

JEFATURA DE INVESTIGACIÓN E INNOVACIÓN

Título del proyecto

Carbon fluxes within the asociation plant – mycorrhizal fungi in Maize crops using stable isotope approaches: Predictions in Climate changes scenarios

Carrera(s): INGENIERÍA AMBIENTAL,

Director del Proyecto:

JAZMIN SALAZAR ORELLANA; 0703228841; ENFERMERÍA; UNIDAD ACADÉMICA DE SALUD Y BIENESTAR; MATRIZ

Colaboradores del Proyecto

Juan Carlos González; 0301116075; Medicina; Unidad Académica de Salud y Bienestar; Matriz

Código de Proyecto: PICCIITT19-53

Cuenca, junio de 2021

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A. DATOS GENERALES DEL PROYECTO

1. TÍTULO
Carbon fluxes within the asociation plant – mycorrhizal fungi in Maize crops using stable isotope approaches: Predictions in Climate changes scenarios
2. CARRERAS
INGENIERÍA AMBIENTAL,
3. MATRIZ, SEDE O EXTENSIÓN
MATRIZ CUENCA

B. INVESTIGADORES PARTICIPANTES EN EL PROYECTO

4. PERSONAL DEL PROYECTO – DIRECTOR DE L PROYECYO	
Función en el proyecto	DIRECTOR DEL PROYECTO
Nombre, Cédula; Carrera; Unidad Académica; Sede o Extensión	
JAZMIN SALAZAR ORELLANA; 0703228841; ENFERMERÍA; UNIDAD ACADÉMICA DE SALUD Y BIENESTAR; MATRIZ	
4.1. Publicaciones con ISSN en los últimos 5 años de más alto nivel y cuartil de la revista:	
Título del artículo,; revista; ISSN; volumen; número; año; DOI; cuartil	
Endophytic fungi associated with roots of epiphytic orchids in two Andean forests in southern Ecuador and their role in germination; LANKESTERIANA; 20; 1; 2020; http://dx.doi.org/10.15517/lank.v20i1.41157 ; Q2.	
Specificity of mycorrhizal fungus (Rhizoctonia sp.) In Phaelonopsis sp., Cymbidium sp., Trichocerus antenifer, Oncidium excavatum, and Cyrtochillum sp.; MASKANA; 7; 1; 2016; https://doi.org/10.18537/mskn.07.01.08	
4.2. Libros y capítulos de libro en los últimos 5 años.	
Título del libro o capítulo de libro; editorial; ISBN; número; año; revisión de pares (SI-NO)	
4.3. Proyectos de Investigación desarrolladas en los últimos cinco años de mayor relevancia:	

Nombre del proyecto; Institución; Monto financiado; fecha de inicio; fecha de culminación.

Identification of biogeochemical and hydrological processes in wetlands using stable isotope techniques: comparing paramo and Amazon ecosystems in southern Ecuador for greenhouse gas mitigation ; CEPRA XII- RED CEDIA Call for proposals 2019; \$73951,70; AGOSTO 2019; FEBRERO 2021

Development and biotechnological innovation for the promotion of important agricultural items in food security, exportable competitiveness, and adaptation to climate change; Austro Station National Institution for Agricultural and livestock research- INIAP / National Secretary of Education, Science and Technology - SENESCYT; \$1458753,42; 2012; 2016

5. PERSONAL DEL PROYECTO – COLABORADORES UNIVERSIDAD CATÓLICA DE CUENCA

Función en el proyecto	COLABORADORES UCACUE
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Nombre, Cédula; Carrera; Unidad Académica; Sede o Extensión

Juan Carlos González; 0301116075; Medicina; Unidad Académica de Salud y Bienestar; Matriz

5.1. Publicaciones con ISSN en los últimos 5 años de más alto nivel y cuartil de la revista:

Título del artículo; revista; ISSN; volumen; número; año; DOI; cuartil

Juan Carlos González; Capacidad de carga y presión de uso de la tierra en cuatro sectores de la sub-cuenca del río Déleg, Provincia del Cañar, Ecuador; Facultades de Agronomía Luz;34;2017; Q4.

Juan Carlos González; Análisis de la calidad de vida en el Cantón Déleg, Provincia del Cañar-Ecuador; Revista Venezolana de Gerencia; 75; 2016; Q3

5.2. Libros y capítulos de libro en los últimos 5 años.

Título del libro o capítulo de libro; editorial; ISBN; número; año; revisión de pares (SI-NO)

5.3. Proyectos de Investigación desarrolladas en los últimos cinco años de mayor relevancia:

Nombre del proyecto; Institución; Monto financiado; fecha de inicio; fecha de culminación.

Juan Carlos González; Análisis del deterioro agroecológico y ambiental, bajo un enfoque integrado y complejo, en un área muestra de la subcuenca del río Déleg, provincia del Cañar, República del Ecuador; Universidad Católica de Cuenca; \$18814; 2015.

