally significant differences on the nutrients values. At time 3, *Brassica juncea* plants cultivated in soil+compost+*Brassica juncea* (SCP) had the highest biomass. Soil+technosol+*Brassica juncea* (STP) treatment was the most effective increasing soil pH. Soil+compost+*Brassica juncea* treatment was the one that produced the greatest increase in total carbon. The treatments increased the cation exchange capacity (CEC) at depth 0-15 cm. Both treatments corrected the CEC by increasing the V% and decreasing the A%. The application of technosol and *Brassica juncea* plants, or compost and *Brassica juncea* to a mine soil improved the soil quality.
RESUMEN

Las minas abandonadas suponen una serie de riesgos potenciales para el medio ambiente y la salud humana. La recuperación de este tipo de suelos es difícil. Los suelos de mina generalmente se caracterizan por una falta de estructura, una alta concentración de elementos potencialmente tóxicos y por ser deficientes en nutrientes. Este estudio se llevó a cabo en invernadero en cilindros en los cuales se introdujo el suelo de la balsa de decantación de la antigua mina de cobre de Touro (Galicia, Noroeste de España). Este suelo fue tratado con enmiendas elaboradas con residuos (orgánicos e inorgánicos), compost y un tecnosol, y se cultivó Brassica juncea sobre ellos durante 11 meses. El objetivo del presente estudio fue evaluar el efecto de enmiendas elaboradas con compost y tecnosol sobre las concentraciones de nutrientes a diferentes profundidades en un suelo de mina vegetado con Brassica juncea. Los resultados revelaron que, al final del tiempo experimental, a las profundidades 0-15 y 15-30 cm el suelo enmendado presentaba un pH más alto que el suelo de la balsa de decantación sin tratar (S). En las profundidades 0-15 y 15-30 cm el tratamiento suelo+compost+Brassica juncea (SCP) presentó un contenido más alto de carbono total. El nitrógeno solo se detectó en la profundidad de 0-15 cm y solo en el suelo de la balsa de decantación donde se aplicaron las enmiendas. Suelo+tecnosol+Brassica juncea (STP) y SCP presentaron una saturación de bases (V%) más alta y una saturación de aluminio (Al%) más baja que S y SS (arena). En la profundidad 0-15 cm, generalmente SCP y STP no mostraron diferencias significativas en los valores de nutrientes. En el tiempo 3, las plantas de Brassica juncea cultivadas en SCP presentaron una mayor biomasa. El tratamiento con STP fue el más eficaz a la hora de aumentar el pH del suelo de la balsa de decantación. El tratamiento SCP fue el que produjo el mayor aumento en carbono total. Los tratamientos aumentaron la capacidad de intercambio catiónico (CEC) en la profundidad 0-15 cm. Ambos tratamientos corrigieron la CEC, aumentando la V% y disminuyendo el Al%. Los tratamientos generalmente incrementaron los nutrientes en el suelo de la balsa de decantación (S). La aplicación combinada de compost con Brassica juncea y tecnosol con Brassica juncea a un suelo de mina mejoraron la calidad de dicho suelo.

RESUMO

As minas abandonadas representam um risco para o meio ambiente e para a saúde humana, e essas áreas são de difícil recuperação. Os solos em áreas minerais têm, normalmente, uma estrutura degradada, elevada concentração de elementos potencialmente tóxicos e deficiência de nutrientes. Foi realizado um ensaio em estufa, em cilindros preenchidos com solo de uma bacia de sedimentação procedente da mina de cobre abandonada de Touro (Galiza, noroeste de Espanha). O objetivo do presente estudo foi avaliar o efeito de tratamentos com aplicação de um composto e um Tecnossolo nas concentrações de nutrientes em diferentes profundidades num solo de mina (S) cultivado com Brassica juncea L.. O solo foi tratado com um composto e com um Tecnossolo, ambos elaborados com resíduos (orgânicos e inorgânicos), tendo-se cultivado Brassica juncea durante 11 meses. Os resultados mostraram que, depois de 11 meses de ensaio, nas profundidades 0-15 e 15-30 cm, os solos dos tratamentos (solo+tecnosol+Brassica juncea) STP e SCP (solo+composto+Brassica juncea) tinham pH mais elevado do que os solos sem tratamento (S). Nas profundidades 0-15 e 15-30 cm, SCP apresentou o maior teor de carbono total. O nitrógeno total foi detetado somente na profundidade 0-15 cm e apenas nos solos tratados. Os solos dos tratamentos STP e SCP tiveram maiores valores de saturação de cátions alcalinos e alcalino-térregos (V%) e menores de saturação de alumínio (Al%) do que S e SS (arena). Na profundidade 0-15 cm, o conteúdo de nutrientes em SCP e STP não apresentaram diferenças significativas. No tempo três, as plantas de B. juncea cultivadas no SCP tiveram a maior biomassa. O tratamento STP foi o mais eficaz para aumentar o pH do solo. O tratamento SCP foi o que mais aumentou o C total. Os tratamentos aumentaram a capacidade de troca catiónica (CTC) na profundidade 0-15 cm. Ambos os tratamentos corrigiram a CTC mediante o aumento da V% e a diminuição da Al%. De uma forma geral, os tratamentos aumentaram o conteúdo de nutrientes no solo de mina. A aplicação combinada de um Tecnossolo e plantas de B. juncea, ou composto e B. juncea a um solo de mina melhorou a sua qualidade.
1. Introduction

Mine soils usually present unfavourable conditions for plant growth due to their physicochemical characteristics, such as extreme pH, high salinity, low water retention capacity, high metal(loid) concentrations, and deficiencies in soil organic matter and soil fertility (Wang et al. 2017). Mine residues can produce acid mine drainage (in our case: \( \text{FeS}_2 + \frac{7}{2} \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + 2 \text{SO}_4^{2-} + 2 \text{H}^+ \)), a dangerous source of water contamination (Barrie and Hallberg 2005) and it has been demonstrated that those pose significant risks to the environment and human health (Yang et al. 2012; Sánchez-López et al. 2015). Therefore, there is a need to develop strategies to reduce the impact of mining residues spread on mine landscapes to guarantee ecosystem reclamation (Moreno-Barriga et al. 2017).

In the mine of Touro (Galicia, NW Spain) copper was extracted for 15 years, from 1973 to 1988. This zone belongs to the most important group of copper mineralization in Galicia, associated with the basic massif of Santiago de Compostela (Galicia, Spain). The Touro mine is located in a formation of granular Precambrian amphibolites mineralized with metal sulphides, pyrite and pyrrhotine, with significant levels of chalcopyrite and minor amounts of blende and other metal sulphides (Calvo de Anta et al. 1991). Once copper extraction ended, another company extracted material for road construction (Forján et al. 2014). The soils formed on the settling pond and on the mine tailing of the depleted copper mine of Touro have unfavourable conditions such as extremely acid pH values, high metal concentrations, low organic matter content, degraded structure and low nutrient contents (Asensio et al. 2014a; Rodríguez-Vila et al. 2017).

The stabilisation of metals in polluted soils is a promising research area for restoring degraded soils and reducing phytotoxicity of metals. However, the remediation process of mining areas is influenced by the presence of a variety of metals and soil characteristics (Ma et al. 2015; Ali et al. 2017a). Numerous amendments have been used to immobilise metals, reduce their bioavailability and increase the soil fertility to facilitate the establishment of plants in polluted soils. They include amendments made from waste such as compost and technosol. However, the application of these types of amendments can have problems such as high metal concentrations, the excess or deficiency of nutrients and the presence of non-biodegradable materials.

The creation of technosols using different organic and inorganic waste materials can be a sustainable strategy to reclaim mine areas and reduce the contamination spread on the environment. Technosol parent material is a material produced or exposed by human activity that otherwise would not occur at the Earth’s surface (IUSS Working Group WRB 2015). Technosols are dominated by human made materials, and their properties and pedogenesis are highly influenced by their technical origin (Moreno-Barriga et al. 2017).

Badly managed organic waste can lead to serious sanitary and environmental problems such as soil, air, and water pollution (Pellejero et al. 2017). Composting of organic waste products is considered to be one of the most economical, practical, and environmentally beneficial management options (Arslan et al. 2016; Azim et al. 2017). The multiple benefits of adding compost to the soil include increasing the amount of nutrients, organic matter, microbial activity and soil vegetation (Rodríguez-Vila et al. 2016). Composting is a biooxidative process that involves the mineralization and partial humification of organic matter, giving rise to a stabilized final product, free of phytotoxicity and pathogens and with certain humic properties (Azim et al. 2017).

Phytoremediation is the use of plants and microorganisms associated with soil to eliminate or reduce pollutants in the different environmental matrices (air, soil and water) (Compant et al. 2010; Rascioa and Navari-Izzo 2011). It is a technology friendly to the environment and that can be used to extract or immobilize metals. Phytoremediation uses chemical, physical and biological processes to eliminate, degrade, transform, or stabilize the pollutants present in air, soil and water. In addition, through these processes, the mobility and bioavailability of metals and their entry into the food chain is
considerably reduced (Chirakkara et al. 2016; Kushwaha et al. 2016; Pinto et al. 2015). *Brassica juncea* is a hyperaccumulator and fast growing plant member of the *Brassicaceae* family used for the phytoremediation of contaminated soils (Ali et al. 2017b).

A greenhouse experiment was carried out in cylinders with the mine soil from the settling pond of the depleted copper mine of Touro (Galicia, Spain) treated with compost or technosol made from waste, and planted with *Brassica juncea* (Indian mustard) plants for 11 months. The effects of the different treatments were studied at three depths. The aim of the study was to evaluate the effect of compost and technosol amendments on nutrient concentrations at different depths in a mine soil planted with *Brassica juncea*.

### 2. Materials and Methods

#### 2.1. Soil sampling and amendments

The sampled zone is located at the depleted copper mine of Touro, north western Spain (8° 20' 12.06" W 42° 52' 46.18" N). The climate in this zone is oceanic with precipitation reaching 1886 mm per year (with an average of 157 mm per month) and a mean daily temperature of 12.6 °C. In order to carry out the study, one soil and three amendments were selected. The soil was collected from the settling pond (S) at the Touro mine, and was comprised of waste material resulting from the flotation of sulphides during copper processing. The pool has been dry since 1988, and is considered to be soil according to the latest version of the IUSS Working Group WRB (2015). Samples were taken at 5 different zones of the settling pond and were mixed, at depths between 0-50 cm.

The three amendments were:

- Sand consisting of washed sea sand provided by the company Leboriz S.L.U. (control).
- Technosol (T) provided by the company Tratamientos Ecológicos del Noroeste (T.E.N.). The technosol (T) consisted of a mixture of 60% purification plant wastes, 10% aluminium company wastes (from Padrón, La Coruña, Spain) 5% ash (from Ence, a cellulose company in Pontevedra, Spain), 10% wastes from the agri-food industry (canning companies and Ecogal), and 5% purification plant sand (sand fraction). The percentages do not add up to 100%, due to the privacy policy of the companies. The company provided a few indicative percentages.
- Compost (C) was made from horse and rabbit manure mixed with grass cuttings, fruit and seaweed, which was provided by the company Ecocelta Galicia S.L. (Ponteareas, Pontevedra, Spain).

#### 2.2. Greenhouse experiment

The greenhouse experiment was carried out in cylinders to try to reflect the top 45 cm of soil; the cylinders are made of PVC with a depth of 50 cm and a diameter of 10 cm. A porous mesh was introduced into the cylinders, and the settling pond soil into the inner. Mesh was used for the settling pond soil was not in contact with the PVC for a long period of time. The cylinders are filled with: i) Settling pond soil (S, negative control), ii) Settling pond soil and sand (SS, neutral control), iii) and the treatments:

- Settling pond soil + technosol + vegetated with *Brassica juncea* (STP).
- Settling pond soil + compost + vegetated with *Brassica juncea* (SCP).

Technosol, compost and sand were deposited in the surface of the soil. The total weight of each cylinder was 3.5 kg. The experiment was carried out over 11 months at a controlled temperature and humidity (temperature of 22 ± 2 °C, and 65 ± 5% relative air humidity). A total of 48 cylinders, 12 cylinders of each treatment were prepared and distributed randomly (S, SS, STP, SCP). Randomly, three cylinders of each type were withdrawn at 3 different times: Time 1 = 3 months, Time 2 = 7 months, Time 3 = 11 months. The meshes were removed from the cylinders and processed for analysis at 3 different depths: the first from 0-15 cm, the second from 15-30 cm, and the third from 30-45 cm. The cylinders were watered to field capacity throughout the experiment.
2.3. Soil, technosol and compost analysis

The settling pond soil samples collected from the cylinders were air dried, passed through a 2 mm sieve and homogenized prior to analysis. Soil pH was determined using a pH electrode in 1:2.5 water to soil extracts (Porta 1986). Total soil carbon (TC) and total nitrogen (TN) were determined in a LECO CN-2000 module using solid samples. Exchangeable cations were extracted with 0.1 M BaCl₂ (Hendershot and Duquett 1986) and their concentrations determined by ICP-OES (Optima 4300 DV; Perkin-Elmer). Pseudototal metal contents were extracted with aqua regia by acid digestion in a microwave oven (Milestone ETHOS 1, Italy). Metal concentrations were determined by ICP-AES (Optima 4300 DV; Perkin-Elmer).

2.4. Harvested biomass and height of *Brassica juncea*.

*Brassica juncea* plants were pre-germinated in seedbeds until they grew two fully expanded leaves, and were then transferred to the cylinders (STP, SCP). The plants were harvested in the same state of maturity (the state of maturity chosen was before flowering), for comparison in the same physiological state of development in the three times (Time 1 = 3 months, Time 2 = 7 months, Time 3 = 11 months). Growth was allowed under greenhouse-controlled conditions, with a photoperiod of 11:13 light/dark, temperature of 22 ± 2 °C and 65 ± 5% relative air humidity. At the end of each time period, the height of the plants was measured, and they were carefully washed with deionised water. Fresh biomass was weighed immediately, and dry mass was assessed after oven-drying for 48 h at 80 °C and cooling at room temperature.

2.5. Statistical analysis

All of the analytical determinations were performed in triplicate. The data obtained were statistically treated using version 19.0 of the programme SPSS for Windows. Analysis of variance (ANOVA) and a test of homogeneity of variance were carried out. In case of homogeneity, a post-hoc least significant difference (LSD) test was carried out. If there was no homogeneity, Dunnett’s T3 test was performed. A correlated bivariate analysis was also carried out between TC and the content of Ca, K, Mg, and Na.

3. Results

3.1. General characteristics of the settling pond soil (S), sand (SS), compost (C) and technosol (T)

The compost (C) had higher pH values, total carbon (TC) and total nitrogen (TN) contents than S, SS and T (P < 0.05) (Table 1). The technosol (T) had higher CEC than S, SS and C (Table 1).

The pseudototal Cu concentration in the settling pond soil was higher than in T, C and SS (P < 0.05) (Table 1). The technosol had the highest pseudototal concentration of Pb (Table 1). The compost had higher pseudototal concentration of Ni and Zn than S, SS and T (Table 1).

3.2. Evolution of pH at three depths and over the 11-month period

On the one hand, at depths 0-15 and 15-30 cm, STP and SCP had higher pH than S at the end of experimental time (Figures 1A, 1B). On the other hand, at depth 0-15 and 15-30 cm, STP had higher pH values than SCP at the end of experimental time (Figures 1A, 1B). At depth 30-45 cm at time 3, STP and SCP had higher pH than S, moreover these treatments did not show significant differences in the pH values (P < 0.05) (Figure 1C).
3.3. Evolution of total carbon (TC) at three depths and over the 11-month period

At depths 0-15 and 15-30 cm at time 1, STP had higher total carbon contents than SCP, SS and S (P < 0.05) (Figures 2A, 2B). At times 2 and 3, SCP had the highest TC contents (Figures 2A, 2B). At depth 30-45 cm at times 1 and 2, SCP had the highest TC contents (Figure 2C).

At time 3 SCP and STP did not show significant differences in the pH values (Figure 2C), but the treatments had higher TC contents than S and SS (Figure 2C).

3.4. Evolution of Total Nitrogen (TN) at the three different depths over the time

The TN was only detected at depth 0–15 cm and only in the treated settling pond soil. At time 1, the settling pond soil amended with technosol and vegetated with Brassica juncea (STP) had the highest TN values (Figure 3). At time 2, the settling pond soil treated with compost and Brassica juncea (SCP) had the highest TN values (Figure 3). At time 3, these treatments did not show significant differences in the TN values (Figure 3).

3.5. Evolution of the cation exchange capacity (CEC), base saturation (V%), and aluminium saturation (Al%) at three depths and over the 11-month period

At depth 0-15 cm, the treatment STP had generally the highest CEC over the time (P < 0.05) (Table 2). STP and SCP had higher V% and lower Al% than S and SS (Table 2).

At depth 15-30 cm, at time 1 SCP had the highest CEC (P < 0.05) (Table 2). At times 2 and 3, S and SS had higher CEC than STP and SCP (P < 0.05) (Table 2), but SCP had generally higher V% over the time at this depth (Table 2).

At depth 30-45 cm, at time 1 SCP had the highest CEC (P < 0.05) (Table 2). At times 2 and 3, S and SS had higher CEC than STP and SCP (P < 0.05) (Table 2).

At time 3, S was the treatment with the highest CEC, but STP and SCP had the highest V% (Table 2).

### Table 1. Characteristics of the mine tailing (S), sand (SS), technosol (T) and compost (C)

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>SS</th>
<th>T</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.73 ± 0.07d</td>
<td>3.78 ± 0.14c</td>
<td>6.04 ± 0.05b</td>
<td>6.47 ± 0.02a</td>
</tr>
<tr>
<td>TC (g kg⁻¹)</td>
<td>1.93 ± 0.15c</td>
<td>2.76 ± 0.60c</td>
<td>256 ± 2.51b</td>
<td>276 ± 2.49a</td>
</tr>
<tr>
<td>TN (mg kg⁻¹)</td>
<td>u.l.</td>
<td>0.10 ± 0.01c</td>
<td>17.67 ± 0.50b</td>
<td>21.33 ± 1.02a</td>
</tr>
<tr>
<td>Ca</td>
<td>13.3 ± 0.02c</td>
<td>9.65 ± 0.05d</td>
<td>7785 ± 0.15a</td>
<td>6455 ± 153b</td>
</tr>
<tr>
<td>K</td>
<td>6.40 ± 0.89c</td>
<td>u.l.</td>
<td>2687 ± 0.08b</td>
<td>3041 ± 46.5a</td>
</tr>
<tr>
<td>Mg (mg kg⁻¹)</td>
<td>216 ± 2.10c</td>
<td>1.82 ± 0.02d</td>
<td>1997 ± 0.25a</td>
<td>1038 ± 14.9b</td>
</tr>
<tr>
<td>Na</td>
<td>7.4 ± 0.90d</td>
<td>9.75 ± 0.05d</td>
<td>2805 ± 0.03a</td>
<td>987 ± 12.4b</td>
</tr>
<tr>
<td>CEC (cmol(+), kg⁻¹)</td>
<td>6.11 ± 0.05c</td>
<td>0.13 ± 0.05d</td>
<td>76.61 ± 0.04a</td>
<td>53.54 ± 1.07b</td>
</tr>
<tr>
<td>Cu</td>
<td>637 ± 2.08a</td>
<td>46.6 ± 0.96d</td>
<td>226 ± 5.13b</td>
<td>193 ± 1.14c</td>
</tr>
<tr>
<td>Pb</td>
<td>16.10 ± 1.00c</td>
<td>10.10 ± 0.27d</td>
<td>89.65 ± 1.52a</td>
<td>26.67 ± 0.96b</td>
</tr>
<tr>
<td>Ni</td>
<td>16.41 ± 1.01c</td>
<td>8.41 ± 0.52d</td>
<td>26.32 ± 0.57b</td>
<td>49.70 ± 1.71a</td>
</tr>
<tr>
<td>Zn</td>
<td>65.40 ± 2.51c</td>
<td>18.75 ± 0.85d</td>
<td>340 ± 5.50b</td>
<td>403 ± 3.33a</td>
</tr>
</tbody>
</table>

Means ± SD. For each row, different letters in different samples means significant differences (n = 3, P < 0.05). u.l.: undetectable level.
Figure 1. Evolution of the pH at three depths and over the 11-month period of experiment. S (settling pond soil), SS (settling pond soil + sand), STP (Settling pond soil + technosol + Brassica juncea), SCP (settling pond soil + compost + Brassica juncea). For each time, different letters in different samples mean significant differences (n = 3, ANOVA; P < 0.05). Error bars represent standard deviation.
Figure 2. Evolution of Total Carbon (TC) at the three depths and over the 11-month period of experiment. S (settling pond soil), SS (settling pond soil + sand), STP (settling pond soil + technosol + *Brassica juncea*), SCP (settling pond soil + compost + *Brassica juncea*). For each time, different letters in different samples mean significant differences (n = 3, ANOVA; P < 0.05). Error bars represent standard deviation.
3.6. Evolution of nutrients at three depths and over the 11-month period

At depth 0-15 cm, at the end of experimental time the treated soils had higher nutrient content (Ca, K, Mg, Na) than S, but SCP and STP did not show generally significant differences in the nutrients values (Table 3).

At depth 15-30 cm, at time 3 SCP had the highest K values (Table 3). STP and SCP had higher Ca values than S and SS (Table 3). S had the highest Mg values (Table 3). S, SS and SCP did not show significant differences in the Na values, which were higher than in STP (Table 3).

At depth 30-45 cm, at time 3 the K content was not detectable at this depth (Table 3). The soil from the settling pond had the highest Ca and Mg contents (Table 3). Finally, the treated soils (STP and SCP), SS and S did not show significant differences in the contents of Na (Table 3).

3.7. Harvested biomass of Brassica juncea over the 11-month period

The Brassica juncea plants were not capable of growing in the settling pond soil (S) and in the neutral control (SS), so these treatments were not represented in the Figure 4. At time 1 and 2, Brassica juncea plants cultivated in STP had higher biomass than Brassica juncea cultivated in SCP, but at time 3 Brassica juncea cultivated in SCP had the highest biomass (Figure 4).
Table 2. Evolution of the cation exchange capacity (CEC), base saturation (V%), and aluminium saturation (Al%) at three depths and over the 11-month period of experiment. S (settling pond soil), SS (settling pond soil + sand), STP (Settling pond soil + technosol + *Brassica juncea*), SCP (settling pond soil + compost + *Brassica juncea*).

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>SS</th>
<th>SCP</th>
<th>STP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CEC</strong></td>
<td>0.84 ± 0.05b</td>
<td>0.94b</td>
<td>34.7 ± 0.08b</td>
<td>36.5 ± 0.77a</td>
</tr>
<tr>
<td><strong>V%</strong></td>
<td>35.7</td>
<td>27.8</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Al%</strong></td>
<td>65.4</td>
<td>72.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>CEC</strong></td>
<td>3.61 ± 0.04c</td>
<td>5.05b</td>
<td>28.7 ± 0.69a</td>
<td>28.4 ± 1.28a</td>
</tr>
<tr>
<td><strong>V%</strong></td>
<td>37.5</td>
<td>36.2</td>
<td>99.9</td>
<td>100</td>
</tr>
<tr>
<td><strong>Al%</strong></td>
<td>64.7</td>
<td>64.0</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td><strong>CEC</strong></td>
<td>10.78 ± 0.47c</td>
<td>5.86c</td>
<td>31.4 ± 3.40b</td>
<td>39.3 ± 0.08a</td>
</tr>
<tr>
<td><strong>V%</strong></td>
<td>43.4</td>
<td>41.8</td>
<td>99.9</td>
<td>100</td>
</tr>
<tr>
<td><strong>Al%</strong></td>
<td>57.1</td>
<td>58.1</td>
<td>0.01</td>
<td>0</td>
</tr>
</tbody>
</table>

For each row, different letters in different samples mean significant differences (n = 3, ANOVA; P < 0.05). u.d. undetectable level. Standard deviation is represented by ±.
Table 3. Evolution of nutrients (mg kg⁻¹) in three depths and along the 11 months of experiment. S (settling pond soil), SS (settling pond soil + sand), STP (settling pond soil + technosol + Brassica juncea), SCP (settling pond soil + compost + Brassica juncea).

<table>
<thead>
<tr>
<th>Depth 0-15 cm</th>
<th>S</th>
<th>SS</th>
<th>STP</th>
<th>SCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 1</td>
<td>Ca 9.51 ± 1.74b</td>
<td>6.53 ± 0.26b</td>
<td>4349 ± 3.04a</td>
<td>4818 ± 93.11a</td>
</tr>
<tr>
<td></td>
<td>K 9.18 ± 0.07d</td>
<td>7.89 ± 0.41c</td>
<td>1888 ± 21.90a</td>
<td>1511 ± 37.89b</td>
</tr>
<tr>
<td></td>
<td>Mg 12.01 ± 2.66b</td>
<td>11.4 ± 0.09b</td>
<td>655 ± 0.65a</td>
<td>662 ± 13.75a</td>
</tr>
<tr>
<td></td>
<td>Na 25.75 ± 2.20c</td>
<td>21.75 ± 0.51c</td>
<td>553 ± 9.46b</td>
<td>615 ± 19.93a</td>
</tr>
<tr>
<td>Time 2</td>
<td>Ca 30.60 ± 0.31c</td>
<td>38.73 ± 1.05c</td>
<td>3870 ± 93.39b</td>
<td>4358 ± 208a</td>
</tr>
<tr>
<td></td>
<td>K 1.68 ± 0.69c</td>
<td>2.12 ± 0.51c</td>
<td>1210 ± 25.24a</td>
<td>699 ± 16.74b</td>
</tr>
<tr>
<td></td>
<td>Mg 128 ± 1.10c</td>
<td>184 ± 0.57b</td>
<td>533 ± 10.42a</td>
<td>479 ± 21.40a</td>
</tr>
<tr>
<td></td>
<td>Na 22.36 ± 0.14c</td>
<td>17.81 ± 1.24c</td>
<td>366 ± 1.210a</td>
<td>176 ± 2.43b</td>
</tr>
<tr>
<td>Time 3</td>
<td>Ca 94.84 ± 4.29b</td>
<td>58.40 ± 0.01c</td>
<td>4942 ± 533a</td>
<td>5647 ± 194a</td>
</tr>
<tr>
<td></td>
<td>K 1.59 ± 0.70c</td>
<td>0.54 ± 0.42c</td>
<td>210 ± 4.33a</td>
<td>51.56 ± 2.17b</td>
</tr>
<tr>
<td></td>
<td>Mg 103 ± 1.10c</td>
<td>148 ± 3.21a</td>
<td>40.79 ± 0.06c</td>
<td>88.49 ± 8.13d</td>
</tr>
<tr>
<td></td>
<td>Na 14.20 ± 0.92b</td>
<td>11.90 ± 0.48b</td>
<td>101 ± 12.46a</td>
<td>125 ± 3.53a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Depth 15-30 cm</th>
<th>S</th>
<th>SS</th>
<th>STP</th>
<th>SCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time 1</td>
<td>Ca 6.47 ± 0.14c</td>
<td>8.18 ± 1.13c</td>
<td>324 ± 21.36a</td>
<td>228 ± 1.26b</td>
</tr>
<tr>
<td></td>
<td>K 8.30 ± 0.87b</td>
<td>14.76 ± 0.42b</td>
<td>205 ± 12.75a</td>
<td>209 ± 17.10a</td>
</tr>
<tr>
<td></td>
<td>Mg 8.64 ± 0.56c</td>
<td>9.18 ± 0.76c</td>
<td>120 ± 40.91a</td>
<td>44.60 ± 3.08b</td>
</tr>
<tr>
<td></td>
<td>Na 21.22 ± 1.21b</td>
<td>23.87 ± 2.54b</td>
<td>123 ± 66.50a</td>
<td>124 ± 8.98a</td>
</tr>
<tr>
<td>Time 2</td>
<td>Ca 28.85 ± 4.60d</td>
<td>45.53 ± 9.96c</td>
<td>229 ± 3.81a</td>
<td>105 ± 13.01b</td>
</tr>
<tr>
<td></td>
<td>K 1.59 ± 0.70c</td>
<td>0.54 ± 0.42c</td>
<td>210 ± 4.33a</td>
<td>51.56 ± 2.17b</td>
</tr>
<tr>
<td></td>
<td>Mg 103 ± 18.61a</td>
<td>32.21a ± 0.06c</td>
<td>40.79 ± 16.74d</td>
<td>28.27 ± 1.57b</td>
</tr>
<tr>
<td></td>
<td>Na 20.60 ± 0.54c</td>
<td>16.70 ± 2.33d</td>
<td>91.84 ± 0.70a</td>
<td>28.27 ± 1.57b</td>
</tr>
<tr>
<td>Time 3</td>
<td>Ca 110 ± 2.71b</td>
<td>123 ± 7.29b</td>
<td>333 ± 31.11a</td>
<td>248 ± 21.50a</td>
</tr>
<tr>
<td></td>
<td>K u.d</td>
<td>u.d</td>
<td>u.d</td>
<td>10.72 ± 0.83a</td>
</tr>
<tr>
<td></td>
<td>Mg 452 ± 4.74a</td>
<td>430 ± 3.45b</td>
<td>135 ± 4.54c</td>
<td>112 ± 8.74c</td>
</tr>
<tr>
<td></td>
<td>Na 10.60 ± 0.13a</td>
<td>11.50 ± 0.58a</td>
<td>8.71 ± 0.32b</td>
<td>11.81 ± 0.02a</td>
</tr>
<tr>
<td>Depth 30-45 cm</td>
<td>S</td>
<td>SS</td>
<td>STP</td>
<td>SCP</td>
</tr>
<tr>
<td>Time 1</td>
<td>Ca 7.01 ± 0.18c</td>
<td>5.38 ± 0.75c</td>
<td>160 ± 40.99a</td>
<td>80.40 ± 6.50b</td>
</tr>
<tr>
<td></td>
<td>K 7.50 ± 0.19c</td>
<td>7.26 ± 0.40c</td>
<td>70.35 ± 10.94b</td>
<td>109 ± 4.59a</td>
</tr>
<tr>
<td></td>
<td>Mg 13.90 ± 0.03c</td>
<td>6.72 ± 2.12d</td>
<td>168 ± 23.76a</td>
<td>32.01 ± 1.91b</td>
</tr>
<tr>
<td></td>
<td>Na 20.89 ± 0.62b</td>
<td>23.11 ± 2.70b</td>
<td>82.07 ± 24.75a</td>
<td>90.11 ± 2.36a</td>
</tr>
<tr>
<td>Time 2</td>
<td>Ca 55.44 ± 1.79b</td>
<td>54.19 ± 10.12b</td>
<td>47.03 ± 1.20c</td>
<td>95.77 ± 0.39a</td>
</tr>
<tr>
<td></td>
<td>K 1.00 ± 0.37c</td>
<td>0.27 ± 0.27c</td>
<td>96.49 ± 1.74a</td>
<td>71.12 ± 0.08b</td>
</tr>
<tr>
<td></td>
<td>Mg 102 ± 4.67b</td>
<td>144 ± 4.57b</td>
<td>17.70 ± 0.15c</td>
<td>165 ± 2.56a</td>
</tr>
<tr>
<td></td>
<td>Na 19.69 ± 1.36b</td>
<td>6.09 ± 3.10c</td>
<td>46.81 ± 1.67a</td>
<td>42.80 ± 4.71a</td>
</tr>
<tr>
<td>Time 3</td>
<td>Ca 184 ± 5.66a</td>
<td>150 ± 0.26b</td>
<td>71.01 ± 0.26c</td>
<td>34.20 ± 1.63d</td>
</tr>
<tr>
<td></td>
<td>K u.d</td>
<td>u.d</td>
<td>u.d</td>
<td>u.d</td>
</tr>
<tr>
<td></td>
<td>Mg 606 ± 18.54a</td>
<td>465 ± 0.49b</td>
<td>192 ± 0.49c</td>
<td>109 ± 8.59d</td>
</tr>
<tr>
<td></td>
<td>Na 10.31 ± 0.56a</td>
<td>11.30 ± 0.19a</td>
<td>9.34 ± 0.19a</td>
<td>10.45 ± 1.07a</td>
</tr>
</tbody>
</table>

For each row, different letters in different samples mean significant differences (n = 3, ANOVA; P < 0.05). u.d. undetectable level. Standard deviation is represented by ±.
4. Discussion

4.1. Evolution of the pH at three depths and over the 11-month period

The sulphide minerals in contact with water and air produce sulphuric acid (Pataca 2004). This process begins with the oxidation of pyrite, key in the oxidation-leaching process, which generates Fe$^{3+}$ ions and sulfuric acid, which in turn contributes sulfate ions (SO$_4^{2-}$) and H$^+$ to the system. At the same time, ferric sulfate [Fe$_2$(SO$_4$)$_3$] is a key element in the oxidation processes that occur in the rest of the sulphides (Johnson and Hallberg 2005). The soil S has a predominance of waste resulting from the flotation of sulphides during copper processing and this was the origin of the low pH of the settling pond soil.

At depth 0-15 cm and 15-30 cm, at time 3 STP had the highest pH values. This might be due to the fact that although the technosol had a lower pH than the compost (Table 1) the technosol presented a higher CEC. This higher CEC of the technosol could influence the increase of pH values in STP. The lower pH increase in SCP treatment at these depths may be due to compost losing its effect before the other types of amendments since compost has a less recalcitrant effect than other amendments such as technosols or biochar, as demonstrated by Walker et al. (2004). At depth 30-45 cm, SCP and STP increased the pH in the settling pond soil but the treatments did not present significant differences on the pH values.

The increase of the pH values produced by the treatments in all the studied depths is important because soils with pH below 3.5 are strongly limited for plant production (Bendfeldt et al. 2001).
4.2. Evolution of the Total Carbon (TC) at the three depths and over the 11-month period

At depths of 0-15 cm and 15-30 cm, SCP treatment increased the TC more than the treatment STP, because compost had a higher TC content than technosol (Table 1). In addition, as compost loses stability over time (Walker et al. 2004), this can produce an increase on TC at lower depths by the migration of carbon due to leaching. At depth 30-45 cm pH showed a similar trend and there were no significant differences between the treatments. The improvement in carbon content through the application of STP and SCP treatments is very important since the carbon content is an important factor in the quality of a soil (Bendfeldt et al. 2001; Vega et al. 2005). This improvement in quality was reflected in the SCP treatment where a positive correlation (0.97, P < 0.01) between TC and Brassica juncea biomass was obtained.

In this experiment, a positive correlation was obtained between TC and the content of Ca (0.97, P < 0.01), K (0.78, P < 0.01), Mg (0.83, P < 0.01), Na (0.67, P < 0.01). These data coincide with those obtained by Lal et al. (2006) who concluded that the soil carbon is important for the recovery of degraded soils, as the increase of the soil organic carbon produce an increase on the concentration of nutrients in bioavailable form.

4.3. Evolution of the Total Nitrogen (TN) at the three depths and over the 11-month period

On mine degraded soils, nitrogen is a major limiting factor and addition of TN fertilizer becomes a common practice to maintain healthy growth and persistence of vegetation (Li 2006). The S soil presented a not detectable TN content, for this reason the treatments STP and SCP were applied and they increased those contents in the depth 0-15 cm. However, there were no significant differences on nitrogen contents between the treatments.

This increase of TN content is due to the residues from which the treatments were assembled. In the case of technosols, these were residues from purification plant waste, ash, and waste from the agri-food industry (Canet et al. 2007; Pérez-Esteban et al. 2012; Smith 2009; Weber et al. 2007). In the case of compost, these were residues from horse and rabbit manure mixed with grass cuttings, fruit and seaweed, which have high TN contents (Canet et al. 2007; Khan et al. 2009; Pérez-Esteban et al. 2012; Miller et al. 2003).

In addition, this increase of TN caused by the treatments can be influenced by the presence of Brassica juncea plants. Some non symbiont nitrogen-fixing bacterial communities are usually associated with certain species of Brassica; this proliferation of bacteria is the result of various exudates released by the root (Germida et al. 1998; Misko and Germida 2002). Due to these factors, Brassica juncea plants can provide significant amounts of nitrogen to the soil, as demonstrated by Zhou et al. (2012).

4.4. Evolution of the cation exchange capacity (CEC) (cmol (+) kg⁻¹), base saturation (V%), and aluminium saturation (Al%) at the three depths over the 11-month period

At depth 0-15, in general the treatment STP improved the CEC more than the treatment SCP. This is possibly due to the technosol having a higher CEC than compost, and compost is an amendment that can lose stability after 31 days (Walker et al. 2004). At depth 0-15 cm, STP and SCP had an average base saturation (V%) of 100%, which indicates that the binding sites of the soil in these treatments are saturated with Ca²⁺, K⁺, Mg²⁺, and Na⁺. Authors such as Pérez-Esteban et al. (2012) showed that the application of amendments made from residues increases the cation exchange capacity.

At depths 15-30 cm and 30-45 cm, at the end of the experimental time the treatments did not increase the CEC in comparison with S. However, the V% is higher in SCP and STP than in S, which indicates that the treatments increased the basic cations. This improvement of the CEC increasing the V% and decreasing the Al% is important since CEC can be an obstacle to recovering a degraded soil because CEC is a major controlling agent of nutrient availability for plant growth, soil pH, and the soil reaction to fertilisers and other ameliorants (Hazleton and Murphy 2007).
4.5. Evolution of nutrients at the three depths and over the 11-month period

The treatments SCP and STP increased the nutrients in S, but this effect in the depths 15-30 cm and 30-45 cm was lost by deepening the profile and over the time. The increase of nutrients provided by amendments made from residues in degraded mine soils was previously reported by Asensio et al. (2014b). At depth 0-15 cm, there were no significant differences on nutrient content between the treatments. This increase in nutrient content was due to the residues used to produce the amendments. On the one hand, technosol whose residues contain high levels of Ca, K and Mg (Canet et al. 2007; Pérez-Esteban et al. 2012; Smith 2009; Weber et al. 2007) and on the other hand, compost made from waste (Canet et al. 2007; Khan et al. 2009; Pérez-Esteban et al. 2012; Miller et al. 2003). The increase of nutrients at depths 15-30 cm and 30-45 cm was lower, this could be due to the acidic pH and lack of organic matter at greater depths. Authors such as Pérez-Esteban et al. (2012) already detailed the relationship between nutrient increase with CEC and organic matter. In addition, in this experiment the positive effect of the increase of the nutrient contents on the biomass of *Brassica juncea* was reflected on positive Pearson correlations with values of *r* = 0.99 (*P* < 0.01), *r* = 0.88 (*P* < 0.05), *r* = 0.99 (*P* < 0.01) for K, Mg and Na, respectively.

4.6. Harvested biomass of *Brassica juncea*

*Brassica juncea* cultivated in S and in SS did not grow over time. These treatments showed deficiencies in TN, TC, nutrient content and low pH or CEC. Wong (2003) studied this difficulty in establishing stable plant cover in mine soils, and states that degraded mine soils are human-made habitats which experience a wide range of problems for establishing and maintaining vegetation. In STP and SCP the *Brassica juncea* plants grew throughout the entire experimental period. The application of the treatments SCP and STP amended with residues improved the conditions of S allowing the development of *Brassica juncea* plants. The improvement of soil condition by organic amendments was already demonstrated by authors such as Wong (2003). At the end of the experiment time, the biomass produced by *Brassica juncea* plants was higher in SCP than in STP. The highest biomass of the *Brassica juncea* harvested in the SCP treatment may be due to the higher TC content of the SCP treatment compared to the STP treatment, since in the other parameters analysed there are practically no significant differences between treatments. In addition, in the SCP treatment, a positive correlation was obtained between the TC and the *Brassica juncea* biomass (0.97, *P* < 0.01).

5. Conclusions

The application of technosol and *Brassica juncea* plants, or compost and *Brassica juncea* to a mine soil improved the soil quality. At the end of the experimental time, STP treatment was more effective than SCP increasing the pH values. In general, SCP treatment produced a greater increase of TC content. Both treatments increased TN content but there were no significant differences between the two treatments. Both treatments increased CEC at depth 0-15 cm. However, throughout the experimental time both treatments corrected the CEC by increasing the V% and decreasing the Al%. The treatments SCP and STP increased the nutrients in S, but this effect in the depths 15-30 cm and 30-45 cm was not observed over the time. At the end of experimental time *Brassica juncea* plants cultivated in SCP had higher biomass than *Brassica juncea* grown in STP. The application of technosol and *Brassica juncea* plants, or compost and *Brassica juncea* to a mine soil improved the soil quality.
REFERENCES


• Lal R. 2006. Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands. Land Degrad Dev. 17:197-209.


• Smith R. 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environ Int. 35:142-156.


Medium-term impact of post-fire emergency rehabilitation techniques on a shrubland ecosystem in Galicia (NW Spain)

Impacto a medio plazo de las técnicas de rehabilitación de emergencia post-incendio sobre un ecosistema de matorral en Galicia (NO de España)

Received: 13.12.2017 | Revised: 16.03.2018 | Accepted: 21.03.2018

ABSTRACT

The aim of this work was to study the effectiveness of two post-fire emergency rehabilitation techniques (seeding and mulching) for reducing soil erosion as well as their effects on the soil quality; therefore in the field, experimental plots of unburnt soil, burnt soil, burnt soil plus seeding and burnt soil plus mulching were established. Soil samples were collected from the A horizon and a wide range of physical, chemical and biological soil properties were analyzed to evaluate soil quality. The effect of fire on the vegetation cover was observed after one year and changes in soil properties persisted even after four years. The phospholipid fatty acids pattern showed that in the medium-term (8-48 months after the fire), the fire may modify the soil microbial communities by altering the plant community via plant-induced changes in the soil environment. No effect of seeding or mulching on the vegetation cover was observed. The mean efficiency in preventing soil erosion between 8 and 12 months after the fire and the application of the treatments was 11% for seeding and 65% for mulching. These stabilization treatments had a minor influence on the post-fire soil quality in the medium term (48 months); therefore, taking into account its effectiveness for reducing soil erosion, the mulching treatment is recommended as the best post-fire stabilization technique.

RESUMEN

El objetivo de este trabajo fue estudiar la eficacia de dos técnicas emergentes de rehabilitación post-incendio (siembra y acolchado) para reducir la erosión del suelo así como sus efectos sobre la calidad del suelo. Para ello, en la experiencia de campo se implementaron las siguientes parcelas: suelo no quemado, suelo quemado, suelo quemado más siembra y suelo quemado más acolchado. Se tomaron muestras de suelo del horizonte A y un amplio rango de propiedades físicas, químicas y biológicas fueron analizadas para evaluar la calidad del suelo. El efecto del fuego sobre la cubierta vegetal fue detectado un año después del incendio y los cambios sobre las propiedades del suelo persistieron incluso 4 años después del mismo. El patrón de los ácidos grasos de los fosfolípidos demostró que a medio plazo (8-48 meses después del incendio) el fuego puede modificar las comunidades microbianas del suelo por alteración de las comunidades vegetales a través de los cambios inducidos en el suelo. No se observó ningún efecto de la siembra ni del acolchado sobre la cubierta vegetal. La eficiencia media para la prevención de la erosión del suelo durante el periodo de 8 y 12 meses después del incendio y la aplicación de los tratamientos fue del 11% para la siembra y del 65% para el acolchado.
el acolchado. Estos tratamientos de estabilización tuvieron una menor influencia sobre la calidad del suelo post-incendio, a medio plazo; por lo tanto, teniendo en cuenta su eficacia, se recomienda el acolchado como la mejor técnica de estabilización post-incendio.

RESUMO

O objetivo deste artigo foi estudar a eficácia para reduzir a erosão do solo de duas técnicas emergentes de reabilitação pós-fogo (sementeira e mulching), bem como seus efeitos sobre a qualidade do solo. Assim, na experiência de campo, foram implementadas as seguintes parcelas: solo não queimado, solo queimado, solo queimado mais sementeira e solo queimado mais mulch. As amostras de solo foram retiradas do horizonte A e uma ampla gama de propriedades físicas, químicas e biológicas foram analisadas para avaliar a qualidade do solo. O efeito do fogo sobre a cobertura da vegetação foi observado um ano após o incêndio e as alterações nas propriedades do solo persistiram até quatro anos depois. O padrão dos ácidos gordos dos fosfolípidos mostrou que, a médio prazo (8 a 48 meses após o incêndio), o fogo pode modificar as comunidades microbianas do solo por alteração das comunidades vegetais através de mudanças induzidas no solo. Não foi observado efeito da sementeira ou da aplicação do mulch na cobertura vegetal. A eficiência média para a prevenção da erosão do solo durante o período de 8 e 12 meses após o incêndio e a aplicação dos tratamentos foi de 11% para a sementeira e de 65% para o mulching. Estes tratamentos de estabilização tiveram, no médio prazo, uma menor influência na qualidade do solo pós-fogo; assim, tendo em consideração a sua eficacia na redução da erosão do solo, o mulching é recomendado como a melhor técnica de estabilização pós-fogo.

1. Introduction

In the last decades, fire has become a major disturbance of forest ecosystems by destroying the vegetation cover, causing soil degradation and increasing soil losses by erosion (Chandler et al. 1983). Fires can affect physical, chemical and biological soil properties to a varying extent; usually they can produce loss of nutrients by volatilization and leaching, partial or total combustion of soil organic matter (SOM), increase of hydrophobicity, alteration of aggregate stability, deterioration of soil structure and marked alterations in the number, activity and composition of the soil microbial communities (Neary et al. 1999; Certini 2005). Consequently, fires can produce a reduction in the soil quality and cause huge economic and ecological damage that will probably be worse in the foreseen scenario of climate change (Shakesby 2011).

Galicia (NW Spain) and the north of Portugal are the most affected areas by forest wildfires worldwide, with the greatest number of fires per hectare or inhabitant (Carballas et al. 2009). Throughout recent decades, this area has been affected by a temporal and spatial change in fire regime, resulting in a dramatic annual increase in the surface burnt by wildfires due to human action, changes in land use, policies and climatic fire risk. Most of these wildfires occur in forest and shrubland areas, which are often located on sloping terrain, in soils with moderate erodibility mainly due to high capacity of soil infiltration, which is related to sandy texture, high SOM content and high stability of soil aggregates (Benito et al. 2010; Varela et al. 2010). However, these factors, together with post-fire meteorological conditions (abundant high-intensity rainfall events in the autumn period, immediately after summer
wildfires), tend to increase runoff and erosion processes of different orders of magnitude in the soil surface horizon (Díaz-Fierros et al. 1990; Vega et al. 2005; Fernández et al. 2008). In Galicia, in the last 42 years 250,000 forest fires have been registered affecting 1,711,000 ha (86% of its forest area) and causing dramatic effects (Carballas et al. 2009). In 2006, two thousand fires occurred in 12 days, affecting 75,000 ha approaching urban centers, although most of them were concentrated in small villages and affected 50% of their forest surface (Carballas et al. 2016); and in October 2017, 1,300 ha were affected in only 1 day (MAPAMA 2018). They caused serious off-site damages by runoff and deposition of sediments in downstream terrestrial and aquatic ecosystems as a consequence of torrential rainfall occurring at the end of the summer (Carballas et al. 2016).

When the vegetation cover is partial- or completely destroyed by the fire, the interception of rainfall decreases, causing destruction of aggregates and dispersion of the fine particles that clog the pores; this decreases the infiltration capacity of water in the soil and increases the runoff and soil loss due to water erosion (Carballas et al. 2016). Therefore, in some burnt areas very susceptible to erosion, emergency stabilization treatments should be applied as early as possible to accelerate re-vegetation or provide a soil cover with plant material, as naturally occurs with needles or leaves of the unconsumed canopy, to reduce post-fire soil erosion (Cerdá and Doerr 2008). Straw mulching and herbaceous seeding are often the techniques recommended for this purpose; however, rigorous quantitative studies concerning their effects on the yield of sediments coming from the burnt hillslopes are scarce and have been made mostly in the USA (Robichaud et al. 2000, 2006; Wagenbrenner et al. 2006; Groen and Woods 2008), in the north and central areas of Portugal (Prats et al. 2012, 2016), in the semiarid region of Spain (Bautista et al. 1996; Badía and Martí 2000; Kribeche et al. 2013), and in some other countries (Prosdocimi et al. 2016). Recently, the Galician Forest Service has initiated a post-fire stabilization program to reduce the potential increase in runoff and erosion. Although the research on the effectiveness of different post-fire erosion mitigation techniques has increased (Vega et al. 2013), results on their effects on soil quality are still necessary. The first results of a recent study performed under field conditions following a high severity wildfire in a shrubland ecosystem in the NW of Spain showed that both rye seeding and straw mulching did not modify the quality of the burnt soil and significantly reduced the post-fire hillslope erosion rates over the first four months after their application (Díaz-Raviña et al. 2012), which can be interpreted as a control of soil quality at short-term. Although these results are promising, it is necessary to study the effects of these treatments on the vegetation recovery, soil quality and erosion control at the medium-term, particularly during the first year, when the risk of erosion is greater.

The effects induced by wildfires on soil quality are highly variable and depend on fire severity, soil type, topography and post-fire conditions (climatology and recovery of the soil-plant system), among other factors, and so in some cases the results obtained in one area cannot be compared to those produced in another. Soil quality has been defined as the capacity of a specific kind of soil to function, with naturally or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and soil quality, and support human health and habitation (Karlen et al. 1997). Several parameters have been proposed for the assessment of quality of burnt soils but most investigations analyze soil parameters separately (Cerdá and Jordán 2010).

On the other hand, most studies or even reviews concerning this topic, performed by diverse researchers, are focused on the short-term effects on diverse soil properties (Neary et al. 1999; Certini 2005; Mataix-Solera et al. 2009). However, to date, studies performed on the same soil combining the information of fire effects on physical, chemical and biological properties and therefore on the overall soil quality of burnt soils are scarce (those performed mainly by our research group). In addition, despite the fact that microorganisms are known to be the main responsible agent for soil functioning, their detailed study, including aspects related with their biomass, activity and diversity, were not taken into account in most of these investigations (Mataix-Solera et al. 2009). Hence, for the same soil we have to attempt to combine the results from different soil properties, including
the biological ones, for a comprehensive understanding of the wildfire effects as well as the effects of the soil rehabilitation treatments on soil quality.

The aim of the present study, therefore, was to evaluate the medium-term (8-48 months) impact of a wildfire (comparing the burnt soil versus an unburnt soil; i.e. control) and two different post-fire stabilization treatments (comparing the burnt soil with seeding and mulching versus the burnt soil) on the vegetation and the soil quality of a shrubland located in the NW of Spain as well as to determine the efficacy of the treatments to control post-fire erosion (8-12 months). This is one of the few studies that combine: (a) the recovery of the vegetation cover; (b) the evaluation of the overall soil quality (physical, chemical and biological properties) after the fire and application of the soil rehabilitation treatments; and (c) the effectiveness of these techniques for post-fire erosion mitigation.

2. Materials and Methods

2.1. Experimental Design

The study was performed in an area of special interest due to its proximity to the Serra do Invernadoiro Natural Park (Laza, Galicia, NW Spain). The climate is temperate (mean annual temperature 6-8 °C) and humid (1600-1800 mm·year\(^{-1}\) of precipitation) as registered at the nearest meteorological station (Cabeza de Manzaneda) during the studied period (first year after the fire). The soil was an Entisol (Udorthent) (Soil Survey Staff 2010) developed over phyllites under a shrubland (Erica spp., Vaccinium myrtillus L., Pterospartum tridentatum Willk., Cistus spp.) reforested with Pinus sylvestris (planted in rows and separated 5 m; mean height of 1.6 m), which was the dominant vegetation prior to the wildfire. The soil is representative of the soils developed over metamorphic rocks and under shrubland vegetation in the temperate humid zone of NW Spain, which have acid pH, high SOM content and a desaturated cation exchange complex (Carballas et al. 2016). This ecosystem, highly susceptible to soil erosion due to the steep relief and high rainfall erosivity, suffered a wildfire in September 2010, affecting 1,700 ha. A hillslope of the burnt and unburnt areas, separated by a firewall, was selected for the study because of its homogeneity in terms of fire severity, vegetation, slope, and the presence of a comparable unburnt control soil near the burnt area (U.T.M. 29T PG34168-71422, 1566 m above sea level and 30% slope, with orientation 245-250° WSW). After the fire the pines died and the prevalence of white ashes over black ones as well as the total consumption of the shrubland (vegetation and soil litter layers) suggested that the fire severity had been from moderate to high (Vega et al. 2013). One week after the wildfire, the field experimental burnt and unburnt areas were delimited by metal fences to avoid soil perturbation by other factors than the rehabilitation treatments (i.e. presence of animals). Nine experimental plots (4 × 20 m each) were established between the pine planting rows across the hillslope in a randomized block design in the burnt area; in the same way, three control plots with the same dimensions and slope position were established in the unburnt area. To study the effect of the wildfire and two selected post-fire stabilization techniques, four treatments were considered in triplicate: unburnt control soil (U); burnt soil (B); burnt soil plus native Secale cereale L. seeds at a rate of 10 g·m\(^{-2}\) (B+S); and burnt soil plus 250 g·m\(^{-2}\) of native wheat straw mulch (B+M); seeds and straw being spread manually immediately after the wildfire at recommended field dose. The straw mulching and the seeding were selected because they are considered the most effective treatments to reduce surface runoff and keep soil in place (Robichaud 2009). Metallic sediment collectors, Gerlach troughs, were located on the downhill portion of the burnt plots to measure soil erosion.

2.2. Soil Sampling

Soil sampling was carried out 8, 12 and 48 months after the wildfire and several physical, chemical and biological properties were analyzed. At each sampling time, from each plot, after removing the litter (4-8 cm thick) of the unburnt plots, the ash layer of the burnt...
plots and also the straw of the mulched plots, 10 soil subsamples from squares (15 cm × 15 cm) uniformly distributed in zig-zag along the plot were taken from the top layer (0-2 cm). They were mixed to form one representative composite soil sample and refrigerated (4 °C) until processing in the laboratory. Additionally, soil samples at 2-5 cm depth were collected 48 months after the wildfire in all treatments and in the same soil surface than those collected at 0-2 cm. This allowed us to determine if the effect of the treatments could be detected in the deeper layer (if this occurs it means that rhizosphere influence and straw incorporation to soil took place). The soil samples were passed through a sieve with a 2 mm diameter mesh; fractions bigger than 2 mm were discarded and the soil fractions < 2 mm were thoroughly homogenized and used in all subsequent analyses. They were divided into: (a) fresh subsamples maintained at -4 °C for the measurement of moisture content, water retention capacity (WRC), hydrosoluble carbon and carbohydrates and biochemical properties; (b) fresh subsamples lyophilized and frozen to -15 °C for the assessment of Phospholipid Fatty Acids (PLFAs) analysis; and (c) air-dried subsamples for analysis of the rest of the physical and chemical soil properties.