6. PERSONAL DEL PROYECTO - COLABORADORES EXTERNOS

Función en el proyecto	COLABORADORES EXTERNOS
------------------------	------------------------

Nombre, Institución

Silvia Parra Suárez; UNiversidad de Bayreuth; Alemania

Rafael Muñoz Tenelema; Instituto Nacional de Investigaciones Agropecuarias INIAP

6.1. Publicaciones con ISSN en los últimos 5 años de más alto nivel y cuartil de la revista:

Título del artículo,; revista; ISSN; volumen; número; año; DOI; cuartil

Silvia Parra; Origin and fate of nitrate runoff in an agricultural catchment (Haeon, South Korea) – Comparison of two extremely different monsoon seasons; Science of the Total Environment; 648;15;2019; doi.org/10.1016/j.scitotenv.2018.08.115; Q1

Silvia Parra; The fate of monsoonal atmospheric nitrate deposition in two forest catchments in Soyang lake watershed, South Korea – A mass balance and stable isotope approach; Biogeochemistry; 142; 2019; https://doi.org/10.1007/s10533-018-0522-2; Q1

Silvia Parra; Relationship between nitrogen isotope ratios of NO₃- and N₂O in vertical porewater profiles through a polluted rain-fed peat bog (Ore Mts., Central Europe); Soil Biology and Biogeochemistry Journal; 123; 2018; https:// doi.org/10.1016/j.soilbio.2018.04.022; Q1

6.2. Libros y capítulos de libro en los últimos 5 años.

Título del libro o capítulo de libro; editorial; ISBN; número; año; revisión de pares (SI-NO)

Silvia Parra; Origin and fate of nitrate runoff in an agricultural catchment (Haeon, South Korea) – Comparison of two extremely different monsoon seasons; Science of the Total Environment; 648;15;2019; doi.org/10.1016/j.scitotenv.2018.08.115; Q1

Silvia Parra; The fate of monsoonal atmospheric nitrate deposition in two forest catchments in Soyang lake watershed, South Korea – A mass balance and stable isotope approach; Biogeochemistry; 142; 2019; https://doi.org/10.1007/s10533-018-0522-2; Q1

Silvia Parra; Relationship between nitrogen isotope ratios of NO₃- and N₂O in vertical porewater profiles through a polluted rain-fed peat bog (Ore Mts., Central Europe); Soil Biology and Biogeochemistry Journal; 123; 2018; https:// doi.org/10.1016/j.soilbio.2018.04.022; Q1

6.3. Proyectos de Investigación desarrolladas en los últimos cinco años de mayor relevancia:

Nombre del proyecto; Institución; Monto financiado; fecha de inicio; fecha de culminación.

Silvia Parra; Identification of biogeochemical and hydrological processes in wetlands using stable isotope techniques: comparing paramo and Amazon ecosystems in southern Ecuador for greenhouse gas mitigation; CEPRA XII- RED CEDIA Call for proposals 2019; \$73951,70; Agosto 2019; Febrero 2021.

Silvia Parra; Isotope constraints on microbial N₂- fixation in ombrotrophic peat bogs; CZECH Geological Survey; 35000€; Marzo 2017; Marzo 2018

Silvia Parra; Complex TERRain and ECological HeterogeneityEvaluating ecosystem services in production versus water yield and water quality in mountainous landscapes (TERRECO);DFG; \$50000; Abril 2017; Abril 2018

C. ESTUDIANTES PARTICIPANTES EN EL PROYECTO

7. PERSONAL DEL PROYECTO – ESTUDIANTES	
Función en el proyecto	ESTUDIANTES COLABORADORES EN EL PROYECTO
Nombre; Cédula; Carrera; Unidad Académica; Sede o Extensión	
Christian Andrés Galarza Riera; 0105846596; Agronomía; Ciencias Agropecuarias; Matriz	

D. CENTRO DE INVESTIGACIÓN INVOLUCRADOS Y BENEFICIARIOS

8. CENTRO Y GRUPO DE INVESTIGACIÓN					
Centro de Investigación CIITT					
Grupo de Investigación INGENIERÍA AMBIENTAL,					
9. LÍNEA Y ÁMBITO DE INVESTIGACIÓN INSTITUCIONAL					
Para información sobre las líneas de investigación dirigirse al enlace Líneas y Ámbitos de Investigación Institucionales ,					
Línea de Investigación: Territorios, Naturalezas y Tecnología					
Ámbito de Investigación: Estudios ecosistémicos					
10. CAMPO, DISCIPLINA Y SUBDISCIPLINA UNESCO					
Código del campo y de la disciplina según UNESCO en el enlace SKOS					
Campo	24	Disciplina	2499	Sub disciplina	2499

11. PROGRAMA:	
En caso de que el proyecto sea parte de un programa.	
12. TIEMPO DE EJECUCIÓN DEL PROYECTO	
Duración del proyecto en meses	24
13. FINANCIAMIENTO DEL PROYECTO	
Monto financiamiento UCACUE	\$ 18000
Monto otras fuentes de financiamiento	\$ 33500
Monto Total del financiamiento Proyecto	\$ 51500

14. REQUIERE AVAL Y/O PERMISO DEL COMITÉ DE BIOÉTICA Y EL MINISTERIO DE SALUD PÚBLICA	
NO	
Justificación:	

15. BENEFICIARIOS DEL PROYECTO	
Comunidad Universitaria (docente investigadores, estudiantes), Investigadores del INIAP; Agricultores de Maíz del cantón Pindal, provincia de Loja.	