2.3. Vegetation Monitoring

The percentage of soil covered by vegetation was estimated in 10 subplots, each one covering an area of 1 m², uniformly distributed and systematically selected along each burnt plot, that were photographed at each sampling time, immediately and 4, 8 and 12 months after the fire. The images were analyzed in the laboratory, using a Photoshop 5.0 program. On each image a mesh (26 cm x 18 cm) with 10 x 14 squares was placed and the square numbers fully covered by straw mulching and plants were registered. Then, the total average percentage of ground cover by the dominant herbaceous and shrub species was calculated for each plot.

2.4. Erosion Measurements

Soil erosion was assessed by the sediments arrived to the burnt plots by runoff, collected at two sampling times, 8 and 12 months after the fire, in metallic collectors, Gerlach throughs, with a modification of the method as previously indicated by Díaz-Raviña et al. (2012).

2.5. Physical, Physicochemical and Chemical Soil Properties

The soil properties monitored were the following: (a) physical: granulometric composition, aggregate stability, water repellency, moisture content and WRC; (b) physicochemical: pH (in water and KCl) and electrical conductivity (EC); and (c) chemical: total carbon (C) and nitrogen (N) contents and hydrosoluble C and carbohydrates. The methods described by Guitián-Ojea and Carballas (1976) were utilized to determine: granulometric composition, moisture, WRC, pH and EC. The aggregate stability and the water repellency were measured following the procedure described by Díaz-Raviña et al. (2012). The total C and N contents, and the different labile fractions of the SOM, hydrosoluble C and carbohydrates [soluble carbon (WSC) and carbohydrates (WSCH), hot water extractable carbon (HWC) and carbohydrates (HWCH)] were analyzed following the techniques previously described by Lombao et al. (2015b).

2.6. Biological Soil Properties

The biochemical properties [β-glucosidase and urease activities, microbial biomass C (Cmic), soil respiration, specific respiration rate or metabolic quotient (qCO₂, ratio between soil respiration and Cmic) and bacterial activity] were determined as described by Lombao et al. (2015b). The microbial community structure and the total and relative bacterial and fungal biomass were estimated by PLFA analysis using the procedure and nomenclature described by Frostegård et al. (1993). The total amount of 10Me18:0, 10Me17:0 and 10Me16:0 PLFAs were used as an indicator of the actinobacteria biomass (ActPLFA). The sum of the PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9t, i17:1ω8t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0) was used as an index of the bacterial biomass.
(BactPLFAs), and the quantity of the 18:2ω6 PLFA was used as an indicator of the fungal biomass (FungPLFAs). The i14:0, i15:0, i16:0 and 10Me18:0 PLFAs are predominantly found in Gram-positive (G+) bacteria, and the cy17:0, cy19:0, 16:1ω7c and 18:1ω7 PLFAs characterize Gram-negative (G−) bacteria.

2.7. Statistical Analyses

All results were obtained from triplicate determinations and were expressed on the basis of oven-dry (105 °C) weight of soil. Mean values of three field replicates (values ± SE, standard error) were used to compare the different soil treatments. The values of the variables determined on samples collected at 0-2 cm depth at different times (8, 12, 48 months) after the fire were analyzed by Two-way Analysis of Variance (ANOVA 2) to determine the percentage of variation attributable to the soil treatment (U, B, B+S, B+M) and to the sampling time. Likewise, the data of samples collected 48 months after the fire at two depths, 0-2 cm and 2-5 cm, were analyzed by ANOVA 2 to determine the percentages of variance attributable to the soil treatment and to the depth. The significant differences among the different treatments at each sampling time and those for the same treatment at different depths were determined with the Bonferroni’s test for the multiple comparisons at \( p < 0.05 \). The comparison of the B soil versus the U soil give us the wildfire effect and the comparison of B+S and B+M versus B give us the rehabilitation treatments effect; nevertheless, the significant highest values of the total ground cover corresponded to B+M because the wheat straw mulching covered 76-90% of the soil surface in the same period.

3. Results

3.1. Vegetation Monitoring

The results of ANOVA 2 showed a significant effect of the time elapsed from the fire on the total vegetation cover, explaining 89% of variance, and on the percentage of the ground cover for most plant species analyzed, explaining 44-59% of variance for herbaceous species and 67-88% for shrub species. The effect of the treatment was only observed on \( \text{Luzula lactea} \) L. and \( \text{Agrostris} \) spp. species (15-17% of variance) and on \( \text{Vaccinium myrtillus} \) L. (40% of variance). Similar results were obtained for data of the plant cover dynamics, the effect of the time elapsed from the fire explaining 20-90% of variance and the treatment a further 2-25% of variance only on \( \text{Luzula lactea} \), \( \text{Agrostis} \) spp. and \( \text{Pterospartum tridentatum} \) Willk.

The recovery of the vegetation cover did not happen during the first four months after the fire (percentage of the ground cover lower than 12%), slightly increased between the 4th and the 8th months (values lower than 18%), and reached values of 36-40% between the 8th and the 12th months (Table 1). The \( \text{Secale cereale} \) L. sown covered 5.4%, 7.1% and 1.9% of the soil surface 4, 8 and 12 months after the fire, respectively. During the first eight months after the fire, the significantly \( (p < 0.05) \) higher (12-18%) and lower (5-10%) percentages of the ground cover by plants corresponded to B+S and B+M treatments, respectively; nevertheless, the significant \( (p < 0.05) \) highest values of the total ground cover corresponded to B+M because the wheat straw mulching covered 76-90% of the soil surface in the same period.
Table 1. Percentages of the ground cover by plants and plant cover dynamics for the principal plant species in the burnt soils and 4, 8, and 12 months after the fire and application of the post-fire stabilization techniques (Mean values of three field replicates ± SE). Treatments: B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. For the same sampling time different letters denote significant differences (p < 0.05) among treatments.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Time (months)</th>
<th>Treatment</th>
<th>Ground cover (%)</th>
<th>Species (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B+S</td>
<td>B+M</td>
<td>B</td>
</tr>
<tr>
<td>Total vegetation</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luzula lactea L.</td>
<td>7.4 ± 0.3 b</td>
<td>12.0 ± 0.3 c</td>
<td>4.5 ± 1.1 a</td>
<td></td>
</tr>
<tr>
<td>Festuca spp.</td>
<td>4.7 ± 0.4 a</td>
<td>4.4 ± 0.4 a</td>
<td>2.6 ± 0.7 a</td>
<td>66.5 ± 8.1 b</td>
</tr>
<tr>
<td>Agrostis spp.</td>
<td>1.6 ± 0.7 a</td>
<td>1.5 ± 0.3 a</td>
<td>1.2 ± 0.1 a</td>
<td>19.2 ± 7.9 a</td>
</tr>
<tr>
<td>Total vegetation</td>
<td>12.4 ± 1.8 b</td>
<td>18.3 ± 0.7 b</td>
<td>9.7 ± 0.0 a</td>
<td></td>
</tr>
<tr>
<td>Luzula lactea L.</td>
<td>8.7 ± 1.1 a</td>
<td>7.4 ± 1.1 a</td>
<td>6.4 ± 1.6 a</td>
<td>72.8 ± 5.2 b</td>
</tr>
<tr>
<td>Festuca spp.</td>
<td>2.1 ± 0.5 a</td>
<td>2.4 ± 0.3 a</td>
<td>1.9 ± 0.1 a</td>
<td>17.2 ± 3.8 a</td>
</tr>
<tr>
<td>Agrostis spp.</td>
<td>0.9 ± 0.1 b</td>
<td>0.7 ± 0.3 b</td>
<td>0.5 ± 0.9 b</td>
<td></td>
</tr>
<tr>
<td>Pterospartum tridentatum Willk.</td>
<td>0.1 ± 0.1 a</td>
<td>0.5 ± 0.2 a</td>
<td>0.1 ± 0.1 a</td>
<td>0.4 ± 0.4 a</td>
</tr>
<tr>
<td>Cistus spp.</td>
<td>0.5 ± 0.2 a</td>
<td>0.7 ± 0.3 a</td>
<td>0.5 ± 0.0 a</td>
<td>3.5 ± 1.1 a</td>
</tr>
<tr>
<td>Erica spp.</td>
<td>0.1 ± 0.1 a</td>
<td>0.1 ± 0.0 a</td>
<td>0.1 ± 0.1 a</td>
<td>0.4 ± 0.4 a</td>
</tr>
<tr>
<td>Vaccinium myrtillus L.</td>
<td>0.1 ± 0.1 a</td>
<td></td>
<td>0.1 ± 0.0 a</td>
<td></td>
</tr>
<tr>
<td>Total vegetation</td>
<td>35.6 ± 3.7 a</td>
<td>36.5 ± 5.0 a</td>
<td>39.9 ± 2.7 a</td>
<td></td>
</tr>
<tr>
<td>Luzula lactea L.</td>
<td>10.0 ± 1.1 a</td>
<td>9.3 ± 1.9 a</td>
<td>6.5 ± 1.7 a</td>
<td>31.1 ± 2.1 a</td>
</tr>
<tr>
<td>Festuca spp.</td>
<td>3.6 ± 0.7 a</td>
<td>4.1 ± 1.4 a</td>
<td>4.3 ± 1.2 a</td>
<td>10.6 ± 1.6 a</td>
</tr>
<tr>
<td>Agrostis spp.</td>
<td>0.1 ± 0.1 a</td>
<td></td>
<td>0.2 ± 0.2 a</td>
<td></td>
</tr>
<tr>
<td>Pterospartum tridentatum Willk.</td>
<td>10.0 ± 1.6 a</td>
<td>6.8 ± 2.6 a</td>
<td>11.3 ± 0.2 a</td>
<td>27.1 ± 1.6 b</td>
</tr>
<tr>
<td>Cistus spp.</td>
<td>4.2 ± 1.6 a</td>
<td>5.5 ± 2.0 a</td>
<td>6.5 ± 0.8 a</td>
<td>10.4 ± 3.3 a</td>
</tr>
<tr>
<td>Erica spp.</td>
<td>7.7 ± 0.5 a</td>
<td>8.9 ± 4.1 a</td>
<td>11.3 ± 3.3 a</td>
<td>20.6 ± 1.3 a</td>
</tr>
</tbody>
</table>
The total vegetation cover did not show significant differences among treatments 12 months after the fire. In the burnt soils (B, B+S, B+M), the most abundant plant species (21-73% of the total vegetation) was the pioneer species *Luzula lactea* L. following by other herbaceous species, mainly *Festuca* spp. (11-30%) and *Agrostis* spp. (0.2-14%). The contribution of the shrub species *Pterospartum tridentatum* Wilk., *Cistus* spp. and *Erica* spp., which represent 0.1-5% of the total vegetation, to the regeneration of the vegetation of the burnt soils did not occur until 8 months after the fire and was very low. However, they were the most abundant plant species (58-68%) in the burnt soils one year after the fire.

### 3.3. Physical, Physicochemical and Chemical Soil Properties

The values of all these physical, physicochemical and chemical soil properties of the unburnt and burnt soils, 8, 12 and 48 months after the fire, are shown in [Figure 2](#) and [Table 2](#). Except for the physical properties (granulometric fractions, aggregate stability) the results of ANOVA 2 for the 0-2 cm depth soil samples showed in all cases a significant effect of the soil treatments, explaining 38-83% of the variance, a slight effect of the sampling time that explained 4-36% of variance while the interaction of soil treatments x sampling time explained 6% and 36% of variance in the moisture content and the EC, respectively. During the study period, comparing the B soil

### 3.2. Sediment Yield

The sediments collected from the B, B+S and B+M treatments in the periods of 4 to 8 months and 8 to 12 months after the fire were 36 g·m⁻², 32 g·m⁻² and 9 g·m⁻², respectively, in the first period; and 9 g·m⁻², 8 g·m⁻² and 4 g·m⁻², respectively, in the second period ([Figure 1](#)). Therefore, the efficiency of the seeding and mulching treatments in reducing the soil loss was 11% and 75%, and 11% and 56% respectively for the first and the second evaluation periods. For both periods (T8 and T12) the ANOVA 1 showed no significant effectiveness for B+S and significant effectiveness (*p* < 0.05) for B+M. One year after the fire and application of the treatments, the accumulated sediment yields were 249 g·m⁻², 168 g·m⁻², and 35 g·m⁻² for B, B+S and B+M soils, respectively. In all the burnt soils B, B+S and B+M the biggest sediment production occurred during the first 4 months reaching 85%, 76% and 63%, respectively, of the total sediments ([Figure 1](#)).

### 3.2. Sediment Yield

The sediments collected from the B, B+S and B+M treatments in the periods of 4 to 8 months and 8 to 12 months after the fire were 36 g·m⁻², 32 g·m⁻² and 9 g·m⁻², respectively, in the first period; and 9 g·m⁻², 8 g·m⁻² and 4 g·m⁻², respectively, in the second period ([Figure 1](#)). Therefore, the efficiency of the seeding and mulching treatments in reducing the soil loss was 11% and 75%, and 11% and 56% respectively for the first and the second evaluation periods. For both periods (T8 and T12) the ANOVA 1 showed no significant effectiveness for B+S and significant effectiveness (*p* < 0.05) for B+M. One year after the fire and application of the treatments, the accumulated sediment yields were 249 g·m⁻², 168 g·m⁻², and 35 g·m⁻² for B, B+S and B+M soils, respectively. In all the burnt soils B, B+S and B+M the biggest sediment production occurred during the first 4 months reaching 85%, 76% and 63%, respectively, of the total sediments ([Figure 1](#)).
with the U soil, we observed: a) no appreciable changes in the granulometric fractions, aggregate stability and water repellency; b) an increase in soil pH (0.5 units); and c) a significant reduction (11-70%) in the moisture content, WRC, EC and total C and N contents (Table 2). Likewise, the labile fractions of the SOM (WSC, HWC, WSCH, HWCH) were drastically reduced (50%) by the wildfire and post-fire erosion processes (Figure 2). The data showed that the physical (moisture content and WRC), physicochemical and chemical properties, particularly the SOM labile fractions of the burnt soils, did not recover 4 years after the fire. Conversely, no effect of the B+S and B+M treatments was observed on the analyzed properties compared with the corresponding ones of the B soil (Table 2). The results of ANOVA 2 for the samples collected 48 months after the fire at 0-2 cm and 2-5 cm depth also showed a significant effect of the soil treatments on the analyzed properties (Table 2), which explained between 59% and 92% of variance, respectively, while the depth only explained 4-22% of variance. The results of the analyzed variables showed a clear effect of the burning, comparing B versus U, while no effect of the rehabilitation treatments was observed comparing B+S and B+M with B in both layers. An effect of the depth was observed on the pH of some burnt soils as well as on total C and N contents and all analyzed SOM labile fractions (WSC, HWC, WSCH, HWCH) of the unburnt soils (Table 2, Figure 2).

Figure 2. Organic matter labile fractions [(water soluble and extractable carbon (WSC and HWC) and carbohydrates (WSCH and HWCH)] in the different soil treatments 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA 2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire at two depths, 0-2 cm (A) and 2-5 cm (B), according to an ANOVA 2 (T2, treatment; D, depth; T2xD, interaction treatment x depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p < 0.05$) between different depths.
Table 2. Soil physical, physicochemical and chemical properties analyzed in the different study soils, 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatment: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denote significant differences ($p < 0.05$) between different depths.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Depth (cm)</th>
<th>Time (months)</th>
<th>Treatment</th>
<th>U</th>
<th>B</th>
<th>B+S</th>
<th>B+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>0-2</td>
<td>8</td>
<td>25 ± 3</td>
<td>30 ± 2</td>
<td>29 ± 5</td>
<td>32 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>24 ± 5</td>
<td>30 ± 2</td>
<td>31 ± 3</td>
<td>31 ± 6</td>
<td></td>
</tr>
<tr>
<td>Silt (%)</td>
<td>0-2</td>
<td>8</td>
<td>56 ± 3</td>
<td>53 ± 3</td>
<td>54 ± 5</td>
<td>51 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>57 ± 1</td>
<td>54 ± 1</td>
<td>53 ± 3</td>
<td>52 ± 5</td>
<td></td>
</tr>
<tr>
<td>Clay (%)</td>
<td>0-2</td>
<td>8</td>
<td>20 ± 0</td>
<td>17 ± 1</td>
<td>17 ± 1</td>
<td>17 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>20 ± 2</td>
<td>17 ± 1</td>
<td>16 ± 1</td>
<td>17 ± 1</td>
<td></td>
</tr>
<tr>
<td>Aggregate stability (%)</td>
<td>0-2</td>
<td>8</td>
<td>96 ± 1</td>
<td>84 ± 8</td>
<td>87 ± 2</td>
<td>92 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>96 ± 2</td>
<td>90 ± 4</td>
<td>89 ± 3</td>
<td>90 ± 7</td>
<td></td>
</tr>
<tr>
<td>Water repellence</td>
<td>0-2</td>
<td>8</td>
<td>Very severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>0-2</td>
<td>8</td>
<td>35 ± 2</td>
<td>29 ± 2</td>
<td>31 ± 6</td>
<td>31 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>26 ± 2</td>
<td>13 ± 3</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>40 ± 1</td>
<td>15 ± 1</td>
<td>19 ± 4</td>
<td>17 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>36 ± 2</td>
<td>19 ± 1</td>
<td>22 ± 3</td>
<td>20 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water retention at field capacity (g kg⁻¹)</td>
<td>0-2</td>
<td>8</td>
<td>872 ± 29</td>
<td>537 ± 28</td>
<td>565 ± 49</td>
<td>579 ± 65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>770 ± 74</td>
<td>501 ± 33</td>
<td>460 ± 83</td>
<td>488 ± 45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1035 ± 13</td>
<td>530 ± 26</td>
<td>622 ± 65</td>
<td>687 ± 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>958 ± 39</td>
<td>636 ± 19</td>
<td>698 ± 34</td>
<td>699 ± 64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHwater</td>
<td>0-2</td>
<td>8</td>
<td>3,4 ± 0,1</td>
<td>4,2 ± 0,2</td>
<td>4,2 ± 0,1</td>
<td>4,0 ± 0,1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>3,7 ± 0,0</td>
<td>4,1 ± 0,1</td>
<td>4 ± 0,1</td>
<td>4,1 ± 0,1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>3,6 ± 0,0</td>
<td>4,3 ± 0,0  bc</td>
<td>4,1 ± 0,0 bc</td>
<td>4,4 ± 0,0 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>3,5 ± 0,0</td>
<td>4,0 ± 0,0  b*</td>
<td>3,9 ± 0,0 b*</td>
<td>4,0 ± 0,0 b*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHKCl</td>
<td>0-2</td>
<td>8</td>
<td>2,5 ± 0,0</td>
<td>2,9 ± 0,1</td>
<td>2,9 ± 0,1</td>
<td>2,9 ± 0,1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>2,6 ± 0,0</td>
<td>2,9 ± 0,0</td>
<td>2,9 ± 0,0</td>
<td>2,9 ± 0,0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>2,3 ± 0,0</td>
<td>2,8 ± 0,0</td>
<td>2,8 ± 0,0</td>
<td>2,9 ± 0,0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>2,4 ± 0,0</td>
<td>2,6 ± 0,0  a*</td>
<td>2,5 ± 0,0 a*</td>
<td>2,6 ± 0,0 a*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electric conductivity (μS cm⁻¹)</td>
<td>0-2</td>
<td>8</td>
<td>51 ± 5</td>
<td>27 ± 1</td>
<td>25 ± 4</td>
<td>26 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>23 ± 2</td>
<td>25 ± 3</td>
<td>30 ± 1</td>
<td>30 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>74 ± 2</td>
<td>22 ± 2</td>
<td>31 ± 8</td>
<td>26 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>82 ± 4</td>
<td>29 ± 2</td>
<td>35 ± 1</td>
<td>27 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total C (g kg⁻¹)</td>
<td>0-2</td>
<td>8</td>
<td>239 ± 11</td>
<td>143 ± 12</td>
<td>154 ± 13</td>
<td>157 ± 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>216 ± 24</td>
<td>140 ± 12</td>
<td>132 ± 26</td>
<td>139 ± 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>266 ± 4</td>
<td>121 ± 13</td>
<td>154 ± 2</td>
<td>171 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>195 ± 17</td>
<td>116 ± 9</td>
<td>129 ± 2</td>
<td>136 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N (g kg⁻¹)</td>
<td>0-2</td>
<td>8</td>
<td>12 ± 1</td>
<td>8 ± 1</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>10 ± 1</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>11 ± 0</td>
<td>6 ± 0</td>
<td>7 ± 1</td>
<td>8 ± 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>8 ± 0        b*</td>
<td>5 ± 0        a</td>
<td>7 ± 0 ab</td>
<td>6 ± 0 a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.4. Biological Properties

The values of the biochemical properties of the unburnt and burnt soils, 8, 12 and 48 months after the fire are shown in Figure 3. The results of ANOVA 2 performed for the biochemical properties of samples collected at 0-2 cm depth indicated that the soil treatments explained 8-72% of variance and the sampling time explained 18-70% of variance, whereas...
Figure 4. Total microbial biomass (TotPLFAs) and biomass of the specific groups: fungi (FungPLFA), bacteria (BactPLFA), actinobacteria (ActPLFA), $G^+$ bacteria (Bact$G^+$PLFA), $G^-$ bacteria (Bact$G^-$PLFA) and Fungi/Bacteria and $G^-$ bacteria/$G^+$ bacteria ratios of the different study soils, 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques (mean values of three field replicates ± SE). Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected at 0-2 cm depth, according to an ANOVA 2 (T1, treatment; S, sampling time; T1xS, interaction treatment x sampling time). Percentages of variance explained by significant factors ($p < 0.05$ level) for samples collected 48 months after the fire at two depths, 0-2 cm (A) and 2-5 cm (B), according to an ANOVA 2 (T2, treatment; D, depth). For the same sampling time different lowercase letters denote significant differences ($p < 0.05$) among treatments. For samples collected 48 months after the fire, * denotes significant differences ($p > 0.05$) between different depths.
the interaction of treatments x sampling time explained only 4-25% of variance. During the study period, most biochemical properties were still disturbed 4 years after the fire but a different behaviour was observed depending on the property considered. For all sampling times a clear negative effect of the burning on urease activity, microbial C and soil respiration was observed. A similar trend was exhibited by these properties since in the B soil they did not recover and the magnitude of the reductions (40-63%) versus their values in the U soil still persisted 4 years after the fire. In general, an opposite behaviour of the fire was observed for bacterial activity and qCO₂, showing in the B soil values significantly higher than those in the U soil 8 months after the wildfire. This behaviour tended to disappear after one year and remained so for up to 48 months after the fire. For β-glucosidase activity a negative effect was found only 4 years after the fire. No significant effect of the mulching and seeding treatments versus the B soil was observed on the biochemical properties during the study period except on qCO₂, 8 months after the fire, showing values in the B soil significantly higher than those exhibited by B+S and B+M. It should be highlighted that this trend disappeared in the rest of the sampling times.

Likewise, the results of ANOVA 2 for samples collected 48 months after the fire at 0-2 and 2-5 cm depths also indicated an effect of the soil treatments, which explained 20-73% of variance, an effect of the depth that explained 11-47% of variance while the interaction of treatments x depth explained a further 7-21% of variance. In the 2-5 cm layer, a clear negative effect of the burning was observed on urease activity, microbial C and soil respiration 48 months after the fire, with the magnitude of the reductions being comparable to those observed in the surface layer. As in the surface layer, no significant effect of the stabilization treatments (seeding, mulching) versus the burnt control was observed in the 2-5 cm layer during the study period. Forty-eight months after the fire, a significant effect of the depth was only observed on all biochemical properties analyzed in the unburnt soil, on urease activity in the B+M treatment, on microbial C and bacterial activity in the B+S treatment and on qCO₂ in all treatments except the B+M treatment (Figure 4).

The total microbial biomass (TotPLFAs) data, as well as those of the different specific microbial groups (fungi, bacteria, actinobacteria, G⁺ bacteria and G⁻ bacteria) of the unburnt and burnt soils, 8, 12 and 48 months after the fire, are shown in Figure 4. The results of ANOVA 2 for samples collected at 0-2 cm depth showed a significant influence of both the treatments and the sampling time on all variables analyzed, the treatments explaining 3-69% of variance and the sampling time 9-94% of variance, these factors not being independent since the interaction of treatment x sampling time explained a further 5-19% of variance. The TotPLFAs were positively and significantly correlated with the biomass of the specific microbial groups (fungi, bacteria, actinobacteria, G⁺ bacteria and G⁻ bacteria) of the B soil versus the U soil were still significantly higher than those of the burnt soil 8 months after the fire. No significant effect of mulching and seeding treatments versus the B soil was observed on the biochemical properties during the study period except on qCO₂. 8 months after the fire, showing values in the B soil significantly higher than those exhibited by B+S and B+M. It should be highlighted that this trend disappeared in the rest of the sampling times.

On the other hand, the results of ANOVA 2 for samples collected 48 months after the fire at 0-2 cm and 2-5 cm depths showed a significant effect of the soil treatments that explained 42-85% of variance and a depth effect for only the Fungi/Bacteria ratio that explained 38% of variance. An effect of the burning was observed in the 2-5 cm layer, whose magnitude was similar to that observed in the 0-2 cm layer. For all the studied variables, no effect of the B+S and B+M treatments compared with the B soil was observed at both depths. Similarly, no effect of the depth on these variables was detected, except for the fungi/bacteria ratio of the soil treated with mulching (B+M).
The PCA performed with the whole PLFAs data (Figure 5) showed that the main differences in the PLFA pattern were due to the sampling time. The first component that explained 45% of variance, mainly distinguished the samples collected at 48 months from the rest of the samples collected 8 and 12 months after the wildfire, although with the B+M treatment being consistently different from the B control also along this component (along PC1, p < 0.001 from ANOVA). The second component that explained 18% of variance was related to the wildfire effect, differentiating the unburnt samples (U8, U12 and U48) from the burnt ones. The samples collected 48 months after the fire with negative values along PC1, were mainly characterized by high concentrations of the 18:2ω6 and 18:1ω9 PLFAs, characteristic of fungi, and the monosaturated i17:1ω8, 18:1ω7 and 19:1a PLFAs. The unburnt samples, with negative values along PC2, were mainly characterized by high concentrations of the saturated fatty acids 16:0, 17:0 and 18:0. The data clearly indicated that fungi were the microbial group more affected by the wildfire, as indicated by the values of the fungal/bacteria ratio and the distribution of the variables along PC1 in the PCA (Figures 4 and 5).

Figure 5. Results of the PCA performed for the whole PLFAs data set of the different soils collected 8, 12 and 48 months after the wildfire and application of the post-fire stabilization techniques. Treatments: U, unburnt soil; B, burnt soil; B+S, burnt soil plus seeding; B+M, burnt soil plus straw mulching. Scores ± SE of three field replicates (I) and loading plots (II). To analyze in more detail the effect of the treatments at each sampling time, a PCA was also performed separately (Figure 6). Similar results were observed independently of the sampling time (8, 12 and 48 months) indicating that the main differences in the PLFA pattern were due to the soil burning.
To analyze in more detail the effect of the treatments at each sampling time, a PCA was also performed separately (Figure 6). Similar results were observed independently of the sampling time (8, 12 or 48 months) indicating that the main differences in the PLFA pattern were due to the soil burning. For samples collected at 8 and 12 months after the fire, the first component, which explained 30-51% of variance, differentiated the unburnt samples from the burnt ones while the second component, explaining 15-23% of variance, differentiated the soil with mulching (B+M) from the other burnt soils (B, B+S). For samples collected 48 months after the fire, the first component, which explained 37% of variance, also separated the unburnt samples (U) from the burnt ones (B, B+S, B+M), whereas the second component, explaining 30% of variance, differentiated samples collected at 0-2 cm from samples collected at 2-5 cm depth. The unburnt samples, with negative values along PC1, were mainly characterized by high concentrations of the saturated 16:0, 17:0 and 18:0 PLFAs; and the samples collected at 2-5 cm depth, with positive values along PC2, were characterized by high concentration of the 18:1, cy19:0, i16:1, 10Me16:0b and a17:0 PLFAs. It should be highlighted that in the 0-2 cm layer...
the B+M treatment was different from the other burnt samples (B, B+S) along PC1 (p < 0.01 from ANOVA); however, this effect was not observed in the 2-5 cm layer (Figure 6).

4. Discussion

4.1. Effect of the Wildfire

The fire destroyed 100% of the vegetation cover in the studied soils. The recovery of the vegetation did not occur during the first 4 months (probably due to the unfavorable winter conditions) but took place mainly between the 8th and the 12th months after the fire, reaching a mean value of 38% of the vegetation cover one year after the fire, a value similar to that obtained by Barreiro et al. (2015) but lower than the 70% of vegetation cover registered by Vega et al. (2014) in experimental fires of the temperate humid zone. This behavior can be explained by the very low mean temperatures registered during the study period in the experimental plot area (-0.1 °C-1.3 °C between the 3th and 7th months after the fire and 6.7 °C–11.7 °C in the following months) as well as by the lower severity of the experimental fires. In our study the herbaceous cover represented 93-100% and 32-44% of the total vegetation cover the first 8 months and one year after the fire, respectively. Luzula lactea L. was the most abundant herbaceous species, particularly in the first 8 months after the fire, which could be attributed to the pioneer behavior of this type of plant whose rapid growth is favored by fire, the grasses Festuca spp. and Agrostis spp. following in importance. The shrub cover showed an opposite trend representing 4-7% and 56-68%, 8 months and one year after the fire, respectively. Erica spp. was the most abundant species (data not shown). This is line with data of Dubuy and Vallejo (2008), who consider that in a burnt Mediterranean area the vegetation formations are dominated by the sprouting species, which are more resilient to fire than those dominated by germinator plants.

At the end of the study period, 4 years after the fire, all burnt soils reached values of 60-80% of vegetation cover (data not shown). Earlier investigations (Fernández and Vega 2014b; Fernández et al. 2016a) showed that two years after wildfires, the vegetation had reached values equivalent to those reported for unburnt shrubland areas in the NW Spain, possibly reflecting both the re-sprouting capability of shrub species and favorable post-fire conditions. In our study, high temperatures were reached during the fire and therefore the pines died, the rest of the vegetation was destroyed and later on the shrubland exhibited lower growth and different species composition, which could also be associated with different soil characteristics. This indicates that although the vegetation cover recovers, it will need much more than 4 years to reach the same development as in the unburnt soil (Robichaud et al. 2013; Morgan et al. 2014).

A short-term study performed in the same experimental plots revealed that immediately after the wildfire, the combustion caused a marked decrease in the content of SOM and related properties (soil moisture, WRC), increases in soil pH, EC, and sand fraction, accompanied by decreases in the clay and silt fractions, and no changes in the aggregate stability and water repellency (Díaz-Raviña et al. 2012). The results obtained in our study 8, 12 and 48 months after the fire indicated that in most of the physical, physicochemical and chemical properties analyzed, the initial changes induced by the wildfire (Díaz-Raviña et al. 2012) were still maintained after 4 years, except in the soil texture, which tended to recover its initial granulometric composition (Table 2). The SOM labile fractions (WSC, HWC, WSCH and HWCH) were drastically reduced in the burnt soils during the study period, mainly due to observed changes in vegetation and microbial activity (Nannipieri et al. 2003; Certini 2005) and post-fire erosion processes (Gómez-Rey et al. 2013). These labile fractions did not reach the values...
of the unburnt soil 4 years after the fire, which confirms what has been reported for cellulosic and non-cellulosic carbohydrates of forest soils affected by wildfires of different severity, located in the same area, in the short-term (Martín et al. 2009), and for microbial and extractable C and N in the medium-term (Prieto-Fernández et al. 1998).

In the short-term study cited above, the microbial biomass, urease and bacterial activity values were markedly reduced immediately after the passage of the fire while the respiration and qCO₂ increased and the β-glucosidase activity was not affected. Our results obtained at 8, 12 and 48 months after the fire showed that most biochemical properties were still modified 4 years after the fire and depending on the soil property considered, a different behavior was observed. The microbial C, the soil respiration and the urease activity did not recover, the bacterial activity and qCO₂ did, while the β-glucosidase activity showed a variable and inconsistent trend (Figure 3). This behavior can be explained on the basis of the different information obtained from the diverse microbial indices (overall microbial biomass, overall metabolic activity, specific enzyme activities) and also to the different sensitivity of the analyzed parameters to detect the impact of fires (Certini 2005; Mataix-Solera et al. 2009; Díaz-Raviña et al. 2010; Lombao et al. 2015a,b). According Eivazi and Bayan (1996) lower temperatures than those reached in different soils after repeated prescribed burnings for several years have a long-term accumulative effect on the microbial C and the soil enzymes related to the cycles of C, N and P but did not affect the pH and organic C. The fact that the burning effects were observed on the labile fractions of SOM (WSC, HWC, WSCH, HWCH) and the biochemical properties, such as urease, Cmic and soil respiration, showed that these properties are the best indicators in the medium-term of the changes in the soil quality induced by fire.

The total microbial biomass, estimated by PLFA, and the biomass of specific microbial groups were reduced immediately after the fire (Díaz-Raviña et al. 2012) and as shown by our results this effect still persisted 4 years later (Figure 4). Immediately after the fire, the burnt soils exhibited lower Fungi/Bacteria and G⁻ bacteria/G⁺ bacteria ratios than those in the corresponding unburnt soils, showing that the fungi and the G⁻ bacteria have a higher sensitivity to heating and/or they did not proliferate following post-fire conditions (Carballas et al. 2009; Bárcenas-Moreno et al. 2011; Holden et al. 2013; Barreiro et al. 2015). The trend of these ratios disappeared 4 years after the fire (Figure 3). Our results of the biomass of specific groups and the PLFA pattern showed that fungi play an important role in the recovery of these burnt soils since high concentrations were obtained in samples collected 48 months after the fire (Figures 5 and 6). The importance of fungi can be explained because they contribute much more to the microbial biomass than bacteria, increase soil C sequestration (Six et al. 2006) and soil aggregation (Helfrich et al. 2015), which will be especially important after a high severity forest fire when the C is drastically reduced and both soil structure and soil microbial biomass are strongly affected.

In general, a significant effect of the sampling time was also observed for the soil properties analyzed that was more accentuated for most labile fractions of the SOM (Cmic, WSC and WSCH) and the enzyme activities. These results are in accordance with marked seasonal fluctuations observed in properties of different forest soils located in the same area, which are associated with above ground vegetation effects and variation in climatic conditions (Díaz-Raviña et al. 1993, 1995; Martín et al. 2011). However, it should be highlighted that the changes induced by the sampling time were of a minor order of magnitude compared to the changes induced by the wildfire as is clearly indicated by the percentages of variance explained by ANOVA 2. Therefore, taking into account the magnitude of the changes and the spatial and temporal variation of most chemical and biochemical properties, the combined interpretation is that the soil conditions did not recover 4 years after the fire. This is consistent with data from other authors, who found that the recovery of soil microflora affected by wildfires (Prieto-Fernández et al. 1998) and prescribed burnings (Fritze et al. 1993) occurs between 10 and 13 years after the fire.

In the unburnt samples, a depth effect was observed on total C, pH, labile fractions of
SOM (WSC, HWC, WSCH, HWCH) and all biochemical properties analyzed (β-glucosidase, urease, Cmic, respiration, qCO₂ and bacterial activity), the effect being more accentuated in the properties related to the activity of soil microorganisms (Table 2, Figures 2 and 3). For most soil properties analyzed (physical, chemical, biological) no effect of depth was generally observed for all burnt samples (B, B+S, B+M), likely due to the fact that the fire tends to homogenize the surface (0-5 cm) of the soil samples as well as to the removal of the burnt soil surface (wind, lixiviation, erosion). In contrast, a depth effect was observed on qCO₂ in B and B+S treatments, on Cmic and bacterial activity in B+S treatment and on urease and Fungi/Bacteria ratio in B+M treatment. The fire modified these properties in the 0-5 cm depth but was more noticeable in the first 2 cm, which support the data of other authors (Certini 2005; Zhan et al. 2005) showing that the burning could influence the enzyme activities and chemical properties of soil not only of the upper layers but also of the lower ones.

In summary, compared with the corresponding unburnt soil, significant reductions in the values of most physical, physicochemical, chemical and biochemical properties as well as changes in the microbial community structure were observed 4 years after the fire. This can be explained by the high temperatures reached during the fire as well as by unfavorable post-fire conditions (climate, vegetation, soil environment, erosion) and is consistent with studies performed in the same area by several authors (Prieto-Fernández et al. 1998; Carballas et al. 2009; Martín et al. 2009; Lombao et al. 2015a) showing a reduction of the soil quality and therefore a degradation of the soil following high severity wildfires. Therefore, effects on both soil and plants are concordant, indicating lasting changes induced by the wildfire on the soil-plant system 4 years after the fire event.

4.2. Effect of Post-Fire Stabilization Treatments

The vegetation recovery reached values between 36% and 40% one year after the fire in all burnt soils (B, B+S, B+M). However, during the first 8 months, as compared with the burnt treatment (B), the seeding had only a slight positive effect on the vegetation cover. This was probably because the germination percentage of the sown rye seeds was low due to the low temperatures registered during the period of germination and growth of the rye, or to the low dose of rye applied (10 g·m⁻²) in our case compared to the doses used by other authors (30-35 g·m⁻²) (Albadalejo-Montoro et al. 2000; Badía and Martí 2000) or to the competence with native plants (Beyers 2009). Our results are consistent with those obtained in the same area by Barreiro et al. (2015) in a shrubland affected by an experimental fire and application of the same treatments at similar doses as well as with other studies (Kim et al. 2008; Vega et al. 2014) showing neutral effects of seeding and mulching on the vegetation cover. In contrast, Fernández et al. (2016b) found that the straw mulching favored the recovery of the plant cover in the first year after the fire by conserving the soil moisture. Nevertheless, in our study, the mulching showed lower vegetation regeneration (< 10% of vegetation cover during the first 8 months) and the same occurred in B+S at the end of the study period. During the first year after the fire, the vegetation regeneration in the mulching treatment increased from 5% to 40% while the ground cover by the straw decreased from 90% to 53%, likely due to the natural regrowth of vegetation cover, the straw incorporation into soil and its removal by wind and overland flow (Wagenbrenner et al. 2006). A similar behavior was observed by Badía and Martí (2000) although two years after the fire and application of the treatments. However, the total ground cover in the mulching treatment remained 85-93% lower than those of Badía and Martí (2000) but it was effective to protect the soil in the B+M treatment (Robichaud et al. 2010).

In general, our results showed that the mulching and seeding did not seem to affect the plant diversity of the burnt soils in the first year after the fire although other researchers reported different effects of these treatments on the vegetation recovery and the plant community composition in the semiarid and temperate-humid zones of Spain (Badía and Martí 2000; Fernández et al. 2016b) and in soils of USA (Wagenbrenner et al. 2006; Morgan et al. 2014). A marked and significant effect of the time elapsed since the fire on the recovery of the vegetation cover and
the plant community composition was observed independently of the treatment applied, showing a continuous increase of the total vegetation cover and the shrub cover, accompanied by a reduction of the herbaceous cover. This may be related to the soil availability of nutrients, especially N, after the fire, which can lead to the displacement of some plant species by other ones (Morgan et al. 2014).

Most of the soil surface was bare in the burnt soil during the first 4 months following the wildfire and therefore the vegetation could not mitigate the impact of the rainfall erosivity. The total precipitation during the first year after the fire was 1055 mm, distributed as follow: 468 mm in the first 4 months, 398 mm between the 4th and the 8th months, and 189 mm between 8th and 12th months. The total rainfall produced between the 4th and the 8th months was quite similar to that produced during the first 4 months after the fire; however, the sediment yields were very different (36 g·m⁻² versus 204 g·m⁻², respectively) (Figure 1). This seems to indicate that the sediment yield was not related to the total rainfall. After one year the accumulated soil loss from the burnt soil was 249 g·m⁻² of sediment, a value that lies in the reported range for burnt soils of the temperate-humid zone using experimental plots of similar size (Díaz-Fierros et al. 1987; Vega et al. 2013).

For all treatments, the accumulated sediment yield during the period between 0 and 12 months after the fire was similar to that obtained in the first 4 months (Figure 1). Cumulative sediment yield values corresponding to the burnt soils in the first year after the fire indicated that the mulching treatment was the most effective for controlling post-fire erosion since, compared to the burnt control, the soil losses were reduced by 86% whereas the seeding reduced the soil losses only by 33% (Figure 1).

Treatment effectiveness and especially soil loss diminished over time, particularly when the vegetation cover reached around 40%. In the medium term, between the 4th and 12th months after the fire, the mean efficiency for seeding and mulching was 11% and 71%, respectively, while in the short term, during the first 4 months, higher values were obtained (37% for seeding and 89% for mulching). Our efficiency values of the soil stabilization treatments fall within the reported values obtained in different contexts such as fire-affected areas, agricultural lands, rangeland and anthropic sites (Badía and Martí 2000; Fernández et al. 2012, 2016a; Prats et al. 2012; Fernández and Vega 2014a; Vega et al. 2014, 2015; Prosdocimi et al. 2016) and are, in part, concordant with the results for the vegetation cover obtained during the present study (18-40% from the 4th to the 12th months and lower than 12% during the first 4 months). The lower sediment yield in B+M relative to the B control plots was attributed to the immediate protection of soil, with around 90% ground cover in the mulch plots. Likewise, the lower sediment yield produced in B+S between 4 and 12 months after the fire can be explained by the protection of the vegetation cover and by the fact that, according to Díaz-Raviña et al. (2012), in the temperate humid zone (Galicia, NW Spain) the erosion is mainly produced by the saturation excess in the form of overland flow, and as the maximum WRC was not reached, the erosion was more reduced than should be expected according to precipitation values. We consider these results as an important contribution for land managers. For instance, when the natural ground cover with needles from the dead burnt pines is around 40% or higher, the implementation of seeding or mulching should not be done due to the high cost/benefit ratio, as was stated by Cerdá and Doerr (2008) and Prats et al. (2016). In addition, in our study seeding is not recommended due to its low efficiency as compared with that of mulching. Post-fire seeding ineffectiveness can be due to the steep slope (seeds can be washed away by water) and to the failure of germination and the establishment of seeded plants (unfavorable conditions such as temperature, humidity, etc.). This is also consistent with data of Beyers (2009), who point out that although non-native annual and perennial grasses commonly have been used to provide temporal ground cover until native plants are re-established, grass seeding compete with native vegetation and often do not effectively reduce the erosion.

The physical, physicochemical and chemical properties were not affected by seeding and mulching (Table 2), as happened in the same experimental area at the short term (4 months) (Díaz-Raviña et al. 2012). The same trend was observed by Gómez-Rey and González-Prieto...
(2014) one year after the fire in the contents of macronutrients (N, P, Ca, Mg and K) and extractable trace elements (Al, Fe, Mn, Cu, Zn, Co and Bo). Likewise, in line with this, no effect of mulching regardless of its application form and dose were found for most physicochemical and chemical soil properties, including the labile C pools (WSH and WSCH) (Lombao et al. 2015b), which are considered to degrade rapidly and may be an immediate source of energy for microorganisms (Nannipieri et al. 2003). However, in a study performed in a prescribed fire, Gómez-Rey et al. (2013) found that mulching and seeding had slight but significant positive effects on $\delta^{15}$N and extractable K, Mg and Ca.

The analyzed biochemical properties of burnt soils were not affected in the medium-term by the stabilization treatments (Figure 3), as occurred at the short-term (Díaz-Raviña et al. 2012); however, an influence of the sampling time was observed on most of them. The results are also consistent with other studies in Galician soils developed under scrub, oak or pines that have suffered controlled fires and wildfires of different severity to which seeding, straw mulching (independently of the form of application), bark mulching and log erosion barriers have been applied (Fernández et al. 2011; Fontúrbel et al. 2012, 2015; Lombao et al. 2015a). These studies, after monitoring for 1-3 years, showed a higher effect of the fire than that of the post-fire rehabilitation techniques on the microbial biomass and activity as well as a great variability of these properties with the sampling time (Fontúrbel et al. 2015; Lombao et al. 2015b). This behavior is related, to a large extent, to the meteorological conditions (temperature, humidity), the availability of the substrate from the plant remains, and the vegetation. In contrast, in a study performed in the same experimental plots, Gómez-Rey and González-Prieto (2015) found a significant effect of both sampling time and mulching treatment on gross N mineralization and NH$_4^+$ immobilization rates of the soil.

The results of the PLFA pattern clearly showed that the main factor determining the composition of the microbial communities was the time elapsed after the wildfire, followed in order of importance by the fire and in a much lesser extent by the soil depth. In general, the results also showed the absence of any medium-term response of the microbial community to mulching and seeding. It should be highlighted, however, that the microbial community composition in the B+M soil at 0-2 cm depth was different than those exhibited by B and B+S soils four years after the fire. This is consistent with above results showing a significant effect of depth on urease and Fungi/Bacteria ratio in the B+M treatment likely due to the incorporation of straw in the first 0-2 cm depth, as well as with the decline of the straw mulching coverage found in the B+M treatment. In line with these results, several authors showed that mulching could affect the soil microclimate (soil moisture and temperature) (Bautista et al. 2009; Ferreira et al. 2015; Fernández et al. 2016b) and the C and N availability (Huang et al. 2008), which in turn can affect the microbial community structure. In fact, it has been shown that the incorporation of plant material with higher C/N ratio into the soil, such as wheat straw used as mulching, is more favorable to fungal than to bacterial growth (Rousk and Bååth 2007; Barreiro et al. 2016). Our PLFA data also seem to indicate that in the medium-term the vegetation rather than the fire was a key factor in determining the evolution of the soil microbial communities since initially the vegetation was totally destroyed and the natural regrowth increased progressively to reach a vegetation cover of 36-40% and 60-80% (data not shown), 12 and 48 months after the fire, respectively (the performed PCA clearly differentiated samples collected at 48 months from the rest of the samples; Figure 5).

The vegetation is crucial for soil formation and differences in plant residues quality and quantity (Hart et al. 2005), including root exudates (Grayston et al. 1998), can influence different soil properties such as C, N and P content and pH. Therefore, the role of the vegetation, litter and vegetation debris, as a source of C to the soil, can determine the quantity and quality of the SOM and can condition soil microbial biomass, activity and diversity (Wardle 1992; Grayston et al. 1998; Garveva et al. 2004). This is consistent with the post-fire vegetation dynamics as drivers of the soil microbial community structure and function (Hart et al. 2005). In other words, changes in the plant community structure in the years following the fire constitute a more dominant
driver of soil microbiota than the wildfire itself. Bárcenas-Moreno et al. (2016) also found that the post-fire microbial recolonization process was different depending on the plant community studied. In the same line, Barreiro et al. (2016) in a recent laboratory study showed a clear effect on bacterial and fungal growth, particularly the latter, as well as on the PLFA pattern of a soil with different incorporated chopped materials (wheat straw, coconut fiber, *Eucalyptus* bark and wood chips). However, our PLFA pattern also differed due to the wildfire (the burnt soils were separated from the corresponding U soil) and even the stabilization treatments (the B+S soil was closely grouped with the corresponding B soil and was clearly separated from B+M) (see Figures 5 and 6). The clear differences observed between the unburnt and burnt soils in the PLFA pattern could be related to differences in the soil pH, the quantity and quality of the SOM, soil microbial properties and post-fire vegetation recovery. This is consistent with other studies performed in the same zone showing a clear effect of both prescribed fires and wildfires on the PLFA pattern (Díaz-Raviña et al. 2006, 2012; Barreiro et al. 2010, 2016; Lombao et al. 2015a). In general, in our study no effect of the depth was detected on the chemical and biochemical properties and PLFAs biomass of the burnt soils (B, B+S, B+M); nevertheless, marked and significant differences with soil depth were observed in the physicochemical, chemical and biochemical properties but not in the PLFAs biomass of the U soil. However, a depth effect was observed on the microbial structure (PLFA pattern), which is in agreement with other studies (Fritze et al. 2000; Fierer et al. 2003; Lombao et al. 2015a; Barreiro et al. 2016); this is related to the decrease of the SOM content that is determinant for soil microorganisms (Fritze et al. 2000; Martinizadeh et al. 2008) and, in a lesser extent, with variations in soil different niches (aeration, moisture, temperature, pH, available C and nutrients, etc.) (Nannipieri et al. 2003; Certini 2005). The data clearly showed the sensitivity of the PLFA analysis to detect the impact of different soil disturbances (Frostergåd et al. 2011) as well as its usefulness as an integrative tool to analyze the relative importance of different factors considered (fire, post-fire stabilization treatments, time, depth) on the microbial community composition and hence on the soil quality of these burnt soils. This lipid biomarkers technique is a rapid, inexpensive and both sensitive and reproducible analysis to explore environmental effects on soil microbiota. Therefore, we consider that PLFA analysis can be a good routine and complementary tool for helping land managers to take decisions concerning the implementation of determined forest practices on burnt soil ecosystems.

5. Conclusions

The vegetation cover was destroyed by the fire and the plant regeneration increased progressively until a 40% cover of the burnt soil surface was reached one year after the fire, the herbaceous and the woody species being dominant at short- and medium-term, respectively. Both seeding and mulching, especially the latter, were effective at reducing post-fire soil erosion at medium-term although their magnitude was lower than that observed during the first 4 months after their application. One year after the fire, the effectiveness of seeding and mulching to reduce post-fire erosion was 33% and 86%, respectively. In the medium term, 4 years after the fire, the initial soil quality had not recovered as indicated by the values of the whole set of physical, chemical and biological soil properties analyzed. The main factor determining the composition of the microbial communities was the time elapsed after the wildfire, followed in order of importance by the fire and to a much lesser extent by the soil depth. The PLFA pattern showed that the fire may modify the soil quality by altering the composition of the plant community via plant-induced changes in the soil environment due to the close relationships between the above (plant communities) and below ground communities (soil microbial communities) living in the burnt soil. In general, seeding and mulching did not modify the vegetation cover or the soil quality. However, the microbial community composition (PLFA pattern) of the soil treated with mulching at 0-2 cm depth was different from the burnt soil community and this effect was likely positive. Therefore, taking into account the absence of negative effects on the soil quality as well as its efficiency, mulching is the recommended rehabilitation technique.
6. Acknowledgements

This study was supported by the Consellería de Medio Rural de la Xunta de Galicia (08MRU-002400PR) and by the Ministerio de Ciencia e Innovación (AGL2008-02823), Spain. A. Barreiro and A. Lombao are recipients of FPU grants from Spanish Ministry of Education (AP2010-2284, AP2010-1900). The authors wish to acknowledge to Mª Cruz Mato for helping in identifying some plant species.

REFERENCES

The medium-term impact of post-fire emergency rehabilitation techniques on a shrubland ecosystem in Galicia (NW Spain)


Efecto de enmiendas orgánicas y azufre en propiedades químicas y biológicas de un suelo sódico

Effect of organic amendments and sulfur on chemical and biological properties of a sodic soil

RESUMEN

Los suelos salinos generan una elevada preocupación en los sistemas productivos de regiones áridas y semiáridas del planeta, ya que provocan un descenso en el rendimiento de los cultivos debido al efecto osmótico, al exceso de Na de cambio presente, que origina degradación de la estructura del suelo, y al efecto del ión específico (Na, Cl, B) que puede causar toxicidad y desequilibrios en la nutrición de las plantas. Es por esto que se deben buscar alternativas de manejo para mejorar la productividad de estos suelos. Con este fin, se evaluó el efecto de la aplicación de abono verde de Prosopis juliflora (GM), vermicompost de residuos de palma de aceite (VERM) y azufre mineral (S°) sobre las propiedades químicas y biológicas de un suelo sódico Typic Haplustept con cultivo de Zea mays, utilizando como tratamientos aplicaciones únicas o mezcladas de 3 t de materia orgánica ha⁻¹ de GM o VERM y 1,4 t ha⁻¹ de S°. Se determinaron las propiedades biológicas biomasa microbiana del suelo (BMS), respiración microbiana del suelo (RMS) y la actividad de las enzimas fosfatasa (alcalina y acida), proteasa y actividad hidrolítica del suelo mediante la hidrólisis del diacetato de fluoresceína (FDA); además, se estudiaron algunas propiedades químicas del suelo asociadas a sodicidad. El efecto sobre las propiedades biológicas del suelo dependió del tipo de insumo utilizado y su composición, dadas las diferencias que se encontraron entre tratamientos. De forma general, la aplicación de enmiendas orgánicas afectó positivamente a la RMS, BMS y a la actividad hidrolítica del suelo, mientras que la aplicación de S° aumentó la BMS pero disminuyó la RMS y la actividad enzimática de fosfatases, proteasas e hidrolasas. Las variables químicas estudiadas presentaron también diferencias significativas entre tratamientos, siendo aquellos tratamientos con presencia de S° los que presentaron diferencias significativas en el mayor número de variables. En conclusión, las aplicaciones de enmiendas orgánicas mezcladas con S° afectan de forma positiva a las propiedades biológicas y químicas de suelos sódicos de la región Caribe Colombiana, pues se estimula la BMS y se reduce la saturación de sodio.