E. DESCRIPCIÓN DE LA PROPUESTA

16. RESUMEN DEL PROYECTO

Microorganisms, including mycorrhizae, a symbiotic association between the mycelium of a fungus and the roots of plants, provide various ecosystem services such as i) enlargement of the root system, ii) solubilization of minerals, making them assimilable by plants, and iii) tolerance of plants to periods of drought. Mycorrhiza – Plant symbiosis is a fundamental relationship for its development and establishment in an ecosystem.

Considering the critical role of microorganisms in the agroecosystems, here we propose to characterize the composition and functions of the microbial communities of the rhizosphere and soil in maize crop (*Zea mays*) and the contributions of mycorrhizal fungi in the flow of carbon in different phenological states using stable C13 isotope techniques under a climate change scenario such as drought. The sampling and fieldwork will take place in maize cultivation plots located in Pindal canton within Loja province.

The information generated will contribute to the specific knowledge about the importance and prevalence of these microorganisms in the current agroecosystems and their potential use as an alternative to the indiscriminate use of agrochemicals and the effects of climate change.

17. PALABRAS CLAVES

Maize, Carbon, microbial communities, Mycorrhizal fungi, Stable Isotopes,

18. PLANTEAMIENTO DEL PROBLEMA Y JUSTIFICACIÓN

Microbial activities are considered of great importance within the dynamics in natural and agroecological soils, such as their role in the soil's nutrients cycles, mineralization, and immobilization, which consequently entails efficient use of these nutrients plants. The symbiotic relation between mycorrhiza and plants provides a series of ecosystem services that researchers in recent years have addressed. As an example, suppression of invasive plants in agricultural systems (1), pathogens elimination (2), herbivory protection (3), tolerance to extreme salinity, drought, and flood conditions (4) (5), and high soil temperatures (6).

Despite all the ecosystem services that mycorrhizal fungi provide, they are exposed to different adverse factors such as incorrect agricultural practices, excessive inorganic fertilization, tillage, and burn that can be translated to significant ecosystem problems decrease in fungal colonization. Furthermore, if we add to this the effects of climate change, the scenario is not very promising.

Although there has been substantial scientific progress in the topic, added to this the community disposition towards the conservation of agroecosystems, much remains unknown. There are uncertainties about the diversity of these communities, their composition and richness, how they are related to plant adaptation, the flow of nutrients, the impact on stabilizing the soil's organic matter, and the carbon sequestration.

Therefore, it is essential to address questions related to the existing relationships between the soil microbiota and plants, especially under an eminent climate change scenario. Consequently, this proposal aims to a) characterize in detail the rhizosphere and soil microbiota in maize crops, b) identify the role of mycorrhizal fungi in carbon flux, and c) Determine the role of mycorrhizal fungi relationships under a climate change scenario (drought).

Studies in Ecuador have focused on applying mycorrhizal fungi in agricultural systems and determining their possible role in plant development. Lack of information in the ecological characteristics and how agroecosystem influences composition and structure of microbial communities in the rhizosphere and the dynamics of carbon flux according to the phenological states of the plant. At an international level, the situation changes. Some other plant species study the dynamics of this symbiotic relationship and its role in plant physiology, carbon flux, phosphorus solubilization, and tolerance to the effects of climate change.

We have to mention the importance of these project findings for our strategic alliance with the National Agricultural Research Institute-INIAP and the University of Bayreuth to address the study of this type of microbial communities from multiple complementary perspectives.

19. MARCO TEÓRICO Y ESTADO DEL ARTE

Terrestrial ecosystems can sequester carbon through plants and soil. Carbon can be stabilized in the ground by associating with mineral particles (7) by forming compounds that are not very susceptible to microbial degradation and constructing stable aggregates (8).

In natural ecosystems, the rates of soil formation and decomposition of organic matter are kept in balance. Here, the plants play a fundamental role in the conservation and restoration of the soil by retaining carbon and stimulating large microbial masses.

On the other hand, in agricultural ecosystems, management practices can cause alterations, modifying the capacity to capture CO₂. These also affect the emissions of other greenhouse gases such as N₂O and CH₄. (9).

According to the 3rd National Communication on Climate Change (2017), the impacts of climate change on soils are i) the increase in temperature; ii) changes in rainfall in terms of distribution and quantity; iii) the increase in the presence of extreme climatic events such as heat or cold waves, prolonged droughts, and floods and iv) the increase in atmospheric CO₂.

Information on the impacts of climate change in Ecuador is scarce; if not null, significant gaps make it difficult to accurately determine climate trends in all regions (10). The analysis of climate models shows an increase in temperature at the national level of 0.6 °C for the period 2011–2040, up to 2.8 °C at the end of the century, with regional differences (11) (12).

Evidence on the effects of climate change in Ecuador is related to variations in precipitation (13), extensive droughts (14), sea-level rise (15), and retreat of glaciers (16), among others. The effects of climate change on the local communities are related to the availability of water used in agricultural production and drinking water for inhabitants and citizens (17)(18).

Climate change mitigation and adaptation strategies

Mitigation strategies to counter greenhouse gas emissions from the soil are based on practices that improve soil quality, including maintaining the soil vegetation cover for an extended period of the year and using crops that develop a considerable number of roots to reach different soil profiles. Mycorrhizae also have a vital role in these strategies, given their location in the plant-soil interface and their influence on plant physiology and plant communities. Therefore, mycorrhiza fungi must be considered in studies of the impact of climate change on ecosystems. (19). For example, to resist water stress conditions, the plants associate with some mycorrhizal fungus. This symbiotic relationship allows the plant to acclimatize and continue with the assimilation of nutrients in the following stages of development (20).