ABSTRACT

Saline soils cause concern in the productive systems of arid and semi-arid regions of the planet, since they cause a decrease in crop yield due to the osmotic effect, the presence of excess exchangeable Na which causes degradation of the soil structure, and the effect of the specific ion (Na, Cl, B) that can cause toxicity and imbalances in the nutrition of plants. Therefore, it is necessary to look for management alternatives to improve the productivity of these soils. To this end, we evaluated the effect of the application of Prosopis juliflora green manure (GM), palm oil residue vermicompost (VERM) and mineral sulfur (S°), on the chemical and biological properties of a Typic Haplustept sodium soil cultivated with Zea mays, using 3 t of organic matter ha⁻¹ of GM or VERM and 1.4 t ha⁻¹ of S° as unique treatments or mixed applications. The biological properties of soil microbial biomass (BMS), soil
microbial respiration (RMS) and the activity of the enzymes phosphatases (alkaline and acid), protease and hydrolytic activity of the soil through the hydrolysis of fluorescein diacetate (FDA) were determined; as well as some chemical properties of the soil associated with sodicity. The effect on the biological properties of the soil depended on the type of amendment used and its composition, due to the differences found between treatments. In general, the application of organic amendments positively affected the RMS, BMS and the hydrolytic activity of the soil, while the application of S° increased the BMS but decreased the RMS and the enzymatic activity of phosphatases, proteases and hydrolyses. The chemical variables studied also showed significant differences between treatments, with the presence of S° presenting significant differences in the greatest number of variables. In conclusion, applications of organic amendments mixed with S° positively affect the biological and chemical properties of sodium soils of the Colombian Caribbean region, since BMS is stimulated and sodium saturation is reduced.

RESUMO

Os solos salinos constituem elevada preocupação nos sistemas produtivos das regiões áridas e semi-áridas do planeta, já que levam a uma queda no rendimento das culturas devido ao efeito osmótico, ao excesso de Na de troca, que origina a degradação da estrutura do solo, e o efeito do ião específico (Na, Cl, B) que pode causar toxicidade e desequilíbrios na nutrição das plantas. Assim, é necessário procurar alternativas de gestão para melhorar a produtividade desses solos. Com este objetivo, foi avaliado o efeito da aplicação da adubação verde com Prosopis juliflora (GM), vermicomposto de resíduos de óleo palma (VERM) e enxofre mineral (S°), nas propriedades químicas e biológicas de um solo sódico Typic Haplustept com cultivo de Zea mays, usando como tratamentos aplicações únicas ou misturas de 3 t de matéria orgânica ha⁻¹ de GM ou VERT, e 1,4 t ha⁻¹ de S°. Foram determinadas a biomassa microbiana do solo (BMS), a respiração microbiana (RMS) e a atividade das enzimas fosfatase (alcalina e ácida), protease e da atividade hidrolítica do solo através da hidrólise do diacetato de fluoresceína (FDA), bem como algumas propriedades químicas do solo associadas à sodicidade. Em função das diferenças entre os tratamentos, o efeito sobre as propriedades biológicas do solo dependeu do tipo e composição do corretivo utilizado. Em geral, a aplicação de aditivos orgânicos afetou positivamente o RMS, BMS e a atividade hidrolítica do solo, enquanto a aplicação do S° aumentou o BMS mas diminuiu o RMS e a atividade enzimática das fosfatases, proteases e hidrolases. As variáveis químicas estudadas também mostraram diferenças significativas entre os tratamentos, sendo os tratamentos com presença de S° aqueles que apresentaram diferenças significativas no maior número de variáveis. Em conclusão, as aplicações de corretivos orgânicos misturados com S° afetam positivamente as propriedades biológicas e químicas dos solos sódicos da região do Caribe Colombiano, uma vez que a BMS é estimulada e a saturação em sódio é reduzida.

1. Introducción

La salinización de los suelos es uno de los principales problemas de la agricultura en regiones áridas y semiáridas del planeta. El manejo inadecuado del riego y los suelos causa acumulación de sales en la superficie que provocan aumento del pH y de la conductividad eléctrica (CE) del suelo, deficiencias nutricionales y toxicidad por iones como el Na⁺ (Amini et al. 2015), por lo que en estos suelos la vegetación es escasa o nula. Se estima que cerca de 955 × 10⁶ ha de los suelos del planeta se encuentran afectados por algún grado de salinidad y sodicidad (Singh et al. 2013), siendo la superficie estimada para la región Caribe Colombiana de 3.506.033 ha, repartidos en los ocho departamentos administrativos del norte de Colombia que limitan con el océano Atlántico (Pulido 2000).

Los suelos sódicos se caracterizan por presentar propiedades hidráulicas pobres, altos potenciales osmóticos, deficiencias nutricionales y toxicidad por aniones y Na⁺ (Wong et al. 2010). Estas características ocasionan que, en regiones con porcentajes considerables
Efecto de enmiendas orgánicas y azufre en propiedades químicas y biológicas de un suelo sódico

del tipo de suelos, la productividad agrícola
sea baja y las necesidades básicas de los pobla-
dores no sean satisfechas.

Dada la creciente demanda de fibras y combusti-
tibles en el mundo, varios países han desarro-
llado planes de remediación de suelos sódicos
a través del uso de enmiendas minerales u or-
gánicas de bajo costo y disponibles localmente,
along con el cultivo de plantas fitorremediadoras
(Singh et al. 2014; Srivastava et al. 2014). En la
región Caribe Colombiana, el manejo y reme-
diación de este tipo de suelos aún utiliza altas
cantidades de agua y yeso. La escasez de agua
apta para uso agrícola en la región y los altos
costes del yeso, que se usa intensivamente en
la industria, hacen que este manejo sea poco
viable desde el punto de vista económico y de
la sostenibilidad en esta región. En la región Cari-
be Colombiana se encuentra el 23% de las tie-
rras cultivadas de Colombia, donde los cultivos
de maíz tradicional y yuca ocupan el 30,5% de
la superficie (Aguiolera et al. 2013), cultivados co-
múnmente por pequeños productores que dis-
ponen de escasa innovación y pobre desarrollo
agrícola (Garrido et al. 2017), lo que provoca
que la productividad, rendimiento y rentabilidad
de los cultivos sea baja.

En este contexto, el azufre elemental (S°) proce-
dente de la industria petroquímica desarrollada
en la región Caribe Colombiana puede ser usa-
do para el manejo de los suelos afectados por
sales en la región debido a su disponibilidad y
bajo coste. El potencial del S° se debe a que, al
oxidarse a sulfato, contribuye a disminuir el pH
del suelo y disolver sales de calcio, lo que facilita
el desplazamiento del Na+ y la productividad de
los cultivos, al disminuir la conductividad eléctri-
ca (CE), el porcentaje de sodio intercambiable
(PSI) y la razón de adsorción de sodio (RAS)
(Stamford et al. 2002). Por otro lado, el vermi-
compost (VERM), enmienda orgánica producida
por la acción conjunta de lombrices de tierra y
microorganismos sobre residuos orgánicos, por
su fácil producción a cualquier escala, alta carga
microbiana y carbono orgánico oxidable, ha sido
utilizado en el manejo de suelos sódicos para
reducir las condiciones de sodicidad de los mis-
mos (Mogollón et al. 2011). Si el vermicompost
es mezclado con enmiendas minerales o quími-
cas reduce las cantidades requeridas de estas
últimas, lográndose un mayor efecto sobre las
propiedades biológicas y físicoquímicas de sue-
los afectados por sales (Hernandez-Araujo et al.
2013).

De igual categoría que el VERM, la enmienda
verde producida a partir de Prosopis juliflora
(GM), planta fabácea nativa de América que cre-
ce generalmente en zonas áridas, viene siendo
utilizada en la rehabilitación de suelos sódicos
(Singh et al. 2014), debido a que la hojarasca
de este árbol mejora la fertilidad de estos suelos
al estimular la actividad microbiana (Vallejo et
al. 2012).

Generalmente, la remediación de los suelos só-
dicos se basa fundamentalmente en estudios
de las propiedades químicas del suelo (Singh
et al. 2013), obviando las propiedades bioló-
gicas, las cuales están íntimamente ligadas a la
calidad de los suelos debido a su papel en la
degradación de la materia orgánica y los ciclos
biogeoquímicos (Singh 2016). Además, los mi-
croorganismos del suelo presentan una mayor
sensibilidad a los cambios ambientales y del uso
del suelo; por ejemplo, las enzimas fosfatásas
(mineralización del fósforo), proteasa (mini-
ralización del nitrógeno), esterasas y lipasas (hidró-
lisis de ésteres y lípidos) han demostrado ser
sensibles a cambios de pH y CE del suelo (Cald-
well 2005). A pesar de lo anterior, existe poca
información referente al efecto de enmiendas
orgánicas o S° sobre las propiedades biológicas
de suelos sódicos, quizá debido a la existencia
de estudios con resultados contradictorios refe-
rentes al efecto de la salinidad y sodicidad so-
bre la biomasa y la actividad microbiana (Singh
et al. 2013; Celis et al. 2013). Aun así, algunos
autores sugieren realizar estudios sobre la enzi-
mología de suelos sódicos con el fin de enten-
der mejor la ecología del sistema suelo en esas
condiciones (Singh 2016).

En este contexto, la presente investigación tuvo
como objetivo evaluar el efecto de la aplicación
de un abono verde, vermicompost y azufre ele-
mental sobre las propiedades químicas y bioló-
gicas de un suelo sódico Typic Haplustept culti-
vado con maíz.
2. Material y Métodos

La investigación se desarrolló en la finca Mojabobos ubicada en el municipio de El Copey, César- Colombia (10°6’3.91”N; 74°0’17.67”O). El clima del lugar presenta una temperatura media de 27,8 °C, una tasa de evaporación de 4,66 mm día⁻¹ y una precipitación promedio anual de 1030 mm. Los datos pertenecen a una estación meteorológica situada a menos de 1 km de distancia de la finca. El suelo es del orden Inceptisoles clasificado taxonómicamente como Typic Haplustept según la clasificación del USDA (Soil Survey Staff 2014), con un relieve plano con pendientes entre el 0 y 1%, de longitud larga y forma rectilínea. Sus propiedades físico-químicas entre 0 y 32 cm de profundidad (Horizonte Ap) fueron: 48,3% arena, 32,4% limo, 19,3% arcilla, por lo que se clasifica como un suelo de textura franca; pH (1:5) 7,9; CE 2,4 dS m⁻¹; carbono orgánico total 0,72%; CICe 10,3 cmol(+) kg⁻¹; sodio intercambiable 2,3 cmol (+) kg⁻¹; sodio soluble 15,6 mmol(+)/L; RAS 9,87; PSI 17,1%; bicarbonatos en solución 22,6 mmol(-)/L y baja reacción de CaCO₃ por el método cualitativo de reacción con ácido clorhídrico.

2.1. Diseño experimental

Los tratamientos a evaluar consistieron en aplicaciones únicas o mezcladas en la superficie de dos enmiendas orgánicas, VERM y GM, y S° (T3-T9), además de un tratamiento manejado netamente con fertilizantes NPK (T2) y un control netamente sin ningún tipo de fertilizante o enmienda (T1). El suelo fue previamente arado con ayuda de cincel y discos a una profundidad de 20 cm, con el fin de tener condiciones óptimas para la incorporación de las enmiendas y el cultivo de maíz. Las enmiendas fueron pesadas y aplicadas manualmente de forma homogénea sobre la superficie para posteriormente ser incorporadas en el suelo a una profundidad de 5 cm con ayuda de un arado. Los tratamientos establecidos fueron los siguientes:

T1: Control; T2: NPK a dosis de 75-50-50 kg ha⁻¹; T3: GM a dosis de 3 t ha⁻¹ + NPK; T4: S° a dosis de 1,4 t ha⁻¹ + NPK; T5: VERM a dosis de 1,4 t ha⁻¹ + NPK; T6: GM a dosis de 3 t ha⁻¹ + S° a dosis de 1,4 t ha⁻¹ + NPK; T7: GM a dosis de 3 t ha⁻¹ + VERM a dosis de 1,4 t ha⁻¹ + NPK; T8: S° a dosis de 1,4 t ha⁻¹ + VERM a dosis de 1,4 t ha⁻¹ + NPK; y T9: GM a dosis de 3 t ha⁻¹ + S° a dosis de 1,4 t ha⁻¹ + VERM a dosis de 1,4 t ha⁻¹ + NPK.

En total se utilizaron 27 unidades experimentales de tamaño 5 m x 2 m, distribuidas en bloques completos al azar de nueve tratamientos con tres repeticiones cada uno. Los tratamientos (T3-T9) fueron suplementados con fertilizante químico triple 15 y sulfato de amonio (21% N), utilizando la misma dosis que para el tratamiento T2, siguiendo lo recomendado para el cultivo de maíz por Guerrero (1990). La fertilización fue fraccionada en tres dosis a los 5, 20 y 35 días después de la siembra. La densidad de plantas utilizadas fue de 50.000 plantas ha⁻¹ de maíz variedad ICA V-109, dispuestas en 3 filas en todas las parcelas (Figura 1). Para todos los tratamientos las plantas fueron regadas por medio de un sistema de riego por aspersión tres veces a la semana, ajustando la humedad del suelo a capacidad de campo. Las enmiendas fueron aplicadas 15 días antes de la siembra del maíz.

Se utilizó S° micronizado con un contenido de azufre del 97% a una dosis correspondiente al 100% de la cantidad requerida de S° para poder reducir el PSI al 5% en 30 cm de profundidad. Se utilizó VERM producido a partir de residuos de palma de aceite manejados con Eisenia fetida, con pH (1:5) 6,9; 2,5% Ca total; 1,2% N total; 15,6% carbono orgánico oxidable total y relación C/N 11,3. Por último, el abono verde (GM) utilizado se obtuvo de la poda de plantas de Prosopis juliflora que crecían en la zona de estudio, cuyos contenidos foliares fueron: 4,8% N total; 0,98% Ca total; 0,3% P total y 1,8% K total.

2.2. Muestreo y análisis

Se tomaron 3 muestras de suelo de la zona rizosférica en torno al tallo de 5 plantas de maíz, las cuales fueron mezcladas y homogenizadas, para cada una de las 27 unidades experimentales, siguiendo un patrón en forma de “W” en un radio de 15 cm y a una profundidad de 0 a 10 cm en el perfil del suelo. Las muestras de suelo fueron almacenadas a -4 °C para la estimación de las variables biológicas, y otra parte fue secada...
al aire para la determinación de las variables químicas. Las muestras fueron tomadas y analizadas en 4 periodos de muestreo equivalentes a los 15, 30, 50 y 75 días después de sembrado el maíz.

Las variables químicas evaluadas fueron: i) pH de suelo en relación 1:5 (p/v); ii) CE en extracto de pasta saturada; iii) bases solubles en agua medidas por espectrometría de absorción y emisión atómica (Ca$^{+2}$, Mg$^{+2}$ y Na$^{+1}$); iv) carbono orgánico total determinado por el método de combustión húmeda Walkey-Black; v) PSI y capacidad de intercambio catiónico efectiva (CICe), determinados a los 75 días. Todas las variables químicas evaluadas fueron estimadas según los métodos propuestos por el Instituto Geográfico Agustín Codazzi (2006).

Por último, los parámetros biológicos evaluados fueron: vi) actividad fosfatasa ácida y alcalina (EC 3.1.3.2 y 3.1.3.1) determinadas por el método de Elivazi & Tabatabai (1977); vii) actividad proteasa (EC 3.4.2.21-24) por el método de Ladd & Butler (1972) utilizando como sustrato caseína durante 2 h a 50 °C a pH 8.1; viii) actividad FDA por hidrólisis del diacetato de fluoresceína según el método de Adam & Duncan (2001) por determinación colorimétrica de la fluoresceína; ix) carbono de la biomasa microbiana (BMS) por el método de fumigación-incubación de Joegensen (Alef & Nannipieri 1995); x) y respiración microbiana (RMS) estimada en sistema cerrado con solución de hidróxido de sodio 1 N según el método propuesto por Jäggi (Alef & Nannipieri 1995). De igual forma que las variables químicas, estos parámetros fueron determinados en 4 periodos de muestreo (15, 30, 50, 75 días después de la siembra).

Todos los análisis fueron realizados en los Laboratorios de Biología y Agua y Suelos de la Universidad Nacional de Colombia con sede en Bogotá.

2.3. Análisis estadístico

Previo al análisis de los supuestos de normalidad y homocedasticidad de varianzas se utilizó el tests de Shapiro-Wilk y Levene. La determinación de la presencia o ausencia de diferencias significativas entre los tratamientos se llevó a cabo por medio de una ANOVA y en algunos casos se aplicó la prueba de comparación de medias (HSD Tukey – p < 0.05). Los análisis estadísticos fueron realizados con el programa SPSS STATICS 17.0.
3. Resultados

3.1. Propiedades químicas

Se encontraron diferencias significativas en los valores de pH al final del periodo de estudio (75 días) en los tratamientos con S° (T4, T6, T8 y T9), ya que presentaron valores de pH por debajo de 5 con respecto al resto de los tratamientos orgánicos, que alcanzaron valores de pH entre 5,5 y 6,7 (Tabla 1). Del mismo modo, Zidan et al. (1990) encontraron niveles de pH entre 5 a 5,5 en la rizosfera de plantas de maíz que crecieron en soluciones salinas de NaCl y CaCl₂.

La conductividad eléctrica (CE) y la concentración de Ca⁺² y Mg⁺² solubles presentaron comportamientos inversos a lo encontrado para el pH a lo largo del experimento. En los tratamientos con S° se incrementó de forma significativa la salinidad y las concentraciones de Ca⁺² y Mg⁺², presentando valores mayores de 10 dS m⁻¹ de CE, de 50 mmol L⁻¹ de Ca⁺² y 80 mmol L⁻¹ de Mg⁺² (Tabla 1). Por el contrario, T3 y T5 no presentaron diferencias significativas con respecto al control y al abonado con fertilizantes químicos (T1, T2), a pesar de que aplicaciones de enmiendas orgánicas en suelos sódicos pueden aumentar la concentración de Ca⁺² y Mg⁺². Esto pudo estar sujeto a las cantidades de enmienda orgánica aplicada y a la concentración de Ca y Mg de las mismas (Oo et al. 2013). Sin embargo, la mezcla de VERM y GM (T7) presentó la mayor concentración de Ca⁺² y Mg⁺² entre los tratamientos orgánicos.

Todos los tratamientos mostraron tendencia a disminuir el sodio soluble en el tiempo (Tabla 2), lo cual corresponde a lo observado en suelos sódicos manejados con enmiendas orgánicas y/o plantas fitorremediadoras (Gharaibeh et al. 2011; Mahmoodabadi et al. 2012). Esta tendencia a disminuir se debe a los procesos de lixiviación y a la absorción del sodio por las plantas. Aun así, la concentración de Na⁺ mostró diferencias significativas (p < 0,05) entre tratamientos, siendo los tratamientos con S° los que alcanzaron valores más altos (> 8 mmol L⁻¹) a lo largo del experimento frente a los tratamientos orgánicos, que fueron los más bajos (Tabla 2).

La relación de absorción de sodio (RAS) disminuyó de igual manera que el Na⁺ (Tabla 2). El mayor porcentaje de reducción del RAS (> 49%) se produjo en aquellos tratamientos en donde se aplicó enmienda verde sola (T3) o mezclada (T6, T7 y T9) (Tabla 2), posiblemente debido a que la descomposición de la materia orgánica produce cargas negativas que atraen el exceso de iones sodio de la solución, disminuyendo de esta forma la RAS (Singh et al. 2013). Lo anterior coincide con lo observado por Yazdanpanah et al. (2012), los cuales utilizaron enmiendas orgánicas y minerales sobre suelos sódicos, encontrando que los mayores porcentajes de reducción de RAS ocurrieron en los tratamientos en los que se aplicaron residuos vegetales y mezclas órgano-minerales. Por otro lado, la presencia de azufre elemental también contribuyó a disminuir la RAS respecto al tratamiento control (T1) y al tratamiento mineral (T2) (Tabla 2), debido a que la formación de ácido sulfúrico contribuye a la disolución de minerales de calcio y magnesio, lo que aumenta la entrada de Ca⁺² y Mg⁺² en la solución del suelo, disminuyendo de esta forma la RAS (Yazdanpanah et al. 2012).

La aplicación de enmiendas orgánicas no originó efecto significativo en el porcentaje de carbono orgánico (CO) en ningún tratamiento (Tabla 3). Aun así, los tratamientos en los que se utilizó VERM mezclado con S° o GM presentaron los mayores valores de CO (Tabla 3). Esto se debe al efecto del sodio y el ácido sulfúrico sobre la solubilización de la materia orgánica, que al aumentar evita su acumulación en el suelo a corto plazo (Gupta et al. 1988; Wong et al. 2010), a menos que se realicen varias aplicaciones en el tiempo.

En cuanto a la CICe, se observó que los tratamientos de mezclas con S° y enmiendas orgánicas fueron los que presentaron diferencias significativas (p < 0,05) al finalizar el experimento, al presentar valores superiores a 50 cmol(+) kg⁻¹ de suelo (Tabla 3). Esto es debido a que la oxidación del S° contribuye al aumento de la concentración de cationes intercambiables; además, el efecto desnaturizador del ácido sulfúrico sobre la celulosa y la lignina puede aumentar la superficie de contacto de los materiales lignocelulósicos y la capacidad de adsorción de metales divalentes de materiales celulolíticos como los provenientes de Prosopis cineraria, los cuales...
<table>
<thead>
<tr>
<th></th>
<th>pH 15dds</th>
<th>pH 30dds</th>
<th>pH 50dds</th>
<th>pH 75dds</th>
<th>CE 15dds</th>
<th>CE 30dds</th>
<th>CE 50dds</th>
<th>CE 75dds</th>
<th>Calcio 15dds</th>
<th>Calcio 30dds</th>
<th>Calcio 50dds</th>
<th>Calcio 75dds</th>
<th>Magnesio 15dds</th>
<th>Magnesio 30dds</th>
<th>Magnesio 50dds</th>
<th>Magnesio 75dds</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>5,9±</td>
<td>5,5±</td>
<td>5,86±</td>
<td>6,11±</td>
<td>1,12±</td>
<td>2,89±</td>
<td>1,73±</td>
<td>1,42±</td>
<td>15,55±</td>
<td>15,83±</td>
<td>12,68±</td>
<td>10,83±</td>
<td>4,79±</td>
<td>6,73±</td>
<td>3,95±</td>
<td>3,18±</td>
</tr>
<tr>
<td></td>
<td>0,28a</td>
<td>0,82a</td>
<td>0,37a</td>
<td>0,18a</td>
<td>0,32a</td>
<td>1b</td>
<td>0,48a</td>
<td>0,41a</td>
<td>14,9±</td>
<td>3,2±</td>
<td>3,3±</td>
<td>3,3±</td>
<td>4,5±</td>
<td>3,78±</td>
<td>1,9±</td>
<td>1,65±</td>
</tr>
<tr>
<td>T2</td>
<td>6,45±</td>
<td>5,82±</td>
<td>5,39±</td>
<td>5,48±</td>
<td>1,89±</td>
<td>2,31±</td>
<td>2,06±</td>
<td>2,92±</td>
<td>12,78±</td>
<td>16,17±</td>
<td>15,16±</td>
<td>17,5±</td>
<td>5,03±</td>
<td>5,96±</td>
<td>5,34±</td>
<td>7,29±</td>
</tr>
<tr>
<td></td>
<td>0,56a</td>
<td>0,5a</td>
<td>0,29a</td>
<td>0,27a</td>
<td>1,1b</td>
<td>0,65a</td>
<td>0,87a</td>
<td>0,96a</td>
<td>5,92a</td>
<td>2,4±</td>
<td>3,3a</td>
<td>1,57a</td>
<td>2,96a</td>
<td>1,91a</td>
<td>2,41a</td>
<td>2,42a</td>
</tr>
<tr>
<td>T3</td>
<td>4,81±</td>
<td>6,32±</td>
<td>5,25±</td>
<td>5,43±</td>
<td>2,84±</td>
<td>6,78±</td>
<td>3,97±</td>
<td>2,16±</td>
<td>14,84±</td>
<td>16,4±</td>
<td>25,7±</td>
<td>15,7±</td>
<td>24,12±</td>
<td>22,33±</td>
<td>10,37±</td>
<td>6,55±</td>
</tr>
<tr>
<td></td>
<td>0,83a</td>
<td>0,22a</td>
<td>0,48a</td>
<td>0,57a</td>
<td>1,32a</td>
<td>2,45a</td>
<td>0,75a</td>
<td>0,72a</td>
<td>2,64a</td>
<td>2,72a</td>
<td>2,63a</td>
<td>2,7a</td>
<td>3,9a</td>
<td>6,1a</td>
<td>6,58a</td>
<td>1,96a</td>
</tr>
<tr>
<td>T4</td>
<td>3,75±</td>
<td>5,59±</td>
<td>2,66±</td>
<td>3,19±</td>
<td>3,81±</td>
<td>2±</td>
<td>17,08±</td>
<td>13,58±</td>
<td>17,34±</td>
<td>16,02±</td>
<td>59,82±</td>
<td>55,8±</td>
<td>22,28±</td>
<td>6,13±</td>
<td>123,68±</td>
<td>83,38±</td>
</tr>
<tr>
<td></td>
<td>0,32a</td>
<td>0,16a</td>
<td>0,07a</td>
<td>0,46a</td>
<td>0,161a</td>
<td>0,33a</td>
<td>0,17a</td>
<td>0,25a</td>
<td>1,21±</td>
<td>0,7±</td>
<td>0,2a</td>
<td>2,8±</td>
<td>1,23a</td>
<td>5,91a</td>
<td>36,44a</td>
<td>3,33a</td>
</tr>
<tr>
<td>T5</td>
<td>6,75±</td>
<td>5,93±</td>
<td>5,59±</td>
<td>6,18±</td>
<td>1,14±</td>
<td>1,68±</td>
<td>3,83±</td>
<td>1,88±</td>
<td>9,12±</td>
<td>14,58±</td>
<td>14,14±</td>
<td>13±</td>
<td>2,93±</td>
<td>17,14±</td>
<td>5,75±</td>
<td>5,21±</td>
</tr>
<tr>
<td></td>
<td>0,60a</td>
<td>0,4b</td>
<td>0,25a</td>
<td>0,08a</td>
<td>0,29a</td>
<td>0,71a</td>
<td>0,12a</td>
<td>0,17a</td>
<td>2,71±</td>
<td>4,4±</td>
<td>2,2±</td>
<td>1,2a</td>
<td>1,51a</td>
<td>9,3±</td>
<td>1,42±</td>
<td>0,92±</td>
</tr>
<tr>
<td>T6</td>
<td>6,2±</td>
<td>2,91a</td>
<td>2,96±</td>
<td>2,78±</td>
<td>1,41±</td>
<td>10,06±</td>
<td>13,75±</td>
<td>14,62±</td>
<td>14,21±</td>
<td>14,75±</td>
<td>96,45±</td>
<td>50,33±</td>
<td>5,38±</td>
<td>15,77±</td>
<td>138,72±</td>
<td>97,95±</td>
</tr>
<tr>
<td></td>
<td>0,53a</td>
<td>0,13a</td>
<td>0,29a</td>
<td>0,1a</td>
<td>0,16a</td>
<td>1,17±</td>
<td>2,44±</td>
<td>2,1a</td>
<td>2,29±</td>
<td>2,59a</td>
<td>0,3a</td>
<td>5,4a</td>
<td>0,37a</td>
<td>12a</td>
<td>51,3±</td>
<td>18,04a</td>
</tr>
<tr>
<td>T7</td>
<td>5,87±</td>
<td>5,51±</td>
<td>5,43±</td>
<td>6,05±</td>
<td>1,97±</td>
<td>3,03±</td>
<td>3,44±</td>
<td>2,17±</td>
<td>13,32±</td>
<td>17,34±</td>
<td>17,15±</td>
<td>17,15±</td>
<td>3,09±</td>
<td>8,3±</td>
<td>11,16±</td>
<td>20,64±</td>
</tr>
<tr>
<td></td>
<td>0,06a</td>
<td>0,17a</td>
<td>0,25a</td>
<td>0,23a</td>
<td>1,36a</td>
<td>0,22a</td>
<td>1,29a</td>
<td>0,91a</td>
<td>5,19±</td>
<td>1,09²</td>
<td>2,84±</td>
<td>6,7±</td>
<td>0,55a</td>
<td>1,14a</td>
<td>4,25a</td>
<td>4,29a</td>
</tr>
<tr>
<td>T8</td>
<td>3,78±</td>
<td>3,3a</td>
<td>2,84±</td>
<td>3,27±</td>
<td>4,38±</td>
<td>11,48±</td>
<td>18,27±</td>
<td>13,94±</td>
<td>16,66±</td>
<td>61,5±</td>
<td>82,3±</td>
<td>54,13±</td>
<td>15,24±</td>
<td>94,07±</td>
<td>209,06±</td>
<td>105,25±</td>
</tr>
<tr>
<td></td>
<td>0,26a</td>
<td>0,5a</td>
<td>0,28a</td>
<td>0,61a</td>
<td>0,88a</td>
<td>0,83a</td>
<td>0,2a</td>
<td>1,1a</td>
<td>0,39±</td>
<td>8,7±</td>
<td>12,3±</td>
<td>3,1a</td>
<td>1,3a</td>
<td>4,9±</td>
<td>8,25±</td>
<td>28,3±</td>
</tr>
<tr>
<td>T9</td>
<td>6,16±</td>
<td>3,11a</td>
<td>2,71±</td>
<td>4,2±</td>
<td>4,75±</td>
<td>8,88±</td>
<td>14,6±</td>
<td>15,72±</td>
<td>18,73±</td>
<td>56,24±</td>
<td>74,42±</td>
<td>52,6±</td>
<td>5,21±</td>
<td>71,04±</td>
<td>135,83±</td>
<td>97,49±</td>
</tr>
<tr>
<td></td>
<td>1,31a</td>
<td>0,1a</td>
<td>0,02a</td>
<td>0,89a</td>
<td>1,39a</td>
<td>0,23a</td>
<td>1,15a</td>
<td>2,0a</td>
<td>0,22a</td>
<td>4,9±</td>
<td>2±</td>
<td>3,47±</td>
<td>0,6±</td>
<td>14,6±</td>
<td>31,08b</td>
<td></td>
</tr>
</tbody>
</table>