Plant's ability to survive in dry soils is associated with the possibility of staying further dehydration. As the ground loses moisture and the water potential decreases, the plants must also decrease their water potential to maintain a favorable gradient in water flow towards the root (21). Studies showed that maize plants (*Zea mays* L.) mycorrhized by *Glomus intraradices* and *G. mosseae* subjected to drought recover faster (22)(23). They also present higher values of water potential in the leaf and CO₂ assimilation rate than non-mycorrhized maize. Several studies have described how this mycorrhizal symbiotic relationship improves the water status of various plant species when exposed to a water deficit by increasing the relative assimilation of water, transpiration, and CO₂ exchange rates and efficiency in using water. (24).

Mycorrhiza Fungi

The growth of the human population and climate change put pressure on plant production. The crops are exposed to water and caloric stress, etc. (25). As climate change does not only mean increased temperatures,

also human activities have increased the emission of carbon and nitrogen into the atmosphere. This situation addresses whether it is possible for the mycorrhizal symbiotic relationship with plants to succeed in this new scenario. The answer is unpredictable and complex. (26).

The emergence of terrestrial plants occurred alongside mycorrhizal fungi, being fundamental in the colonization of the terrestrial environment by plants (27). Nutritional associations between fungi and plants are considered a relatively stable evolutionary strategy (28). Mycorrhizal symbiosis is present in almost all terrestrial ecosystems, including in aquatic environments, since it is a relationship that occurs in around 80% of the existing plants (28)(29). Nowadays, we know the genes responsible for forming the symbiotic relationship with mycorrhizae in the plants (30).

The role of mycorrhizal fungi is known for its contribution to plant nutrition and its marked influence on the physical structure of the soil that can affect the availability of resources (31) since they function as a link between biological, chemical, and physical factors (28).

Mycorrhizas act as an extension of the plant in the soil and increase the availability of nutrients through the hyphae network (32). They connect two environments, one very heterogeneous like the soil and the other relatively homogeneous like the roots of plants (29). Thus, they play an essential role in the physiology of plants facilitating their growth under adverse conditions (31), increasing their access to nutrients and water in exchange for sugars (33). Besides, mycorrhizas are fundamental role players in the carbon cycle, redistributing it through the soil, facilitating mineralization of organic matter, and stabilizing carbon in stable organic compounds (29).

With the advance of intensive agriculture, mechanization, and the incorporation of fertilizers, there has been a negative impact on mycorrhizae (27). Particularly phosphorus as a limiting nutrient, its depletion worries farmers and decision makers, and overfertilization and its negative impact.

Studies determine that mycorrhizas adapt to scenarios of phosphorus overfertilization, although studies also report the opposite effect (34). In any case, it is most likely that in the future agricultural systems will depend on the flow of nutrients through the mycorrhizal network (27), leading to the robust growth of mycorrhizal plants and making them tolerant to abiotic factors (25). Some crops respond favorably to water stress when their seeds are inoculated with mycorrhizae (32), increasing their ability to reduce the harmful effects of drought on crops (35).

The effects of soil mycorrhizal inoculation were evaluated on leaves chlorophyll content in some cereal crops as maize. The plant responses differ according to the soil nutrients, which vary between positive, negative, and neutral values, depending on the plant-soil combination (36). Fungi are responsible for producing phytohormones and essential role in achieving soil resources through a more excellent absorption of water and nutrients (37).

It has been proven that the incorporation of composted manure and mycorrhiza generates higher yields in forage maize than chemical fertilization (38). It is well known the cultural practice of chemical fertilization favors maize production in intermediate doses in the presence of mycorrhiza fungi (39). The maize production is also preferred with the application of biostimulants and mycorrhizae (40).

Within the groups of mycorrhizal fungi, it has been found that arbuscular mycorrhizae support drought better than ectomycorrhizae, and they better resist low-temperature conditions (41) (42).

The infectivity and effectiveness of mycorrhizal fungi in maize have been tested, predominantly better with native fungi than commercial formulations (43). However, in a study made in maize, they responded better to the use of mycorrhizae exotic compared to native ones when there is a low density of them (44).

Carbon fluxes

In mycorrhizal symbiosis, the energy balance is essential for the associated organisms to benefit each other during the interaction. In mycorrhizal symbiotic systems, the relationship between growth and metabolism depends on the efficiency with which the carbon fixed by the plant is used in response to the association and transfer of carbon to the fungus for its maintenance (45).

When the plant-mycorrhizal fungus interaction is established, the first one transfers to the fungus between 4 and 20% of the net photoassimilates. In comparison, the fungus considerably increases the assimilation of nutrients and can contribute to the plant up to 80% of the phosphorus, 60% of the copper, 25% nitrogen, 25% zinc, and 10% potassium (46).

The metabolism in mycorrhizal plants subjected to water stress is little known. What is known is that the colonization of roots by mycorrhizal fungi possibly increases the metabolic rate by facilitating the use of phosphorus at the expense of the transfer of carbon from the plant (47). The gas exchange rate will probably compensate for the carbon required for the sustenance of mycorrhizal fungi (48).