Los valores representan la media de n = 3 con su respectivo error estándar. Para cada muestreo y parámetro, valores con letras diferentes presentan diferencias estadísticamente significativas (p < 0,05) entre tratamientos, de acuerdo a la comparación de medias de Tukey.
poseen capacidad de absorción de metales a valores de pH ácidos (Sinha et al. 2013). Con respecto al porcentaje de sodio intercambiable (PSI), todos los tratamientos, incluyendo el control, presentaron valores de PSI por debajo del inicial del suelo (< 15) (Tabla 3), lo cual podría deberse al efecto del cultivo de maíz sobre la concentración de sodio intercambiable en el suelo, pues i) aumenta la concentración de $\text{H}^+$, $\text{Ca}^{2+}$ y $\text{CO}_2$ en el suelo, que permite el reemplazo y lixiviación de $\text{Na}^+$ de los sitios de intercambio del suelo (Wong et al. 2010), y ii) debido a que, para el cultivo de maíz, el terreno fue previamente arado y el riego se mantuvo periódicamente, esto permitió que la lixiviación del $\text{Na}^+$ fuera mayor, dado que se mejora la porosidad del suelo y permite un mejor lavado de

<table>
<thead>
<tr>
<th>Tratamiento</th>
<th>CO (%)</th>
<th>CICe (cmol(+) kg⁻¹)</th>
<th>PSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Control)</td>
<td>2,06 ± 0,27¹</td>
<td>30,66 ± 2,70²</td>
<td>2,74 ± 0,11³</td>
</tr>
<tr>
<td>T2 (Químico)</td>
<td>1,96 ± 0,34⁴</td>
<td>32,14 ± 8,48⁵</td>
<td>1,51 ± 0,26⁶</td>
</tr>
<tr>
<td>T3 (Enmienda verde)</td>
<td>1,67 ± 0,44⁶</td>
<td>30,56 ± 2,68⁵</td>
<td>1,28 ± 0,25⁷</td>
</tr>
<tr>
<td>T4 (Azufre elemental)</td>
<td>2,01 ± 0,46⁸</td>
<td>34,36 ± 4,93⁷</td>
<td>0,96 ± 0,48⁹</td>
</tr>
<tr>
<td>T5 (Vermicompost)</td>
<td>2,04 ± 0,22¹⁰</td>
<td>30,24 ± 2,31¹¹</td>
<td>2,80 ± 0,08¹²</td>
</tr>
<tr>
<td>T6 (E. verde + Azufre)</td>
<td>1,92 ± 0,03¹³</td>
<td>82,05 ± 16,91¹⁴</td>
<td>0,56 ± 0,03¹⁵</td>
</tr>
<tr>
<td>T7 (E. verde + Vermi.)</td>
<td>2,58 ± 0,20¹⁶</td>
<td>37,82 ± 2,32¹⁷</td>
<td>1,21 ± 0,27¹⁸</td>
</tr>
<tr>
<td>T8 (Vermi. + Azufre)</td>
<td>2,23 ± 1,09¹⁹</td>
<td>71,67 ± 8,62²⁰</td>
<td>0,89 ± 0,05²¹</td>
</tr>
<tr>
<td>T9 (E. verde + Vermi + Azufre)</td>
<td>2,45 ± 0,27²²</td>
<td>83,43 ± 15,80²³</td>
<td>0,53 ± 0,61²⁴</td>
</tr>
</tbody>
</table>

Los valores representan la media de n = 3 con su respectivo error estándar. Para cada parámetro, valores con letras diferentes presentan diferencias estadísticamente significativas (p < 0,05) entre tratamientos, de acuerdo a la comparación de medias de Tukey.
las sales (Qadir & Oster 2002; Gutiérrez et al. 2016). De igual manera, los tratamientos con S° presentaron los menores valores de PSI (< 1%) (Tabla 3), lo cual podría indicar que en estos tratamientos ocurrió un mayor reemplazo y lixiviación de Na⁺ por la solubilización de minerales del suelo gracias a la formación de ácido sulfúrico.

3.2. Propiedades biológicas

Quince días después de sembradas las plantas de maíz, se encontró que todos los tratamientos presentaron mayor biomasa microbiana (BMS) con respecto a T1 (71.97 µg C g⁻¹ suelo seco) (Figura 2), siendo significativamente (p < 0.05) mayor para T5 y T7. Posteriormente, entre los 30 y 50 días, la mayoría de los tratamientos aumentó la BMS (Figura 2). A los 75 días, los tratamientos T3, T5 y T7 disminuyeron su BMS a valores menores de 150 µg C g⁻¹ suelo seco (Figura 2A), lo cual corresponde a lo también encontrado por Wong et al. (2009) en suelos sodícos enmendados con materia orgánica, donde a medida que disminuye la disponibilidad de sustrato por mineralización decrece la BMS; mientras, en los tratamientos con S° se mantiene en valores significativamente mayores debido a mejores condiciones químicas del suelo, además de que el S° puede ser incorporado en la biomasa microbiana cuando es oxidado inicialmente a SO₄²⁻ y, posteriormente, transformado en formas orgánicas de S, transformación que es alta en suelos alcalinos y temperaturas mayores de 35 °C (Jaggi et al. 1999), parámetros parecidos a los presentados en el área de estudio.

En relación a la RMS, se observó que en los 15 y 30 días, ninguno de los tratamientos presentó diferencias significativas con respecto al control (Figura 2B), manteniendo valores alrededor de ± 86 µg C-CO₂ g⁻¹ suelo seco. En el día 50, la mayoría de los tratamientos tendieron a aumentar la RMS sobre 120 µg C-CO₂ g⁻¹ suelo seco, donde T4, T5 y T8 presentaron diferencias significativas (p < 0.05) con valores por debajo de 80 µg C-CO₂ g⁻¹ suelo seco (Figura 2B). En el día 75 se produjeron los mayores valores de RMS del experimento, en T3 y T5 con 329 y 263 µg C-CO₂ g⁻¹ suelo seco respectivamente, además del aumento de la RMS de forma general en todos los tratamientos (Figura 2B). Al final del experimento los tratamientos orgánicos presentaron tendencia al aumento de la RMS, lo cual puede ser debido al efecto dispersante del sodio sobre la materia orgánica, al aumentar la disolución y disponibilidad de esta para los microorganismos, por lo que dichas poblaciones aumentan su actividad (Wong et al. 2010).

En las Figuras 3A y 3B se presenta la evolución de la fosfatasa alcalina y ácida según tratamientos. La tendencia de la actividad fosfatasa alca-
Disponible, pero a medida que el suelo disminuye su alcalinidad y sodicidad la disponibilidad de P en el suelo aumenta, por lo que la actividad fosfatasa disminuye. Los valores más bajos se encontraron en los tratamientos con S° (<50 µg PNP g⁻¹ suelo seco) (Figura 3A), lo cual podría reflejar inhibición de las fosfatases alcalinas por el efecto de las enmiendas utilizadas sobre el pH y la disponibilidad de fósforo, por disolución de minerales del suelo.

Los tratamientos orgánicos presentaron los mayores valores de actividad fosfatasa ácida (>100 µg PNP g⁻¹ suelo seco) a lo largo del experimento (Figura 3B), lo cual concuerda con lo mencionado por Saha et al. (2008) acerca del efecto positivo de aplicaciones de enmiendas orgánicas sobre la actividad de la fosfatasa ácida. A pesar de lo anterior, se observó que en todos los tratamientos la actividad fosfatasa ácida tendió a disminuir hasta el día 50, lo cual puede deberse a que la producción de esta enzima por parte de las plantas está ligada al estado fenológico de las mismas, dado que depende de la actividad y de la tasa de crecimiento de las raíces (Marschner et al. 2007). Para los tratamientos en los que se utilizó S° los menores valores de actividad (<50 µg PNP g⁻¹ suelo seco) (Figura 3B) pueden deberse al efecto inhibidor del S° sobre ciertas comunidades de microorganismos (Gupta et al. 1988).

Debido a la incorporación de compuestos orgánicos nitrogenados, aplicaciones de enmiendas orgánicas en el suelo presentan generalmente efectos positivos sobre la actividad proteasa de suelos áridos (Dinesh et al. 2004). No obstante, en el presente estudio los tratamientos con enmiendas orgánicas, a excepción de T7, presentaron la mayor parte del experimento baja actividad proteasa (<10 µg tirosina g⁻¹ suelo seco 2 h⁻¹) (Figura 4A), donde la menor actividad para T3 puede relacionarse con lo reportado por Mukhopadhyay & Joy (2010) en suelos cultivados con Acacia auriculiformis y Shorea robusta, donde la alta concentración de fenoles, lignina, polifenoles y taninos disminuye la actividad proteolítica del suelo.

Por otro lado, en el VERM, al ser materia orgánica mineralizada por la acción de lombrices de tierra y microorganismos, la mayor parte del nitrógeno se encuentra en formas mineralizadas (Ali et al. 2015), lo cual también inhibe la actividad proteolítica. De igual manera, los tratamientos con S° presentaron tendencia a disminuir su actividad proteolítica después del día 30.
(Figura 4A), lo cual puede estar relacionado con la reducción del pH de la rizosfera por debajo de 7, lo que conlleva a que disminuya la actividad, estabilidad y producción de las proteasas alcalinas (Ellaiah et al. 2002).

Se observó que T1 y T2 presentaron la mayor actividad hidrolítica en el tiempo al presentar valores mayores a 7 µg fluoreseina g⁻¹ suelo seco 0.5 h⁻¹, contrario a los que se aplicó S⁰, que presentaron tendencia a disminuir su actividad hidrolítica después del día 30 a valores menores a 5 µg fluoreseina g⁻¹ suelo seco 0.5 h⁻¹ (Figura 4B), mientras que los tratamientos orgánicos tienden a aumentar su actividad con el tiempo a valores cercanos a T1 y T2. La mayor actividad hidrolítica en T1 y T2 y menor en los tratamientos con S⁰ probablemente se deba al pH ligeramente ácido presentado en la rizosfera de T1 y T2, mientras que los tratamientos con S⁰ presentaron valores de pH cercanos a 5.5.

3.3. Efecto de enmiendas orgánicas y azufre elemental sobre las variables de desarrollo del cultivo maíz

Todas las variables de desarrollo mostraron respuesta a los tratamientos, siendo las aplicaciones de S⁰ mezclado con enmiendas orgánicas los que presentaron los mayores porcentajes de respuesta (Figura 5), hallándose que la variable longitud aumentó hasta un 42,3%, el peso fresco 46,8% y por último para el diámetro de tallo 13,4% con respecto al control.

Dado que en los suelos afectados por sales la alta concentración de iones tóxicos y el pH alcalino son los principales factores que dificultan el establecimiento y crecimiento de los cultivos en estos suelos (Fageria et al. 2011; Karimizarchi et al. 2014), generalmente se cultivan en estos suelos plantas resistentes a dichas condiciones. A pesar que el maíz es un cultivo moderadamente sensible al estrés por sales, este presenta ciertas estrategias fisiológicas que le permiten crecer en condiciones sódicas a costa de reducir su desarrollo y producción, como son la producción de antioxidantes, regulación estomatal, ajustes osmóticos, exclusión vacuolar de los iones tóxicos (Dias et al. 2004; Farooq et al. 2015), las cuales demandan una alta inversión energética, por lo que la reducción de las condiciones sódicas del suelo, concentración de sodio y el pH del suelo en suelos cultivados con maíz han demostrado, al igual que en este estudio, que se aumenta el desarrollo y producción de las plantas debido a la reducción de las condiciones de estrés y aumento en la disponibilidad de nutrientes (Karimizarchi et al. 2014).

La mayor respuesta en el desarrollo de las plantas de maíz se presentó en los tratamientos (T6, T8 y T9) donde se mezcló el S⁰ con materia orgánica (Figura 5), similar a lo observado por Karimizarchi et al. (2014) en plantas de maíz cultivadas en un suelo alcalino con varias do-
sis de S₉, encontrando que aquellos tratamientos donde la oxidación del azufre fue mayor la biomasa seca de las plantas de maíz aumentó hasta un 45,06%, acompañado además de aumento en la concentración de micronutrientes. Por otro lado, Oo et al. (2013) encontraron que, en suelos sódicos enmendados con enmiendas orgánicas y fertilizantes NPK, la biomasa seca de las plantas de maíz fue mayor en aquellos tratamientos que presentaron los mayores valores de BMS, cationes y menores valores de CE al final del experimento, de igual manera como fue observado en este estudio.

Los tratamientos basados solo en enmiendas orgánicas (T3, T5 y T7) no aumentaron de forma significativa (p < 0,05) los parámetros de crecimiento del maíz (Figura 5), lo cual puede deberse a que en suelos sódicos, la interferencia del sodio en la absorción y transporte de cationes constituye el principal problema nutricional en el cultivo de maíz (Farooq et al. 2015), por lo que el aumento en la disponibilidad de cationes y la capacidad de intercambio catiónico del suelo contribuyen a disminuir el efecto toxico del sodio, lo cual se ve reflejado en mayor altura y ganancias de biomasa por parte de las plantas. Esto concuerda con lo observado por Oo et al. (2013) en plantas de maíz cultivadas en suelos salinos manejados con enmiendas orgánicas, donde los mayores valores de longitud de tallo y peso fresco de las plantas se encontraron en los tratamientos donde la CICe y la concentración de cationes aumentaron.

![Figura 5. Variables de desarrollo del cultivo de Zea mays de un suelo sódico tratado con dos abonos orgánicos (abono verde de Prosopis juliflora y vermicompost) y azufre elemental. A) Longitud plantas, B) Peso plantas, y C) Diámetro tallo plantas. Letras distintas indican diferencias significativas entre tratamientos (p < 0,05).](image)

4. Discusión

4.1. Propiedades fisicoquímicas

La acidificación de la rizosfera en todos los tratamientos se debe a la capacidad de las raíces de maíz de excretar ácidos orgánicos e hidrogeniones en condiciones salinas o sódicas (Zidan et al. 1990), lo cual conlleva que el pH rizosférico sea menor que el del suelo circundante. Aun así, los menores valores de pH rizosférico en los tratamientos con S₉ indican que gran parte del azufre fue oxidado a sulfato, donde dicha
La oxidación fue más rápida cuando el S° se mezcló con enmiendas orgánicas, en comparación a cuando fue aplicado solo. Por otro lado, si bien en la literatura se menciona que aplicaciones de enmiendas orgánicas pueden disminuir significativamente el pH de los suelos sódicos (Mogollon et al. 2011; Singh et al. 2014), esta capacidad de reducción pudo verse interferida por la presencia de carbonatos y bicarbonatos, dado que en suelos con altas concentraciones de carbonatos aplicaciones de materia orgánica no tuvieron efecto significativo sobre el pH del suelo en comparación a la aplicación de S° (Hashemimajd et al. 2012).

Los mayores valores de CE, Ca²⁺ y Mg²⁺ en los tratamientos con S° (Tabla 1) se deben al aumento de Ca²⁺ y Mg²⁺ por la disolución de minerales y el reemplazo del Na⁺ por cationes (Araujo et al. 2015), lo cual aumenta la concentración de cationes en la solución del suelo. No se encontraron diferencias significativas en los tratamientos en los que se aplicaron enmiendas orgánicas para la CE, lo que coincide con varios trabajos en donde se establece que aplicaciones de materias orgánicas contribuyen a disminuir o mantener la CE de los suelos sódicos o salinos, debido al aumento de la lixiviación de las sales (Oo et al. 2013).

Los mayores valores de CICE para los tratamientos de enmiendas orgánicas con S° pudo deberse al efecto desnaturalizador del ácido sulfúrico sobre la celulosa y la lignina, lo cual mejora la capacidad de adsorción de metales divalentes de materiales lignocelulolíticos por aumento de la superficie de contacto de los grupos carboxílicos (Sinha et al. 2013).

4.2. Propiedades biológicas

El mantenimiento de los valores de RMS durante los primeros 30 días en todos los tratamientos puede deberse al efecto inhibidor de la rizosfera del maíz sobre ciertas poblaciones de microorganismos de estrategia r, por la secreción de carboidratos a concentraciones bajas y constantes con el fin de estimular el desarrollo de microorganismos especialistas (Fontaine et al. 2003), los cuales presentan baja tasa de RMS, pero mayor de BMS. El aumento en general de la RMS en todos los tratamientos después de 30 días puede deberse a la disminución del efecto modulador de la rizosfera sobre la microbiota del suelo, pues al ir envejeciendo las plantas disminuye la excreción de carboidratos (Fu et al. 2002) y las raíces de estas pueden ser utilizadas por microorganismos descomponedores.

La tendencia a disminuir la actividad fosfatasa en todos los tratamientos pudo ocurrir como respuesta al aumento de la concentración de fósforo disponible debido a la inhibición competitiva de las fosfatasas por los iones fosfatos (Nannipieri et al. 2011). La menor actividad en los tratamientos con S° puede deberse directamente a la capacidad del S° de inhibir ciertas poblaciones de microorganismos (Gupta et al. 1988) o indirectamente debido a que la oxidación del S° en suelos sódicos y alcalinos aumenta la concentración de fosfatos (Araujo et al. 2015). De igual manera, los tratamientos con GM presentaron bajos valores de actividad fosfatasa alcalina (Figura 3A), debido a la capacidad de este tipo de enmienda de bajar el pH del suelo y aumentar la concentración de fósforo soluble en suelos sódicos (Vallejo et al. 2012; Singh et al. 2014). Por otra parte, se observó que para T9 a los 75 días la actividad de las fosfatasas aumentó de forma significativa a pesar de contar con S°, indicando que en T9 ocurrió inmovilización del fósforo, probablemente en la biomasa microbiana (Saha et al. 2008), dado que para el mismo periodo se presentaron los mayores valores de BMS.

Dada la menor actividad proteolítica en los tratamientos orgánicos, es probable que, para los tratamientos con GM de plantas de *Prosopis juliflora* que crecían en suelo sódico, estas presentaran alto contenido de polifenoles y compuestos antioxidantes, con capacidad de disminuir la actividad enzimática de los suelos (Mukhopadhyay & Joy 2010). Incluso al ser sometidos a pH ácidos, como los tratamientos con S°, el contenido de fenoles y polifenoles pudo aumentar (Sarasvati et al. 2014) a niveles que pueden inhibir el crecimiento o funcionamiento de organismos descomponedores al formar complejos y enlaces con ciertas enzimas microbianas (Reyes-Reyes et al. 2003). Por otra parte, el control y T7 fueron los únicos tratamientos que presentaron tendencia a aumentar la actividad proteolítica en el tiempo (Figura 4A), lo cual puede deberse al aumento en la disponibilidad de sustrato orgánico, provenientes de raíces y material vegetal del...
cultivo de maíz, además que T7 corresponde al tratamiento con mayor cantidad de C aplicado sin S°, por lo que la relación C/N pudo haber sido la más alta entre los tratamientos, lo que favorece la actividad proteolítica en suelos enmendados con enmiendas vegetales (Geisseler & Horwath 2009). La mayor actividad FDA en los tratamientos control y T2 es probable que se deba a que presentaron valores de pH rizosférico cercanos a la neutralidad, mientras que los tratamientos con S°, de menor actividad FDA, presentaron valores ácidos de pH que disminuyen el potencial de hidrolizar FDA de los suelos. Esto es debido a que, al igual que las proteasas, muchas enzimas hidrolíticas de suelos alcalinos presentan especificidad al pH, por lo que los grupos ionizables de las enzimas se ven afectados (Turner 2010). En la literatura también se ha descrito que aplicaciones de enmiendas orgánicas presentan aumentos significativos de la FDA (Silva et al. 2015), lo cual no fue observado en el transcurso del ensayo; lo anterior pudo deberse a que las enmiendas orgánicas utilizadas contuvieron baja concentración de sustratos orgánicos solubles, dado que el VERM corresponde a materia orgánica estabilizada con alto contenido de compuestos recalcitrantes, mientras que la GM puede poseer compuestos fenólicos que inhiben a corto plazo el crecimiento de ciertos grupos microbianos, por lo que la degradación de compuestos recalcitrantes, como ligninas y celulosas, aumenta la actividad heterotrófica en el suelo (Fontaine et al. 2003).

5. Conclusiones

La aplicación de S° mezclado con enmiendas orgánicas influyó de forma significativa en las propiedades biológicas y fisicoquímicas de un suelo sódico cultivado con maíz. En general, la aplicación de S° disminuyó la actividad enzimática del suelo y la RMS, lo cual podría atribuirse a la inhibición de ciertas comunidades de microorganismos descomponedores. Por el contrario, la aplicación de enmiendas orgánicas sin S° aumentó la RMS y la actividad hidrolítica, lo cual podría impedir la acumulación de carbono orgánico en el tiempo. Por otro lado, la aplicación de S°, además de acidificar la rizosfera, tuvo un efecto positivo en la concentración de cationes solubles y la CICe del suelo, lo cual podría contribuir a la lixiviación y el reemplazo de iones sodio de los sitios de intercambio. Lo anterior sugiere que las aplicaciones orgánicominerales con S° podrían ayudar a la reducción de las condiciones sódicas y al establecimiento de cultivos en suelos afectados por sodio.

6. Agradecimientos

Al Departamento Administrativo de Ciencia, Tecnología e Innovación (COLCIENCIAS) por la financiación de este proyecto a través del programa Jóvenes Investigadores 2016 y a la empresa Fertiservicios SAS por facilitar el lugar de muestreo, insumos y maquinaria necesaria para la realización del proyecto.

REFERENCIAS


• Oo AN, Iwai CB, Saenjan P. 2013. Soil properties and maize growth in saline and nonsaline soils using cassava-industrial waste compost and vermicompost with or without earthworms. Land Degr Dev. 26(3):300-310.


Effect of incubation time on transformation rate and chemical forms of phosphorous in calcareous soils along a climotoposequence

ABSTRACT

Soil phosphorous fertility can be significantly affected by the P transformation rate in soils. The present study was designed to evaluate the application of time-dependent P fertilizer in terms of its availability and chemical forms in 23 soils of a climotoposequence. The collected soil samples were incubated with 200 µg g⁻¹ P as KH₂PO₄ for 1, 20, 60, 100, 150, and 200 days at 25 °C and after that, the available and chemical forms of P were determined for each incubation time. A rapid decrease in available P was observed within 20 days after application of P fertilizer. A 200-day application of P led to a decrease in the available P by 15% compared to the one-day application. In addition, the biphasic pattern of transformation rate of available P fitted well to parabolic and power function models. The results of sequential extraction methods showed that in treated and untreated soils, Ca-bound P, residual P, Fe- and Al-bound P were the highest fractions, while the exchangeable form was the lowest. At the end of incubation, on average, only 15% of total P (200 µg g⁻¹) remained as exchangeable-P, while this amount was 40.6% and 28.5% at days 1 and 20, respectively. In addition, among all soil characteristics, mineralogical properties showed the most obvious effects on controlling the chemical forms of P over time.
parabólicas y potenciales. Los resultados de los métodos de extracción secuencial mostraron que en suelos tratados y sin tratar, el P unido al Ca, el P residual y el P unido a Fe y Al fueron las fracciones más abundantes mientras que la forma intercambiable fue la más baja. Al final de la incubación, en promedio, solo el 15% del P total (200 µg g⁻¹) permaneció como P intercambiable, mientras que este porcentaje fue del 40.6% en el día 1 y del 28.5% en el día 20. En cuanto a las características de los suelos, las propiedades mineralógicas fueron las que controlaron de forma más evidente las formas químicas de P con el tiempo.

RESUMO

A fertilidade do solo no que se refere ao fósforo pode ser significativamente afetada pela taxa de transformação das formas de fósforo nos solos. O presente estudo foi desenhado para avaliar a aplicação de um fertilizante de P, em função do tempo, em termos da sua disponibilidade e formas químicas em 23 solos de uma climoptossequência. As amostras de solo recolhidas foram incubadas com 200 µg g⁻¹ P como KH₂PO₄ durante 1, 20, 60, 100, 150, e 200 dias a 25 °C após o que se determinaram as formas disponíveis e químicas de P para cada período de incubação. Observou-se uma rápida diminuição do P disponível nos 20 dias de aplicação do fertilizante. A aplicação de P durante 200 dias levou, em comparação com a aplicação de 1 dia, a uma diminuição de 15% do P disponível. Além disso, o padrão bifásico da taxa de transformação do P disponível ajustou-se bem a modelos de funções parabólicas e potenciais. Os resultados da extração sequencial mostraram que em solos tratados e não tratados, o P ligado ao Ca, o P residual e o P ligado ao Fe e Al foram as frações mais elevadas enquanto a forma de troca foi a mais baixa. Em média, no final da incubação, apenas 15% do P total (200 µg g⁻¹) permaneceu como P de troca, enquanto esta fração correspondia a 40,6% e 28,5% nos dias 1 e 20, respectivamente. Além disso, entre todas as características do solo, a composição mineralógica mostrou ser a mais óbvia no controle das formas químicas do P ao longo do tempo.