Stable (^{13}C) and radioactive (^{14}C) isotope labeling techniques and ^{13}C natural abundance approaches are the best tools available to overcome these difficulties and separate root-derived C from soil-derived C (49). Exposure to either ^{13}C or ^{14}C -labeled CO_2 , in contrast to continuous labeling and the ^{13}C natural abundance approach, allows analysis of the dynamics of C allocation within the plant-soil system

20. OBJETIVOS

To characterize the composition and functions of the microorganisms of the rhizosphere and soil in a maize crop (*Zea mays*) and the contribution of mycorrhizal fungi in the carbon flux in different phenological states using stable ^{13}C isotope techniques.

21. ESPECÍFICOS

- 1) Determine the composition and indices of diversity, of the communities of microorganisms that colonize the soil of the rhizosphere and the cultivation soil (at rest, at the beginning of the cycle and the end of the cultivation cycle).
- 2) Identify possible differences in the composition of the microbial communities that colonize the soil (rest, beginning of the cycle, and end of the cultivation cycle).
- 3) Identify mycorrhizal fungi and their contribution to carbon flux in different phenological stages of corn (V5, V14, R2 to R3, and R3 to R4).
- 4) Evaluate under greenhouse conditions the effect of drought on the plant-mycorrhizal fungus, soil carbon flux.
- 5) Establish in what way the environmental variables and the soil affect the composition these communities of microorganisms.

22. MARCO METODOLÓGICO

Study Area

The study will take place in the Pindal canton of the Province of Loja, located in southern Ecuador. It has a humid tropical climate in the north and hot dry in the south and west. The main crops are maize, bananas, coffee, rice, orange, cane, pineapple, and yucca.

The cantonal area destined for agricultural activities represents 13,441.25 hectares of cultivated area. The maize represents 76% of the total hectares considered under cultivation according to the 2019-2023 Territorial Development and organization Plan.

Sampling

Plots of $10 \times 10 \text{ m}^2$ will be selected for the cultivation of maize in the Pindal canton. In each plot, ten subplots will be located. Then, 1Kg of soil - samples will be collected randomly as i) at soil rest (the period between cultivation periods), ii) soil at the beginning of the cycle, iii) at the end of the cycle, and iv) 300 grams of rhizospheric soil (20 plants) understood as the soil attached to the roots and which is influenced by the exudates. With the subsamples, a composite sample will be formed that will be used for the isolation of microorganisms, extraction of metagenomic DNA from the soil, and physical-chemical analysis.

Physical-chemical analysis

About 1kg of the composite sample will be weighed to perform physical-chemical analysis, including pH, apparent density, OM content, texture, salinity, electrical conductivity, and humidity. Besides the content of carbon, nitrogen, potassium, phosphorus, calcium, magnesium, iron, copper, manganese, and zinc, the complete analysis will be carried out in the Soil Laboratory of the Austro-INIAP Station.

Characterization of microbial communities by massive sequencing

We will use massive sequencing to know the composition and structure of the microbial communities that colonize the rhizospheric soil and the cultivation soil. For this, we extract the total metagenomic DNA from soil and rhizospheric soil samples collected in the field, using a DNA extraction kit (MoBio Power Soil type) following the manufacturer's protocol (50)(51). The quantity and quality of the DNA will be checked by 1% agarose gel electrophoresis. Qubit Fluorometric Quantitation will determine the DNA concentration.

For massive sequencing (NGS), we will send the DNA to Illumina's Novaseq platform from Macrogen (South Korea) for the construction and selection of libraries that allow describing a more reliably and complete scenario of the communities of microorganisms associated with the rhizosphere of maize in various stages (52).

Isolation and culture of microorganisms

We will weigh 1gr of each of the collected and composited samples. Each one will be serially diluted and inoculated in a potato dextrose agar medium with streptomycin sulfate to avoid bacterial colonies; and incubated for several days (53). When pure cultures are available, molecular identification will be carried out.

Molecular identification of fungi isolated from the rhizosphere

To identify the species present in the different samples or those that are capable of growing in the selected culture medium, we will use the molecular identification technique based on polymerase chain reaction (PCR) amplification of the ITS region for its subsequent analysis (54). To do this, once the DNA is extracted from the fungi, universal primers will be used. The sequences obtained will be compared with those deposited in the public and free access databases (GenBank, EMBL-EBI).

Carbon flux analysis using stable isotopes

The carbon isotopic analysis will be carried out under controlled conditions (greenhouse), following the methodology of Zhou, et al. 2020 with an adjustment in the CO₂ flux to be treated from a C₄ plant. The isotopic value of ¹³C will be located in each of the selected phenological states of the maize plant, in the soil, and rhizospheric soil, allowing us to determine the carbon of the microbial biomass, the isolated and inoculated mycorrhizal fungi will form part. In two scenarios: 1) normal conditions and 2) drought conditions.

Experimental Scheme

Question: How many days the plant tolerate the drought with the presence of a mycorrhiza inoculum and without inoculum (blank/control)?

Stages: V5, V14, R2 to R3 and R3 to R4

Inoculum of mycorrhizal fungus: (to be defined, depending on the results of the isolates)

Repetitions: 4 for each phenological state

Question: How does carbon flow between plant-fungus?

Stages: V5, V14, R2 to R3 and R3 to R4

Inoculum of mycorrhizal fungus: (to be defined, depending on the results of the isolates)

Repetitions: 4 for each phenological state

Sampling for isotopic analysis: plant, soil, and rhizosphere soil.

F. IMPACTO DEL PROYECTO

23. CONSIDERACIONES ÉTICAS DE LA PROPUESTA

The strategic partnership with INIAP and the University of Bayreuth will allow us to work without significant inconveniences. Our work likely contributes to decision-making regarding cultivation practices under a climate change scenario. This is important for the Association of Maize Farmers of the Pindal Canton of the Loja province since it is oriented to understand the microbial dynamics of the soil and the potential use of mycorrhizal fungi as growth stimulators and resistance to climate change in some crops

24. RESULTADOS ESPERADOS DEL PROYECTO

1) Determine the composition and indices of diversity, of the communities of microorganisms that colonize the soil of the rhizosphere and the cultivation soil (at rest, at the beginning of the cycle and the end of the cultivation cycle)

The results obtained will allow us to know all the dynamic ecological of the microbial communities studied these soils, information that can be very useful to guide agricultural practices such as burning, fertilization, and the use of agricultural inputs.

2) Identify possible differences in the composition of the microbial communities that colonize the soil (rest, beginning of the cycle, and end of the cultivation cycle)

By analyzing the microbiota of the different soil types, we will know what differentiates the microbial communities. The information will be essential to understand the cause-effect of this type of agricultural practice.

3) Identify mycorrhizal fungi and their contribution to carbon flux in different phenological stages of corn (V5, V14, R2 to R3, and R3 to R4)

The results will allow us to understand the carbon fluxes changes under the different plant phenological states.

4) Evaluate under greenhouse conditions the effect of drought on the plant-mycorrhizal fungus, soil carbon flux.

By understanding the modification of the carbon fluxes with the different phenological stages of maize, we will identify potential critical phases during plant growth and drought stress.

5) Establish in what way the environmental variables and the soil affect the composition these communities of microorganisms

The information will allow contributions to be made on the abiotic factors and the soil that intervene in the composition of this type of microbial community.

The results obtained during the implementation of this project could be helpful to formulate production and mitigation strategies through the potential use of mycorrhizal fungi associated with this or other types of

crops in Ecuador. In such a way, the knowledge of the microbial communities, their dynamics, colonization of the rhizosphere, and soils subjected to agricultural practices will be crucial to formulating effective and innovative treatments.

The activities in this project will not cause any environmental impact.

25. TRANSFERENCIA Y DIFUSIÓN DE RESULTADOS

The project's execution will enhance the institution's capacity to undertake studies on similar topics in the medium and long term and use the methodological tools and the knowledge acquired in agriculture at the local, regional and international levels. All of the above will be reflected in an increase in the scientific productivity of the institution.

The results obtained from the research project will allow the generation of scientific knowledge that will be transferred through scientific articles and disseminate the products directly to the farmers of the study area.

26. REFERENCIAS BIBLIOGRÁFICAS

1. Rinaudo V, Bàrberi P, Giovannetti M, van der Heijden MG. Mycorrhizal fungi suppress aggressive agricultural weeds. *Plant and Soil*. 2010; 333(1-2): 7-20.
2. Lenzemo V, Kuyper TW, Kropff M, van Ast Av. Field inoculation with arbuscular mycorrhizal fungi reduces *Striga hermonthica* performance on cereal crops and has the potential to contribute to integrated *Striga* management. *Field Crops Research*. 2005; 91(1): 51-61.
3. Bennett EM, Peterson GD, & Gordon LJ. Understanding relationships among multiple ecosystem services. *Ecology letters*; 2009; 12(12), 1394-1404.
4. Augé RM. Water relations, drought, and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*. 2001; 11(1): 3-42
5. Fougnes L, Renciot S, Muller F, Plenchette C, Prin Y, de Faria S, Bouvet J, Sylla SN, Dreyfus B, Bâ A. Arbuscular mycorrhizal colonization and nodulation improve flooding tolerance in *Pterocarpus officinalis* Jacq. Seedlings. *Mycorrhiza*. 2007; 17(3): 159-166.
6. Bunn R, Lekberg Y, Zabinski C. Arbuscular mycorrhizal fungi ameliorate temperature stress in thermophilic plants. *Ecology*. 2009; 90(5): 1378-1388.
7. Hassink, J. Preservation of plant residues in soils differing in unsaturated protective capacity. *Soil Science Society of America Journal*. 1996; 60(2), 487-491.
8. Six J, Feller C, Denef K, Ogle S, de Moraes Sa J C, Albrecht A. Soil organic matter, biota and aggregation in temperate and tropical soils-Effects of no-tillage. *Agronomie*. 2002; 22(7-8), 755-775.
9. Alvarez CR, Fernández P L, & Taboada M. A. Relación de la inestabilidad estructural con el manejo y propiedades de los suelos de la región pampeana. *Ciencia del suelo*. 2012; 30(2), 173-178.
10. Nolivos I, Villacís M, Vázquez RF, Mora DE, Domínguez-Granda L, Hampel H. & Velarde E. Challenges for a sustainable management of Ecuadorian water resources. *Sustainability of Water Quality and Ecology*. 2015; 6, 101-106.
11. Vuille M, Franquist E, Garreaud R, Lavado Casimiro WS, & Cáceres B. Impact of the global warming hiatus on Andean temperature. *Journal of Geophysical Research: Atmospheres*. 2015; 120(9), 3745-3757.
12. Urrutia R, & Vuille M. Climate change projections for the tropical Andes using a regional climate model: Temperature and precipitation simulations for the end of the 21st century. *Journal of Geophysical Research: Atmospheres*. 2009; 114(D2).