1. Introduction

Phosphorous one of the most important and highly consumed nutrients for growing plants and other living organisms (Nesme et al. 2014), playing an important role in cellular metabolism in the form of adenosine triphosphate (ATP). Apart from the important role of this element in structural and biochemical compounds of cells, its increasing concentration in natural environments due to excessive use of P fertilizers can lead to environmental problems such as eutrophication of aquatic ecosystems (Devau et al. 2011) and surface waters toxicity (Kier and Kirkland 2013). The vital processes of plants are not feasible without P presence and it is very difficult to access an acceptable concentration of available P in the soil (Bünemann et al. 2010). Total P content is high in most inorganic soils; however, its greater part is found in soil as non-available compounds for the plant (Jalali and Ahmadi Mohammad Zinli 2011). Application of P to soils is done to ensure the sufficient crop yield in many agricultural systems, but the recovery percentage of added P by the plants is very low (10 to 30%) in growing seasons. Due to the sorption, precipitation or transformation to other organic forms, more than 80% of P added to soils would be changed to inactive P and eventually it would be inaccessible for the plant (Holford 1997). In most soils, very low concentrations of non-organic P exist in the soil solution. Phosphate ions tend to be sorbed on positively charged minerals including iron and aluminum oxides (Hinsinger 2001). According to Karaman et al. (2001), physico-chemical characteristic of soil, the type of parent materials, the degree of
weathering, and climatic conditions, have a great impact on the P status in the soil environment. So, a proper management of P fertilizers is required to improve the crop yield plant performance due to low levels and low mobility of P in the soil. P in the soil exists in various geochemical forms including the soil solution, exchangeable phases, organic matter-, Ca-, Fe- and Al-bound phases, and residual phases (Jalali and Ranjbar 2010). The degree of association of P with various geochemical forms in soils is different and depends on various parameters such as type of clay minerals, soil clay content, soil pH, the content of iron and aluminum oxides, calcium ions, and organic matter (OM), oxidation and reduction potential, the presence of competitive ions for surface adsorption, and plant factors (Pierzynski et al. 2005). Dynamic, bioavailability, and chemical behavior of each geochemical forms of P is different and these forms can convert to each other under different conditions. Sequential extraction methods are applied to give qualitative and quantitative information about chemical forms of P (Jalali and Ranjbar 2010) and heavy metals (Saffari et al. 2016) in soils. This method can give us useful information about the mobility, leachability, and transformation of P between chemical forms in soils (Jalali and Ranjbar 2010). Previous studies showed that the availability of P in soil was directly impacted by residence time (Jalali and Ranjbar 2010; Javid and Rowell 2002). P had the highest bioavailability immediately after the application to soils and with increasing time, the bioavailability of P decreased (Jalali and Ranjbar 2010). On the other hand, in order to predict P fertilizer needs and develop efficient and accurate P recommendations, it is necessary to evaluate the residual available P in the soil. Thus, this research attempts to study the impact of application of time-dependent phosphorous fertilizer on the availability of P and its chemical forms in five soil orders (Aridisols, Inceptisols, Alfisols, Histosols and Mollisols) from a climotoposequence.

2. Material and Methods

2.1. Area study, sampling design, and laboratory analysis

The study was conducted across a toposequence transect, near Kerman city in the Kerman province, Iran (Figure 1). The studied climotoposequence covers an area of about 1,200 km². The mean annual precipitation of the area varies between 116.1 and 238.70 mm year⁻¹ and the air temperature ranges from 15.7 °C in the beginning of the transect (north of the transect, 1,840 m above mean sea level) to 10.4 °C in the end of transect (south of the transect, 3,207 m above mean sea level), respectively (Meteorological Organization of Iran 2016). Soil moisture regime (SMR) and temperature regime (STR) in the north of the transect are aridic and mesic that change to xeric and mesic in the south of the transect (Banaei 1998). Two geomorphic surfaces, including a piedmont plain and lowlands of the Lalehzar Mountains were evaluated to determine their P status (Figure 2). Five representative pedons on two geomorphic positions were described.

All soil horizons were described according to Soil Survey Staff (2014) and some chemical and physical properties were determined using standard methods. Soil texture was performed using the hydrometer method (Bouyoucos 1962). pH and EC (electric conductivity) were measured in saturated pastes (in a 1:1 soil and distilled water mixture) and saturated paste extracts, respectively. Percentage of calcium carbonate equivalent (CCE) was determined by acid neutralization (Loeppert and Suárez 1996). Organic matter (OM) content was determined using wet oxidation (Nelson and Sommers 1996). Cation exchange capacity (CEC) was measured by replacing exchangeable cations with sodium acetate (Sumner and Miller 1996). Olsen-P was determined by using a soil to solution ratio 1:20 and 30 min of shaking (Olsen et al. 1982). Phosphorous in the extractant was determined by using the ammonium molybdate-ascorbic acid method described by Murphy and Riley (1962). For mineralogical analysis, some selected soil samples were washed to remove gypsum and soluble salts. Fe-oxides, carbonate, and organic matter were removed by citrate-bicarbonate-
dithionite (CBD), sodium acetate (adjusted pH 5), and H$_2$O$_2$ (30%), respectively (Mehra and Jackson 1960). The clay fraction of the prepared samples was separated by centrifuge (Kittrick and Hope 1963). The centrifuged clay samples (at 750 rpm for 5.4 min) were treated with Mg (1N MgCl$_2$), Mg/ethylene glycol, K (1N KCl), and K/heated at 550 °C for 2 h. The clay minerals were analyzed (Jackson and Barak 2005), using an X-ray diffractometer (Philips, PW 1130/00), using Ni-filtered CuKα radiation (40 kV, 30 mA). Semi-quantitative estimation of clay minerals performed using the (001) peak intensities of the Mg-saturated and glycerol solvated samples (Johns et al. 1954).

2.2. Incubation study and release kinetic of available P

In order to evaluate the effect of aging on the P transformation rate and chemical fractionation in some calcareous soils, all soil samples were treated with P fertilizer (KH$_2$PO$_4$). The soil samples were placed in plastic containers and P was added at the rate of 200 mg kg$^{-1}$ P as KH$_2$PO$_4$. After that, the soil samples were incubated for 1, 20, 60, 100, 150, and 200 days at 25 ± 2 °C. Soil moisture was preserved at field capacity. After each incubation time, sub-samples were air-dried and were analyzed for available P (Olsen et al. 1982) and chemical fractionation of P. Sodium bicarbonate extractant (pH = 8.5) was used for extraction of available P, and concentration of P was determined by the ammonium molybdate-ascorbic acid method (Murphy and Riley 1962). In addition, different kinetic equations (including: zero, 1$^\text{st}$, 2$^\text{nd}$, 3$^\text{rd}$ order, power function, and parabolic diffusion), were also used to describe P release in the studied soils (Table 1). To choose the best-fitted model, a standard error of estimate (SE) was calculated for each equation. Relatively high values of coefficients of determination (R$^2$) and low values of SE were used as criteria for the selection of the best-fitted models. The standard error was calculated as follows:

$$SE = \left[\frac{\sum(S - S')^2}{n - 2}\right]^{0.5}$$

where S and S’ are the observed and estimated amounts of P release in soil at time t, respectively, and n is the number of measurements.

2.3. Fractionation of P
Table 1. Equations fitted to describe P release kinetic

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>( q_t = q_0 - k_0 t )</td>
<td>( k_0 ), zero order rate constant (mg P kg(^{-1}) h(^{-1}))</td>
</tr>
<tr>
<td>First order</td>
<td>( \ln q_t = \ln q_0 - k_1 t )</td>
<td>( k_1 ), first-order rate constant (h(^{-1}))</td>
</tr>
<tr>
<td>Second order</td>
<td>( \frac{1}{q_t} = \frac{1}{q_0} - k_2 t )</td>
<td>( k_2 ), second-order rate constant (h(^{-2}))</td>
</tr>
<tr>
<td>Third order</td>
<td>( \frac{1}{q_t} = \frac{1}{q_0} - k_3 t )</td>
<td>( k_3 ), third-order rate constant (h(^{-3}))</td>
</tr>
<tr>
<td>Simple Elovich</td>
<td>( q_t = \frac{1}{\beta s} \ln (\alpha s \beta s) + \frac{1}{\beta s} \ln t )</td>
<td>( \alpha_s ), initial desorption rate (mg P kg(^{-1}) h(^{-1})); ( \beta_s ), desorption constant (mg P kg(^{-1}) h(^{-1}))</td>
</tr>
<tr>
<td>Parabolic diffusion</td>
<td>( q_t = q_0 - k_p t^{1/2} )</td>
<td>( k_p ), diffusion rate constant (mg P g(^{-1}) h(^{-1}))</td>
</tr>
<tr>
<td>Power function</td>
<td>( q_t = a t^b )</td>
<td>( a ), initial desorption rate constant (mg P kg(^{-1}) h(^{-1})); ( b ), desorption rate coefficient (mg P kg(^{-1}) h(^{-1}))</td>
</tr>
</tbody>
</table>

\( q_0 \) and \( q_t \) are the amount of P extracted from each soil (mg kg\(^{-1}\)) at time zero and \( t \), respectively.

The amount of P in each extracted sample was determined using colorimetric ascorbic acid method (Murphy and Riley 1962).

2.4. Statistical analysis

The regressions and simple correlation were calculated by Microsoft Excel 2010 and SPSS V19.

3. Results

Table 2. Summary of the sequential extraction procedure used in this study

<table>
<thead>
<tr>
<th>g soil: mL solution</th>
<th>Extracting solution</th>
<th>Shaking time (h)</th>
<th>Chemical form of P</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5:30</td>
<td>0.5M NaHCO(_3) (pH 8.5)</td>
<td>16</td>
<td>Soluble and exchangeable</td>
<td>Exch-P</td>
</tr>
<tr>
<td>0.5:30</td>
<td>0.1M NaOH</td>
<td>16</td>
<td>Fe- and Al-bound P</td>
<td>Fe-Al-P</td>
</tr>
<tr>
<td>0.5:30</td>
<td>1M HCl</td>
<td>16</td>
<td>Ca-bound P</td>
<td>Ca-P</td>
</tr>
<tr>
<td>0.5:30</td>
<td>5:2 mixture of concentrated HNO(_3) and HClO(_4)</td>
<td>16</td>
<td>Residual P</td>
<td>Res-P</td>
</tr>
</tbody>
</table>

3.1. Morphology of studied soils

Figure 2 shows the location of each pedon in different geomorphic surfaces along the sequence. The piedmont plain, as the first geomorphic surface observed in this research, was divided in to 2 parts based on SMR. In the first part of this geomorphic surface (P1) the SMR was aridic. On the other side, in the second part of this geomorphic surface, the SMR was xeric (for P2 and P3). P1 was classified as Calcic Haplosalid. A black surface as a result of dissolved organic matter was observed in P1, which showed that this soil is a saline-sodic soil (EC\(_e\): 86.3 dS m\(^{-1}\), pH: 7.4, SAR: 21.19). Existence of a salic horizon and a petrocalcic horizon in P2 is the reason why this soil was classified as Petrocalcic Calcixerept. In the end...
part of rock pediment geomorphic surface with a xeric moisture regime, P3 (Typic Natrixeralf), formation of argillic horizon is as a result of increase in clay along the depth. In addition, an increase in SAR with depth lead to formation of Btk horizon, as a result of clay dispersion. P4 and P5 were located in the lowland geomorphic surface of Lalehzar Mountains. In P4, the soil was classified as a Typic Epiaquoll due to existence of a Mollic epipedon with about 32.5% OM and xeric SMR and saturated water for 6 months of a normal year. In P5, the amount of organic matter (46.3%) was higher than P8 (32.5%). This pedon was known as an organic soil with an intermediate decomposition of organic matter (Typic Haplohemists). Stream flow derived from melting snow and rainfall in lowland did not allow the decomposition of organic matter. Results of this part showed that by moving up along the transect, parent materials, topography, and climate changed, that is why soil properties, as well as soil classifications also changed along the transect.

3.2. Physical and chemical properties of studied soils

Selected chemical and physical properties of the studied soils are shown in Table 3. The soils were alkaline (except for Mollisols). The pH value in these soils ranged from 6.1 to 7.8. The OM and calcium carbonate equivalent (CCE) values ranged from 0.2 to 46.3% and 7.5 to 32.5%, respectively. Higher OM values were observed in Mollisols (with average 12.71%) and Histosols (with average 22.34%), which their conditions did not provide the decomposition of organic matter. The highest and lowest average of CCE values were found in Inceptisols and Histosols, respectively. Available P in different soil types was significantly different. Available P (Olsen P) concentration varied from 1.5 to 138.6 mg kg\(^{-1}\) with an average of 34.12 mg kg\(^{-1}\). Higher values of available P were observed in Histosols and Mollisols (on average: 123.66 and 19.24 mg kg\(^{-1}\), respectively) than in Aridisols, Inceptisols, and Alfisols (on average: 2.04, 5.97, 9.05 mg P kg\(^{-1}\), respectively). However, the total P concentration in all soil samples varied from 248 to 1812 mg kg\(^{-1}\) with an average of 701.739 mg kg\(^{-1}\), but only 20% of the soil samples have an amount of P higher than the critical level (above 18 mg kg\(^{-1}\)). Generally, different variations of physical and chemical properties of present soils would be useful to better understand P status and helpful for the best management of P and soil responses to fertilization.

3.3. P releasing over time
<table>
<thead>
<tr>
<th>Soil number</th>
<th>Profile number</th>
<th>Taxonomy</th>
<th>Geographic coordinates</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>pH</th>
<th>OM</th>
<th>CCE cmol(+) kg⁻¹</th>
<th>Clay</th>
<th>Sand</th>
<th>CEC mg kg⁻¹</th>
<th>Olsen-P mg kg⁻¹</th>
<th>Total P mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1</td>
<td>Aridisols</td>
<td>29º 49'N; 56º 47'E</td>
<td>A</td>
<td>0-5</td>
<td>7.4</td>
<td>0.8</td>
<td>25.2</td>
<td>29</td>
<td>16</td>
<td>19.1</td>
<td>3.5</td>
<td>378</td>
</tr>
<tr>
<td>2</td>
<td>P1</td>
<td>Aridisols</td>
<td>29º 49'N; 56º 47'E</td>
<td>Bk</td>
<td>5-30</td>
<td>7.3</td>
<td>0.5</td>
<td>25.6</td>
<td>25</td>
<td>37</td>
<td>15.5</td>
<td>1.8</td>
<td>356</td>
</tr>
<tr>
<td>3</td>
<td>P1</td>
<td>Aridisols</td>
<td>29º 49'N; 56º 47'E</td>
<td>Bz</td>
<td>30-60</td>
<td>7.5</td>
<td>0.6</td>
<td>19.3</td>
<td>25</td>
<td>75</td>
<td>14</td>
<td>1.8</td>
<td>311</td>
</tr>
<tr>
<td>4</td>
<td>P1</td>
<td>Aridisols</td>
<td>29º 49'N; 56º 47'E</td>
<td>Btn1</td>
<td>60-90</td>
<td>7.7</td>
<td>0.2</td>
<td>16</td>
<td>31</td>
<td>35</td>
<td>17.1</td>
<td>1.5</td>
<td>287</td>
</tr>
<tr>
<td>5</td>
<td>P1</td>
<td>Aridisols</td>
<td>29º 49'N; 56º 47'E</td>
<td>Btn2</td>
<td>90-120</td>
<td>7.6</td>
<td>0.45</td>
<td>15.1</td>
<td>47</td>
<td>35</td>
<td>25.3</td>
<td>1.6</td>
<td>248</td>
</tr>
<tr>
<td>6</td>
<td>P2</td>
<td>Inceptisols</td>
<td>29º 45'N; 56º 46'E</td>
<td>Az</td>
<td>0-20</td>
<td>7.4</td>
<td>0.43</td>
<td>21.7</td>
<td>22</td>
<td>62</td>
<td>14.1</td>
<td>8.5</td>
<td>315</td>
</tr>
<tr>
<td>7</td>
<td>P2</td>
<td>Inceptisols</td>
<td>29º 45'N; 56º 46'E</td>
<td>Bk1</td>
<td>20-70</td>
<td>7.5</td>
<td>0.35</td>
<td>20.2</td>
<td>16</td>
<td>72</td>
<td>11.4</td>
<td>7.5</td>
<td>288</td>
</tr>
<tr>
<td>8</td>
<td>P2</td>
<td>Inceptisols</td>
<td>29º 45'N; 56º 46'E</td>
<td>Bk2</td>
<td>70-100</td>
<td>7.8</td>
<td>0.52</td>
<td>25.5</td>
<td>14</td>
<td>72</td>
<td>9.5</td>
<td>5.8</td>
<td>274</td>
</tr>
<tr>
<td>9</td>
<td>P2</td>
<td>Inceptisols</td>
<td>29º 45'N; 56º 46'E</td>
<td>Bkm</td>
<td>100-132</td>
<td>7.7</td>
<td>0.42</td>
<td>32.5</td>
<td>16</td>
<td>56</td>
<td>10.2</td>
<td>2.1</td>
<td>266</td>
</tr>
<tr>
<td>10</td>
<td>P3</td>
<td>Alfisols</td>
<td>29º 44'N; 56º 46'E</td>
<td>A</td>
<td>0-5</td>
<td>7.4</td>
<td>0.85</td>
<td>12.5</td>
<td>19</td>
<td>75</td>
<td>11.3</td>
<td>14.1</td>
<td>413</td>
</tr>
<tr>
<td>11</td>
<td>P3</td>
<td>Alfisols</td>
<td>29º 44'N; 56º 46'E</td>
<td>Bkz</td>
<td>5-20</td>
<td>7.4</td>
<td>0.38</td>
<td>17.3</td>
<td>21</td>
<td>75</td>
<td>14.2</td>
<td>14.5</td>
<td>389</td>
</tr>
<tr>
<td>12</td>
<td>P3</td>
<td>Alfisols</td>
<td>29º 44'N; 56º 46'E</td>
<td>Bk</td>
<td>30-65</td>
<td>7.5</td>
<td>0.25</td>
<td>16.8</td>
<td>13</td>
<td>81</td>
<td>6.5</td>
<td>5.2</td>
<td>365</td>
</tr>
<tr>
<td>13</td>
<td>P3</td>
<td>Alfisols</td>
<td>29º 44'N; 56º 46'E</td>
<td>Btnk</td>
<td>65-100</td>
<td>7.7</td>
<td>0.2</td>
<td>28.5</td>
<td>23</td>
<td>55</td>
<td>11.7</td>
<td>2.4</td>
<td>378</td>
</tr>
<tr>
<td>14</td>
<td>P4</td>
<td>Mollisols</td>
<td>29º 28'N; 56º 50'E</td>
<td>Ag</td>
<td>0-30</td>
<td>6.1</td>
<td>32.5</td>
<td>10.5</td>
<td>44</td>
<td>34</td>
<td>82.36</td>
<td>22.3</td>
<td>873</td>
</tr>
<tr>
<td>15</td>
<td>P4</td>
<td>Mollisols</td>
<td>29º 28'N; 56º 50'E</td>
<td>Bwg1</td>
<td>30-60</td>
<td>6.4</td>
<td>11.4</td>
<td>9.8</td>
<td>32</td>
<td>46</td>
<td>43.5</td>
<td>20.5</td>
<td>834</td>
</tr>
<tr>
<td>16</td>
<td>P4</td>
<td>Mollisols</td>
<td>29º 28'N; 56º 50'E</td>
<td>Bwg2</td>
<td>60-90</td>
<td>7.5</td>
<td>6.1</td>
<td>9.1</td>
<td>16</td>
<td>66</td>
<td>23.5</td>
<td>21.1</td>
<td>854</td>
</tr>
<tr>
<td>17</td>
<td>P4</td>
<td>Mollisols</td>
<td>29º 28'N; 56º 50'E</td>
<td>Cg1</td>
<td>90-120</td>
<td>6.8</td>
<td>6.05</td>
<td>10.2</td>
<td>18</td>
<td>60</td>
<td>25.8</td>
<td>16.8</td>
<td>812</td>
</tr>
<tr>
<td>18</td>
<td>P4</td>
<td>Mollisols</td>
<td>29º 28'N; 56º 50'E</td>
<td>Cg2</td>
<td>120-150</td>
<td>7</td>
<td>7.5</td>
<td>8.5</td>
<td>22</td>
<td>56</td>
<td>15.7</td>
<td>15.5</td>
<td>789</td>
</tr>
<tr>
<td>19</td>
<td>P5</td>
<td>Histosols</td>
<td>29º 27'N; 56º 37'E</td>
<td>Oe1</td>
<td>0-30</td>
<td>7.5</td>
<td>46.3</td>
<td>15.1</td>
<td>14</td>
<td>36</td>
<td>120.1</td>
<td>122.4</td>
<td>1812</td>
</tr>
<tr>
<td>20</td>
<td>P5</td>
<td>Histosols</td>
<td>29º 27'N; 56º 37'E</td>
<td>Oe2</td>
<td>30-60</td>
<td>7.1</td>
<td>26.7</td>
<td>9</td>
<td>30</td>
<td>28</td>
<td>86.7</td>
<td>102.5</td>
<td>1687</td>
</tr>
<tr>
<td>21</td>
<td>P5</td>
<td>Histosols</td>
<td>29º 27'N; 56º 37'E</td>
<td>Bwg1</td>
<td>60-90</td>
<td>7.1</td>
<td>18.9</td>
<td>12.6</td>
<td>43</td>
<td>5</td>
<td>70.2</td>
<td>138.6</td>
<td>1477</td>
</tr>
<tr>
<td>22</td>
<td>P5</td>
<td>Histosols</td>
<td>29º 27'N; 56º 37'E</td>
<td>Bwg2</td>
<td>90-120</td>
<td>7.2</td>
<td>9.5</td>
<td>7.5</td>
<td>33</td>
<td>32</td>
<td>44.3</td>
<td>120.5</td>
<td>1419</td>
</tr>
<tr>
<td>23</td>
<td>P5</td>
<td>Histosols</td>
<td>29º 27'N; 56º 37'E</td>
<td>Cg</td>
<td>120-150</td>
<td>7</td>
<td>10.3</td>
<td>9.1</td>
<td>26</td>
<td>28</td>
<td>39.1</td>
<td>134.3</td>
<td>1315</td>
</tr>
</tbody>
</table>

OM: organic matter; CCE: calcium carbonate equivalent; CEC: cation exchange capacity; Olsen-P: extracted P by sodium bicarbonate.
Figure 3 shows the amount of P releasing over incubation time in different orders of soils treated with KH$_2$PO$_4$. The trend of P release was the same in all soil samples, so that it showed a very high desorption initially and then decreased gradually. A very significant increase occurred in the available P content one day after application of KH$_2$PO$_4$. The highest transformation rate of added P was observed at the lowest depth of Inceptisols one day after fertilizer application, so that 87 µg g$^{-1}$ (43.5%) of 200 µg g$^{-1}$ added KH$_2$PO$_4$ was transformed to available P form.

On the other hand, the lowest transformation rate of the added P to available form was observed in Histosol topsoil (61.5%) one day after application of KH$_2$PO$_4$. On average, one day after application of KH$_2$PO$_4$ (200 µg g$^{-1}$), the available P in Aridisols, Inceptisols, Alfisols, Mollisols, and Histosols were measured as 49.8%, 51.75%, 50.25%, 56.8% and 55.8%, respectively. The trend of changes in all treated soils indicated a rapid decrease in the available P, 20 days after application of P compared to the one-day application. The largest and the smallest decrease in the available P, 20 days after P application compared to one-day P application, was observed in the Aridisol and Inceptisol orders, respectively. The results indicated that 10%, 12.8%, 9.6%, 10.9% and 8.2% of available P decreased in the Aridisol, Inceptisol, Alfisol, Mollisol, and Histisol orders, respectively, 20 days after P application compared to the 1-day application. On average, 52.4% of P fertilizer added to the studied soils was available one day after application of P, while this form was observed 42%, 20 days after P application.

A slight decrease in the available P was observed in all treated soil samples within 60 days after application of P fertilizer, which led to a decrease by 1.8%, 2.1%, 1.6%, 1.2%, and 1.5% of the available P in the Aridisols, Inceptisols, Alfisols, Mollisols, and Histisols, respectively, compared to the 20-day application of P. The amount of the available P during the 100-day application compared to the 60-day application decreased by 1.3%, 1.15%, 1.5%, 0.68% and 0.54% in the Aridisols, Inceptisols, Alfisols, Mollisols, and Histisols, respectively. Totally, the results show that only 36.5%, 35.6%, 37.4%, 43.9% and 45.53% of the total P added were available in Aridisols, Inceptisols, Alfisols, Mollisols, and Histisols, respectively, 100 days after application of the P fertilizer. The trend of changes in the available P over time in both 150 and 200 days was very gradual and low compared to 60 and 100 days, so that the amount of the available P after 150 and 200 days, compared to 100 days, decreased by 1.3%, 1.3%, 1.4%, 1.6%, 1.1% and 1.1%, 1.8%, 2%, 2.4%, 1.6%, 1.4% in Aridisols, Inceptisols, Alfisols, Mollisols, and Histisols, respectively.

200 days after application of P, decrease in the available P by 15% was observed, compared to the one-day application of P in the studied soils. Due to the high amount of the available P as well as high OM content and lower soil CCE content, Mollisols and Histisols showed the lowest P sorption of added P compared to other orders. Due to the changes in the chemical properties of the studied soils, such as CCE, OM, CEC and kind of clay minerals, the amount of available P released in various depths and profiles of different soil orders were not similar. However, soil samples from deeper horizons contained the least amount of available P, which can be attributed to more P fixation at early times. The average P available in the lowest and uppermost horizons of studied soils was obtained 148 and 127 µg g$^{-1}$, respectively, one day after using the P fertilizer, which decreased to 117 and 97 µg g$^{-1}$, 200 days after application of the P fertilizer.

3.4. P releasing rate over time

Different equations were used to predict the P release rate in the studied soils. The zero, first, second, and third orders equations are linear and simple. However, the P behavior in soil is very complex, so they did not fit the P release as well as predicted in the studied soils (data not shown). Both parabolic diffusion and power function rate equations were chosen as the best ones for fitting to data in this research. The results obtained from R$^2$ and SE values in the prediction of P desorption models in the studied soils indicate the superiority of the power function model to the parabolic equation (Table 4). Figure 4 shows the fitting of data obtained from P desorption in treated soils, in each soil order, using the power function equation. Kuo and Mikkelsen (1980) reported that an increase in the value of “a” and a decrease in the value of “b” in the power function equation probably
indicates an increase in the rate of metal release from soils. As it can be seen in Table 4, coefficient “a” has the highest value in the Mollisol and Histosol soils. On the other hand, both the Aridisol and Alfisol soils had the lowest value of this coefficient. Changes in the amount of this coefficient can help significantly to determine the potential for P release.