13. Pineda, L. E., & Willems, P. Multisite downscaling of seasonal predictions to daily rainfall characteristics over Pacific–Andean River Basins in Ecuador and Peru Using a nonhomogeneous Hidden Markov model. *Journal of Hydrometeorology*. 2016; 17(2), 481-498.
14. Mora DE, & Willems P. Decadal oscillations in rainfall and air temperature in the Paute River Basin—Southern Andes of Ecuador. *Theoretical and Applied Climatology*. 2012; 108(1), 267-282.
15. Federici PR, & Rodolfi G. Rapid shoreline retreat along the Esmeraldas coast, Ecuador: natural and man-induced processes. *Journal of Coastal Conservation*, 2001; 7(2), 163-170.
16. Sklenář P, Kuèerová A, Macková J, et al. Temporal variation of climate in the high-elevation páramo of Antisana, Ecuador. *Geogr Fis e Din Quat*. 2015; 38(1):67–78.
17. Buytaert W, De Bievre B. Water for cities: the impact of climate change and demographic growth in the tropical Andes. *Water Resour Res*. 2012; 48(8):1–13
18. Viviroli D, Dürr HH, Messerli B, et al. Mountains of the world, water towers for humanity: typology, mapping, and global significance. *Water Resour*. 2007; 43(7):1–13
19. Valdés Ramírez, M. El cambio climático y el estado simbiótico de los árboles del bosque. *Revista mexicana de ciencias forestales*. 2011; 2(5), 05-13.
20. Bhoopander G, & Mukerji KG Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions. *Mycorrhiza*. 2004; 14, 307-312.
21. Augé RM. Water relations, drought, and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*. 2003; 11(1): 3-42.
22. Feire-Cruz A, T Ishii, K Kadoya. Effects of arbuscular mycorrhizal fungi on tree growth, leaf water potential, and levels of 1-aminocyclopropane-1-carboxylic acid and ethylene in the roots of papaya under water-stress conditions. *Mycorrhiza*. 2000; 10:121-123.
23. Amerian M R, W S Stewart, H Griffiths. Effect of two species of arbuscular mycorrhizal fungi on growth, assimilation and leaf water relations in maize (*Zea mays*). *Asp. Appl. Biol*. 2001; 63:1-6
24. Ruiz-Lozano JM. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. *New perspectives for molecular studies*. *Mycorrhiza*. 2003; 13:309-317.
25. Malhi Y, Girardin C, Metcalfe DB, Doughty CE, Aragão LE, Rifai S. W. et al. The Global Ecosystems Monitoring network: Monitoring ecosystem productivity and carbon cycling across the tropics. *Biological Conservation*. 2021, 253, 108889.
26. Allen MF, & Allen, EB. Mycorrhizal Mediation of Soil Fertility Amidst Nitrogen Eutrophication and Climate Change. *Mycorrhizal Mediation of Soil*. 2017; 213-231.
27. Hamel C, & Plenchette C. Future Agricultural Practices for Mycorrhiza-Mediated Nutrient Flux. *Mycorrhizal Mediation of Soil*. 2017; 175-186.
28. Abbott L K, & Johnson NC. Introduction : Perspectives on Mycorrhizas and Soil Fertility. *Mycorrhizal Mediation of Soil*. 2017; 93-105.
29. Jansa J. & Treseder KK. Introduction : Mycorrhizas and the Carbon Cycle. *Mycorrhizal Mediation of Soil*. 2017; 343-355.
30. Vigneron N, Radhakrishnan GV, & Delaux P. What have we learned from studying the evolution of the arbuscular mycorrhizal symbiosis ? *Current Opinion in Plant Biology*. 2018; 44, 49–56.
31. Gehring CA. Structure , Moisture , and Salinity. *Mycorrhizal Mediation of Soil*. 2017; 235-240.
32. Casanova-lugo F, Morales-maldonado ER, Biología C, Contaminado S, Kilpel J, Aphalo PJ, et al. Influencia del estiércol composteado y micorriza arbuscular sobre la composición química del suelo y el rendimiento productivo de maíz forrajero (*Zea mays* L .) Influence of composteado manure and arbuscular mycorrhiza on the chemical composition of the s. *Mycorrhizal Mediation of Soil*. 2017; 40(2), 20–29.
33. Pickles BJ, & Simard SW. Mycorrhizal Networks and Forest Resilience to Drought. *Mycorrhizal Mediation of Soil*. 2017; 319-339.

34. Faucon M, Houben D , Reynoird J, Armand R, & Lambers H. Advances and Perspectives to Improve the Phosphorus Availability in Cropping Systems for Agroecological Phosphorus Management. *Advances in Agronomy*. 2015; (134), 51-79.
35. Reza A, Langeroodi S, Osipitan O A, Radicetti E, & Mancinelli R. *Scientia Horticulturae* To what extent arbuscular mycorrhiza can protect chicory (*Cichorium intybus* L .) against drought stress. *Scientia Horticulturae*. 2019; 1-10
36. Buba T, & Yunusa S. Combine Effects of Soil Nutrient Levels and Mycorrhiza Inoculums from Soils under *Parkia Biglobosa* and *Tamarindus Indica* on Chlorophyll Content of Some Cereal and Legume Crops. *Scientific African*. 2020
37. Farias, CP, Carvalho RC, Resende FM, & Azevedo LC. Consortium of five fungal isolates conditioning root growth and arbuscular mycorrhiza in soybean, corn, and sugarcane. *Anais da Academia Brasileira de Ciências*, 2028; 90(4), 3649-3660.
38. Jiménez-Peña N, Sandoval-Villa M, Volke-Haller VH, Pedraza-Santos ME, & Fernández-Herrera E Colonización micorrízica de *Laelia autumnalis* (La Llave & Lex.) Lindl. *Ecosistemas y recursos agropecuarios*. 2018; 5(15), 547-553.
39. Montejo-Martínez D, Casanova-Lugo F, García-Gómez M, Oros-Ortega I, Díaz-Echeverría V, & Morales-Maldonado ER. Respuesta foliar y radical del maíz a la fertilización biológica-química en un suelo Luvisol. *Agronomía Mesoamericana*. 2018; 29(2), 325-341.
40. Pérez-Pérez R, Oudot M, Serrano L, Hernández I, Nápoles M, Sosa D, & Pérez-Martínez S. Rhizospheric rhizobia identification in maize (*Zea mays* L.) plants. *Agronomía Colombiana*. 2019; 37(3), 255-262.
41. Kilpel J, Aphalo PJ, & Lehto T. Temperature affected the formation of arbuscular mycorrhizas and ectomycorrhizas in *Populus angustifolia* seedlings more than a mild drought. 2020; 146, 1-12.
42. Leonardi P, Lotti M, Doati S, Lancellotti E, Amicucci A, & Zambonelli A. Morphological and functional changes in mycelium and mycorrhizas of *Tuber borchii* due to heat stress. *Fungal Ecology*. 2017; 29, 20–29.
43. Lauriano-Barajas J, & Vega-Frutis R. Infectivity and effectivity of commercial and native arbuscular mycorrhizal biofertilizers in seedlings of maize (*Zea mays*). *Botanical Sciences*. 2018; 96(3), 395-404.
44. Moreira FH, Sousa FM, Siqueira JO, Barbosa RH, Barbosa H & Carbone MA. Arbuscular mycorrhizal fungi and colonization stimulant in cotton and maize. *Ciencia Rural*. 2017; 1–8.
45. Ruiz-Lozano J M, Azcón R, & Gomez M. Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: Physiological and nutritional plant responses. *Appl. Environ. Microbiol.* 1995; 61 (2), 456-460.
46. Morgan JAW, Bending GD, & White PJ. Biological costs and benefits to plant-microbe interactions in the rhizosphere. *J. Exp. Bot.* 2005; 56 (417), 1729-1739
47. Tinker PB, Durall DM, & Jones MD. Carbon use efficiency in mycorrhizas: theory and sample calculation. *New Phytol.* 1994; 128(1), 115-122
48. Augé RM, Toler HD, Sams CE, & Nasim G. Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance. *Mycorrhiza*. 2008; 18(3), 115-121.
49. Kuzyakov Y, & Domanski G. Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science*. 2020; 163(4), 421-431.
50. Chica E, Buela L, Valdez A, Villena P, Peña D, & Yarzabal LA. Metagenomic survey of the bacterial communities in the rhizosphere of three Andean tuber crops. *Symbiosis*. 2019; 79(2), 141-150.
51. Mahmoudi N, Slater GF, Fulthorpe RR. Comparison of commercial DNA extraction kits for isolation and purification of bacterial and eukaryotic DNA from PAH-contaminated soils. *Can J Microbiol* 2011; 57, 623–628
52. Shakira G, Atiya A, Mudassar AG, Muhammad I. Metagenomics and its application in soil microbial community studies: biotechnological prospects. *Journal of Animal & Plant Sciences*. 2010; 2, 611- 622.
53. Pérez-madruga Y. Aplicación combinada de quitosano y HMA en el rendimiento de maíz. 2019; 40(4).

54. Wu Z, Hao Z, Zeng Y, Guo L, Huang L, & Chen B. Molecular characterization of microbial communities in the rhizosphere soils and roots of diseased and healthy *Panax notoginseng*. *Antonie van Leeuwenhoek*. 2015; 108(5), 1059-1074.
55. Zhou J, Zang H, Loeppmann S, Gube M, Kuzyakov Y, & Pausch J. Arbuscular mycorrhiza enhances rhizodeposition and reduces the rhizosphere priming effect on the decomposition of soil organic matter. *Soil Biology and Biochemistry*. 2020; 140.

G. ANEXOS

Planilla de anexos del Proyecto

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Número de Archivos: 1

Documentación adicional

Número de archivos: 0



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