3.5. Chemical forms of P in untreated soils

Investigating the chemical forms of P in the studied soils before adding P fertilizer shows the superiority of Ca-P form (Figure 5). The contribution percentage of the Ca-P, Res-P, Fe-Al-P, and Exch-P forms in all studied soils was obtained as 61.9%, 27.1%, 6.5%, and 4.35%, respectively. Among the different orders studied, Aridisols (71.41%) and Histosols (50.2%) had the highest and the lowest amounts of Ca-P, respectively. On the other hand, Histosols had...
the highest amount of Exch-P (9.67%), which was three times more than Exch-P in Mollisols, Alfisols, Inceptisols, and 9 times more than the Aridosol order. Changes in chemical forms of Fe-Al-P and Ca-P did not follow a certain order trough the depth, however both Exch-P and Res-P forms were higher (except Histosols) in the topsoil than subsoil.

### 3.6. Chemical forms of P in treated soils

Investigating the chemical forms of P in the studied soils, one day after application of P fertilizer, showed many changes in different forms of P (Figure 5). The results obtained showed that on average, application of P fertilizer in all studied soils increased Exch-P form from 4.35% to 14.8% and Fe-Al-P from 6.57% to 8.89%. On the other hand, application of P fertilizer in all studied soils decreased Ca-P up to 7% (from 61.9% to 54.9%) and Res-P up to 5.8% (from 27.1% to 21.28%). 40.6%, 14.8%, 39.9%, and 5.34% of total applied P fertilizer entered the Exch-P, Fe-Al-P, Ca-P and Res-P forms, respectively. The highest and lowest change of total phosphorous in the Exch-P form was observed in Histosol and Alfisol soils, respectively. On the other hand, the highest and lowest amounts of P added to Ca-P were observed in Aridosol and Histosol soils, respectively. Because the Exch-P form is considered to be the available form of P for plants as well as it having higher availability for plants and leaching compared to other forms,

### Table 4. Values of two equations in examined soils

<table>
<thead>
<tr>
<th>Soil number</th>
<th>Parabolic diffusion</th>
<th>Power function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$q_0$</td>
<td>$K_p$</td>
</tr>
<tr>
<td>1</td>
<td>112.985</td>
<td>0.059</td>
</tr>
<tr>
<td>2</td>
<td>116.519</td>
<td>0.046</td>
</tr>
<tr>
<td>3</td>
<td>97.763</td>
<td>0.056</td>
</tr>
<tr>
<td>4</td>
<td>97.338</td>
<td>0.049</td>
</tr>
<tr>
<td>5</td>
<td>106.255</td>
<td>0.064</td>
</tr>
<tr>
<td>6</td>
<td>131.432</td>
<td>0.075</td>
</tr>
<tr>
<td>7</td>
<td>125.529</td>
<td>0.061</td>
</tr>
<tr>
<td>8</td>
<td>110.342</td>
<td>0.062</td>
</tr>
<tr>
<td>9</td>
<td>96.047</td>
<td>0.060</td>
</tr>
<tr>
<td>10</td>
<td>131.873</td>
<td>0.059</td>
</tr>
<tr>
<td>11</td>
<td>118.181</td>
<td>0.054</td>
</tr>
<tr>
<td>12</td>
<td>101.389</td>
<td>0.055</td>
</tr>
<tr>
<td>13</td>
<td>102.144</td>
<td>0.053</td>
</tr>
<tr>
<td>14</td>
<td>145.951</td>
<td>0.053</td>
</tr>
<tr>
<td>15</td>
<td>147.961</td>
<td>0.050</td>
</tr>
<tr>
<td>16</td>
<td>145.287</td>
<td>0.053</td>
</tr>
<tr>
<td>17</td>
<td>137.802</td>
<td>0.047</td>
</tr>
<tr>
<td>18</td>
<td>115.908</td>
<td>0.048</td>
</tr>
<tr>
<td>19</td>
<td>248.567</td>
<td>0.036</td>
</tr>
<tr>
<td>20</td>
<td>224.640</td>
<td>0.034</td>
</tr>
<tr>
<td>21</td>
<td>247.395</td>
<td>0.046</td>
</tr>
<tr>
<td>22</td>
<td>229.777</td>
<td>0.045</td>
</tr>
<tr>
<td>23</td>
<td>246.080</td>
<td>0.047</td>
</tr>
</tbody>
</table>

**, *, Significant at 0.01 and 0.05, respectively.
and considering that less than 40% of P fertilizer consumed has transferred to the Exch-P, we can say that more than 60% of the applied fertilizer has changed to non-available form in the studied soils due to their calcareous nature and high pH. However, much of this P is defined as labile and can be released at various stages and introduced as supplier of initial plant needs. Changes occurring in these soils can be very important and critical for fertilizer management.

3.7. The effect of incubation time on chemical forms of P
Figure 5. Chemical forms of P before (untreated soils) and after incubation.
Investigating the effect of incubation time on P chemical forms can provide useful information about fixation status and availability of P. The effect of different times on P chemical forms has been shown in Figure 5. The results obtained show that for a 20-day incubation compared to a one-day incubation, the Exch-P form decreased (from 14.8% to 11.7%), both Fe-Al-P and Ca-P increased (from 8.89% to 9.65% and from 54.9% to 57%, respectively), and Res-P had almost no changes (from 21.2% to 21.5%). Like the effect of incubation time on releasing available P, a severe abrupt change was observed in the amount of available (Exch-P) P after 20-days in all samples. The greatest decrease in Exch-P was observed in the Aridisols (from 16% to 11.3%) and Inceptisols (from 17.8% to 13.5%), and the lowest was observed in Histosols (from 13.4% to 12.2%) and Mollisols (from 10.8% to 8.9%). Moreover, changes made in two forms of Fe-Al-P and Exch-P showed that both the Aridisols and Inceptisols had the largest increase in these forms of P. Changes occurred at days 60, 100, 150 and 200, compared to day 20, were relatively slighter than changes trend at day 20 compared to day 1. A slight decrease in the Exch-P form and a very slight increase in three forms of Ca-P, Fe-Al-P and Res-P were observed in all the soil orders. The greatest decrease of Exch-P and smallest decrease of Res-P form were observed in both Aridisols and Inceptisols 200 days after incubation. On the other hand, the lowest decrease of Exch-P and highest decrease of Res-P were observed in the Mollisols and Histosols. Relative percentage of Exch-P 200 days after incubation showed a decrease by 25.8% (from 40.7 to 14.9%) compared to first day of incubation. On the other hand, 200 days after application of P fertilizer, while this amount was 40.6% and 28.5% at days 1 and 20, respectively.

3.8. Correlation between released P and soil properties

Simple correlations between released P and soil properties show that available P had a significant positive correlation with OM, CEC, initial available P and total P at all desorption times (Table 5). On the other hand, the relationship between CEC, EC and sand with released P was significantly negative at all times. The relation between other soil properties and P desorption was not statistically significant (Table 5).

3.9. Correlation between chemical forms of P and soil properties

The results obtained from relation between chemical forms of P and properties of the untreated soils (without application of P fertilizer) showed that there was a positive and significant correlation between all forms of P and OM, CEC, and total and available P in soils, and a significant negative correlation between EC, sand, and CCE (Table 6). The correlation between chemical forms of P with properties of soils one day after application of P fertilizer was the same as the untreated soils. 200 days after application of P fertilizer made different changes in correlation between chemical forms of P and soil properties, so that no significant correlation was observed except a significant positive correlation between available P (Olsen-P) with Exch-P forms, Fe-Al-P and Ca-P. It seems that chemical forms of P are controlled by other factors over time, like clay minerals.

3.10. Clay minerals and their correlation with P chemical forms

The capacity of soil clay minerals (kaolinite, montmorillonite and illite) has been underestimated for P sorption compared to Fe/Al oxides in many studies. The capacity of P sorption by clay minerals may even exceed the capacity of Fe/Al oxides depending on these minerals’ crystallization (for example, the
### Table 5. Simple correlation coefficient (r) between P chemical forms and soil properties in untreated soils and treated soils after 1 and 200-days incubation time

<table>
<thead>
<tr>
<th>Chemical fractions</th>
<th>pH</th>
<th>OM</th>
<th>CCE</th>
<th>Clay</th>
<th>Sand</th>
<th>EC</th>
<th>CEC</th>
<th>Olsen-P</th>
<th>Total-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>-0.477*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCE</td>
<td>0.600**</td>
<td>-0.447*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>-0.449*</td>
<td>0.222</td>
<td>-0.317</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td>0.314</td>
<td>-0.472*</td>
<td>0.203</td>
<td>-0.701**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>0.341</td>
<td>-0.442*</td>
<td>0.310</td>
<td>-0.082</td>
<td>0.395</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEC</td>
<td>-0.450*</td>
<td>0.976**</td>
<td>-0.456*</td>
<td>0.345</td>
<td>-0.588**</td>
<td>-0.418*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-Olsen-P</td>
<td>-0.238</td>
<td>0.658**</td>
<td>-0.506*</td>
<td>0.237</td>
<td>-0.591**</td>
<td>-0.410</td>
<td>0.729**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total-P</td>
<td>-0.418*</td>
<td>0.826**</td>
<td>-0.633**</td>
<td>0.204</td>
<td>-0.557**</td>
<td>-0.538**</td>
<td>0.860**</td>
<td>0.918**</td>
<td></td>
</tr>
<tr>
<td>†R 1-day</td>
<td>-0.324</td>
<td>0.717**</td>
<td>-0.552**</td>
<td>0.219</td>
<td>-0.576**</td>
<td>-0.416*</td>
<td>0.781**</td>
<td>0.981**</td>
<td>0.944**</td>
</tr>
<tr>
<td>†R 20-days</td>
<td>-0.315</td>
<td>0.718**</td>
<td>-0.546**</td>
<td>0.200</td>
<td>-0.570**</td>
<td>-0.432*</td>
<td>0.780**</td>
<td>0.981**</td>
<td>0.949**</td>
</tr>
<tr>
<td>†R 60-days</td>
<td>-0.323</td>
<td>0.725**</td>
<td>-0.551**</td>
<td>0.201</td>
<td>-0.573**</td>
<td>-0.438*</td>
<td>0.787**</td>
<td>0.980**</td>
<td>0.953**</td>
</tr>
<tr>
<td>†R 100-days</td>
<td>-0.325</td>
<td>0.728**</td>
<td>-0.555**</td>
<td>0.204</td>
<td>-0.575**</td>
<td>-0.442*</td>
<td>0.789**</td>
<td>0.980**</td>
<td>0.955**</td>
</tr>
<tr>
<td>†R 150-days</td>
<td>-0.328</td>
<td>0.728**</td>
<td>-0.557**</td>
<td>0.208</td>
<td>-0.578**</td>
<td>-0.444*</td>
<td>0.790**</td>
<td>0.980**</td>
<td>0.955**</td>
</tr>
<tr>
<td>†R 200-days</td>
<td>-0.328</td>
<td>0.729**</td>
<td>-0.559**</td>
<td>0.211</td>
<td>-0.581**</td>
<td>-0.446*</td>
<td>0.791**</td>
<td>0.980**</td>
<td>0.956**</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 5% level; **Correlation is significant at the 1% level. †R: Released P in different incubation time. Abbreviations as in Table 3. EC: electric conductivity.

### Table 6. Simple correlation coefficient (r) between P chemical forms and soil properties in untreated soils and treated soils after 1 and 200-days incubation time

<table>
<thead>
<tr>
<th>Chemical fractions</th>
<th>pH</th>
<th>OM</th>
<th>CCE</th>
<th>Clay</th>
<th>Sand</th>
<th>EC</th>
<th>CEC</th>
<th>Olsen-P</th>
<th>Total-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exch-P</td>
<td>-0.256</td>
<td>0.677**</td>
<td>-0.535**</td>
<td>0.228</td>
<td>-0.578**</td>
<td>-0.426*</td>
<td>0.744**</td>
<td>0.997**</td>
<td>0.937**</td>
</tr>
<tr>
<td>Fe-Al-P</td>
<td>-0.389</td>
<td>0.809**</td>
<td>-0.614**</td>
<td>0.153</td>
<td>-0.546**</td>
<td>-0.536**</td>
<td>0.831**</td>
<td>0.925**</td>
<td>0.980**</td>
</tr>
<tr>
<td>Ca-P</td>
<td>-0.472*</td>
<td>0.771**</td>
<td>-0.665**</td>
<td>0.148</td>
<td>-0.505*</td>
<td>-0.586**</td>
<td>0.784**</td>
<td>0.889**</td>
<td>0.967**</td>
</tr>
<tr>
<td>Res-P</td>
<td>-0.361</td>
<td>0.839**</td>
<td>-0.553**</td>
<td>0.246</td>
<td>-0.547**</td>
<td>-0.453*</td>
<td>0.884**</td>
<td>0.818**</td>
<td>0.943**</td>
</tr>
</tbody>
</table>

1-day incubation time

| Exch-P             | -0.286 | 0.690** | -0.535** | 0.239 | -0.588** | -0.434* | 0.755** | 0.995** | 0.943** |
| Fe-Al-P            | -0.404 | 0.811** | -0.629** | 0.153 | -0.534** | -0.548** | 0.832** | 0.918** | 0.979** |
| Ca-P               | -0.465*| 0.766** | -0.663** | 0.142 | -0.502* | -0.584** | 0.780** | 0.889** | 0.965** |
| Res-P              | -0.360 | 0.839** | -0.555** | 0.249 | -0.550** | -0.450* | 0.884** | 0.820** | 0.944** |

200-days incubation time

| Exch-P             | -0.097 | 0.009 | -0.304 | 0.078 | -0.229 | -0.152 | 0.057 | 0.574** | 0.372 |
| Fe-Al-P            | -0.164 | 0.015 | -0.279 | 0.236 | -0.366 | -0.147 | 0.071 | 0.506*  | 0.311 |
| Ca-P               | -0.161 | 0.021 | -0.296 | 0.169 | -0.296 | -0.115 | 0.065 | 0.453*  | 0.308 |
| Res-P              | -0.169 | -0.079 | -0.277 | 0.343 | -0.355 | -0.108 | 0.008 | 0.312   | 0.207 |

*Correlation is significant at the 5% level; **Correlation is significant at the 1% level. Abbreviations as in Table 3. EC: electric conductivity.
specific surface area) (Gérard 2016). Therefore, the secondary clay minerals were identified in some soil horizons and reported quantitatively (Table 7) to investigate the effects of clay minerals on chemical forms of P at different times. Palygorskite, chlorite, kaolinite, illite and smectite minerals were found in the Aridisols order. The most abundant mineral in this order was smectite and the least abundant were chlorite and kaolinite. Mineralogical changes in both the Inceptisol and Alfisol orders were similar to the Aridisols, with smectite the most abundant in these orders, and chlorite and kaolinite the least abundant. Palygorskite clay mineral was not found in the Mollisol and Histosol orders due to their high humidity in the slope position, showing that this mineral changed to smectite. Except palygorskite, the minerals present in the three previous orders were also observable in both the Mollisol and Histosol orders.

Generally, from the beginning (Aridisols) to the end of the study area (Histosols), with increasing rainfall and moisture, palygorskite would be destroyed due to instability at high humidity, and the smectite and vermiculite minerals would be prevailing. Also, because the Mollisols and Histosols are located in the lowland geomorphological positions, and due to water from melting snow and relatively high rainfall, the conditions are available for formation of smectite in the form of neoformation. The results obtained from the correlation of clay minerals with P chemical forms in all treated and non-treated samples at different times (one day and 200 days after application of P fertilizer) were somewhat similar, so that illite had a significant negative relation with all P forms (Table 8). Moreover, kaolinite had a significant positive relation with Fe-Al-P before treatment, as well as Fe-Al-P and Ca-P one day after application of P fertilizer, and with Res-P form and total P 200 days after adding P fertilizer. The relation between other clay minerals and chemical forms of P were not statically significant.

### 4. Discussion

According to the results obtained from changes in available P over time, 52.4% (on average) out of total P fertilizer was observed as available one day after application of P fertilizer in all studied soils. The available P decreased severely and reduced to 42% with increasing the

<table>
<thead>
<tr>
<th>Pedon</th>
<th>Soil number</th>
<th>Horizon</th>
<th>smectite</th>
<th>illite</th>
<th>chlorite</th>
<th>Interstratified</th>
<th>kaolinite</th>
<th>palygorskite</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>A</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Bk</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>*</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>Bz</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>Bk1</td>
<td>+++</td>
<td>++++</td>
<td>++</td>
<td>*</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Bk2</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Bkm</td>
<td>++++</td>
<td>++</td>
<td>-</td>
<td>*</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>Bkz</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>Ag</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>*</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>Bwg1</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>*</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>Oe1</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>Oe2</td>
<td>++++</td>
<td>+</td>
<td>-</td>
<td>*</td>
<td>+</td>
<td>*</td>
</tr>
</tbody>
</table>

*: not detected, -: < 10%, +: 10-20%, ++: 20-30%, +++: 30-45%, ++++: 45-55%, +++++: > 55%.  

Table 7. Semi-quantitative analysis of clay minerals in the clay fraction of some soils under study
incubation time (20 days). Passing 200 days from application of P compared to 1 day showed a 15% decrease, on average, in the available P in the studied soils. High concentrations of CCE in all studied soils may be the main reason for significant reduction in available P (less than 50%). P mobility in calcareous soils is controlled by soil properties, which preserves P in the soil strongly and reduces its concentration in soil solution (Delgado et al. 2002). The possible reactions of P in calcareous soils are usually as follows: 1) precipitation in the form of insoluble calcium phosphate such as octacalcium phosphate, hydroxyapatite and apatite carbonate, 2) surface precipitation by free calcium carbonates, and 3) P fixation by calcium-saturated clays. However, in low P concentrations, the exchange process is carried out in the form of sorption silicates and Al-oxyhydroxide on the edge of the minerals.

Reduced phosphate availability by calcium carbonate in calcareous soils is well known (Bertrand et al. 2003). In a study, Jalali (2006) investigated the effect of aging on the available P after fertilizer application in calcareous soils of Iran, and showed that 90 days after application of P fertilizer, only 21% of the applied fertilizer remained available; while in another study by (Jalali and Ranjbar 2010), the same time passing on other regional calcareous soils showed an availability of 32% of the P added to the soil. In a study by Javid and Rowell (2002), it was found that 100 days after adding P fertilizer the availability of P added to the soil was 20-55%. Reduction of the P available over time is a two-phase process, which is very fast at the beginning, and then it proceeds at a slower speed. The fast process may be attributed to sorption of P on aggregates, and the slow one may be attributed to diffusion of P into Fe and Al

Table 8. Simple correlation coefficient ($r$) between P chemical forms and clay minerals in untreated soils and treated soils after 1 and 200-days incubation time ($n = 11$)

<table>
<thead>
<tr>
<th>Clay minerals</th>
<th>Exch-P</th>
<th>Untreated soil</th>
<th>Total-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe-Al-P</td>
<td>Ca-P</td>
<td>Res-P</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>0.420</td>
<td>0.671*</td>
<td>0.631</td>
</tr>
<tr>
<td>Illite</td>
<td>-0.889**</td>
<td>-0.770**</td>
<td>-0.711*</td>
</tr>
<tr>
<td>Chlorite</td>
<td>0.170</td>
<td>0.324</td>
<td>0.394</td>
</tr>
<tr>
<td>Smectite</td>
<td>0.448</td>
<td>0.338</td>
<td>0.264</td>
</tr>
<tr>
<td>Interstratified</td>
<td>-0.343</td>
<td>-0.421</td>
<td>-0.491</td>
</tr>
<tr>
<td>Palygorskite</td>
<td>-0.315</td>
<td>-0.338</td>
<td>-0.374</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaolinite</td>
<td>0.513</td>
<td>0.663*</td>
<td>0.635*</td>
</tr>
<tr>
<td>Illite</td>
<td>-0.890**</td>
<td>-0.756*</td>
<td>-0.717*</td>
</tr>
<tr>
<td>Chlorite</td>
<td>0.187</td>
<td>0.335</td>
<td>0.388</td>
</tr>
<tr>
<td>Smectite</td>
<td>0.444</td>
<td>0.336</td>
<td>0.273</td>
</tr>
<tr>
<td>Interstratified</td>
<td>-0.365</td>
<td>-0.411</td>
<td>-0.487</td>
</tr>
<tr>
<td>Palygorskite</td>
<td>-0.332</td>
<td>-0.366</td>
<td>-0.373</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaolinite</td>
<td>0.454</td>
<td>0.632</td>
<td>0.612</td>
</tr>
<tr>
<td>Illite</td>
<td>-0.873**</td>
<td>-0.765**</td>
<td>-0.761*</td>
</tr>
<tr>
<td>Chlorite</td>
<td>0.115</td>
<td>0.287</td>
<td>0.305</td>
</tr>
<tr>
<td>Smectite</td>
<td>0.508</td>
<td>0.333</td>
<td>0.336</td>
</tr>
<tr>
<td>Interstratified</td>
<td>-0.402</td>
<td>-0.485</td>
<td>-0.496</td>
</tr>
<tr>
<td>Palygorskite</td>
<td>-0.255</td>
<td>-0.277</td>
<td>-0.330</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 5% level; **Correlation is significant at the 1% level.
oxides microspores of solid part of the soil (Wang et al. 2000). In other words, P is adsorbed by soil components through a two-phase process (Sims and Pierzynski 2005). In the early stages, high sorption takes place at high speed as non-specific sorption and ligand exchange on the edges of minerals and short links with Fe and Al crystalline hydroxides and calcium carbonates (depending on the type of soil) (Rayment and Lyons 2011). Following these reactions, P moves at a lower speed through diffusion into internal parts of the soil forms, as well as the surface precipitation and P polymerization at minerals surfaces (Correll 1998). Griffin et al. (2003) investigated the effects of time and application of potassium phosphate on extracted P by Mehlich-3 method, and showed that passing 84 days after application of P fertilizer caused a severe decrease in Mehlich-extracted P and an increase in the contents of Exch-P and Fe-Al-P of the soil. After 20 days of using P fertilizer, the available P content had been reduced by 10%, therefore, it is suggested that fertilization should be carried out in several stages shortly after planting. The availability of P in alkaline and calcareous soils is very low due to the high calcium carbonate and clay content (Tisdale et al. 2002). In the present study, since both the Aridisol and Inceptisol orders had the most available P fixation, the recommendation to use less successive P fertilizer in these soils is more necessary than for Histosols and Mollisols with less P fixation. According to the results, only two parabolic and power function equations were able to adequately fit the available P release data in the studied soils. An investigation on the availability of P release models from calcareous soils in Iran, showed that the parabolic equation was the best predictor equation for P release (Jalali and Ranjbar 2010). The researchers stated that the q₀ (slope) coefficient in this equation represents the release of P during time, and may be introduced as an index of transformation. The smaller index indicates less P release in the studied soils. As given in Table 4, the index is lowest in Aridisols and Inceptisols, indicating a greater transformation index and less P release in these soils. In treated soils, Ca-P (61/9%), Res-P (27/1%), Fe-Al-P (6/5%) and Exch-P (4/35%) forms have the highest and lowest P fractions, respectively. Among different studied orders, Aridisols (71/41%) and Histosols (52%) had the highest and lowest Ca-P respectively. On the other hand, Histosols had the most amount of Exch-P (9/67%), which was three times more than the one in the Mollisol, Alfisol, and Inceptisol orders, and nine times more than the one in the Aridisols order. Jalali and Ranjbar (2010) investigated chemical forms of P in 20 calcareous soils and obtained similar results as in this study. They reported the chemical forms of P as follows: Ca-P forms (63/7%) > Res-P (31/1%) > Fe-Al-P (3/3%) > Exch-P (1/9%). In a study on P chemical forms in Alfisols, Entisols, Mollisols and Vertisols soils in California, Linquist et al. (2011) showed that non-organic and organic P content in Mollisols soils, especially chemical form of P extracted with hydrochloric acid (Ca-P), is more than in other orders. Yang et al. (2013) reported that in epipedon A (15 cm from soil surface), the most labile P in soil orders was in Inceptisols, Entisols, Spodosols, Alfisols, Ultisols, and Oxisols orders, respectively. On average, the application of P fertilizer in all studied soils increased the relative percentages of Exch-P and Fe-Al-P, and reduced the relative percentage of Ca-P and Res-P. From total 200 µg g⁻¹ of the applied P fertilizer, 40.6% has entered to Exch-P, 14.8% has entered to Fe-Al-P, 39.9% has entered to Ca-P part, and the remaining (5.34%) has entered to Res-P part. Unlike the results of this study, Jalali and Ranjbar (2010) showed that adding P to the soil immediately caused more transformed to Ca-P as well as Fe-Al-P parts. The results of the effect of different time on chemical forms of P in various soils showed that the average percentage of the Exch-P form decreased with time and the Ca-P form had the highest P content like untreated soils. Jalali and Ranjbar (2010) showed that the Exch-P decreased and both Ca-P and Fe-Al-P forms increased with time while Res-P remained unchanged. The results of the relationship between chemical forms of P and chemical, physical and mineralogical properties of soils showed that physical and chemical properties of soils in the early stages of P fertilizer addition were more obviously controlling some chemical forms of P, and the correlation decreases over time (200-days) and shows that the other factors mentioned can affect the chemical forms of P. Therefore, we studied the correlation between chemical forms of P and clay minerals. The results showed that there has been no change in the correlation between clay minerals and chemical forms of P over time, and both kaolinite...
and illite minerals got positive and negative meaningful relationships, respectively, with some chemical forms at the beginning and the end of P fertilizer application. The number of hydroxyl groups of 1:1 clays such as kaolinite is more than in 2:1 clays such as montmorillonite. For this reason, the phosphate fixation strength in 1:1 clays is more than in 2:1 clays. In this case, even iron and aluminum ions formed from the breakdown of clay edges are combined with phosphate and make P non-available (Gérard 2016). This can help to justify why kaolinite has a significant correlation with all forms of P except Exch-P. Silicate clays, such as kaolinite, montmorillonite, or illite, have P fixation capability. Hydroxides groups on the surface of clay crystals may be exchanged with hydrogen and phosphate ions and result in P fixation. This may justify the negative correlation of Exch-P form with illite compared to other forms of P.

5. Conclusions

The P reactions added to the studied soils indicated a rapid conversion of available P to relatively less soluble compounds in a short time due to the high adsorption capacities of the studied soils. Investigating the release of available P shows that both the parabolic and power function models have potential to interpret the P release as well. The most extracted chemical forms of P were related to Ca-P in treated and untreated soils. Application of phosphorus fertilizer at first lead to transfer of the highest amount of it to Exch-P form and the least amount to the Res-P form. The relative percentage of the Exch-P form decreased and other parts increased over time. It seems that mineralogical properties can be effective in controlling the P chemical forms of P over longer term compared to the physical and chemical properties.

REFERENCES

• ABanaei MH. 1998. Soil moisture and temperature regime map of Iran. Soil and Water Research Institute, Ministry of Agriculture, Iran.
EFFECT OF INCUBATION TIME ON TRANSFORMATION RATE AND CHEMICAL FORMS OF PHOSPHOROUS IN CALCAREOUS SOILS ALONG A CLIMOTOPOSEQUENCE


