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The Influence of the Cultivation of Different Plant Species and their Associated Microorganisms on Soil Aggregate Stability in Topsoil and Subsoil

Masterarbeit
zur Erlangung des akademischen Grades Diplomingenieur

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Christina Anna Orieschnig, September 2018

Abstract

Diese Masterarbeit beschäftigt sich mit dem Einfluss der Kultivierung verschiedener Pflanzenarten auf die Stabilität von Bodenaggregaten und den Schutz von Soil Organic Carbon (organischem Kohlenstoff im Boden) in diesen Aggregaten sowohl in Topsoil (Oberboden) als auch in Subsoil (Unterboden). Die Forschungen für diese Arbeit wurden im Rahmen von zwei größeren Forschungsprojekten - TERRE und TalVeg in Montpellier, Frankreich, am Forschungsinstitut AMAP/INRA durchgeführt. Die grundlegenden Forschungsfragen sind, welche Arten aus welchen Pflanzenfamilien die größte Verbesserung der Aggregatsstabilität und den besten Schutz des Soil Organic Carbon herbeiführen würden und welche Unterschiede sich bei der Kultivierung auf Topsoil und auf Subsoil zeigen würden. Der Versuchsaufbau umfasste die Bepflanzung von Bodenproben mit zwei Arten für TERRE und mit zwölf für TalVeg. Die Kultivierung für beide Projekte wurde in Töpfen durchgeführt - für TalVeg waren diese im Freiland aufgestellt, für TERRE wurden sie in Pflanzenwachstumskammern mit kontrollierten Umweltbedingungen platziert. Für TalVeg dauerte die Wachstumszeit zwei Jahre an, für TERRE sechs Monate. Um die Forschungsfragen zu beantworten wurden Aggregatsstabilitätstests nach der Methodik von Le Bissonnais (1996 & 1997) durchgeführt, sowie vergleichende Messungen der Respirationsrate in Proben mit intakten Aggregaten und Proben, in denen die Aggregate vorher zerstört wurden.

Die Ergebnisse zeigen, dass für TERRE der Kultivierungserfolg auf Topsoil höher ist als auf Subsoil und, dass ein Effekt auf die Stabilität der Aggregate nur in Topsoil beobachtet werden kann - und dort nur im Vergleich zwischen kultivierten Proben und Proben, die als Kontrolle ohne Bepflanzung mitgeführt wurden. Für TalVeg ist die Aggregatstabilität generell in Töpfen am höchsten, die mit Poaceaen bepflanzt wurden, gefolgt von Fabaceaen. In TERRE kann eine signifikante Differenz zwischen Proben mit intakten und zerstörten Aggregaten nur in der Aggregatsgrößenklasse von 3-0.2 mm festgestellt werden. Der Unterschied in der Respirationsrate zeigt keine signifikante Variation in Abhängigkeit von der Bepflanzung. In TalVeg ist der Unterschied der Respirationsrate zwischen Proben mit ganzen und zerstörten Aggregaten in keiner der untersuchten Aggregatsgrößenklassen signifikant. Allerdings zeigt die Differenz, die beobachtet werden konnte, eine signifikante Abhängigkeit von der Bepflanzung mit mit verschiedenen Pflanzenfamilien und Arten. Hier zeigen die Fabaceaen die größte positive Auswirkung auf den Schutz von Kohlenstoff in den Bodenaggregaten. Die Schlüsse, die man aus diesen Ergebnissen ziehen kann, sind, dass die Kultivierungsdauer einen großen Einfluss auf die Resultate von Aggregatsstabilitätstests und Respirationsexperimenten hat. Für TERRE war die Kultivierungsperiode anscheinend zu kurz, als dass sich differenzierte Effekte ausbilden hätten konnten. Die Resultate für TalVeg zeigen, dass die Poaceaen auf längere Sicht gesehen den größten Effekt auf Aggregatstabilität haben, während die Fabaceaen den höchsten Einfluss auf den Kohlenstoffschutz haben.

Abstract

This MSc thesis focuses on the influence of the cultivation of different species on the stability of soil aggregates and the protection of Soil Organic Carbon in these aggregates in topsoil and subsoil. Research was conducted in the frame of two larger projects - TERRE and TalVeg at INRA/AMAP Montpellier. The basic research questions are, which species from which plant families would have the highest impact on aggregate stability and the protection of Soil Organic Carbon and what the differences of the cultivation of the same species on topsoil and subsoil that had been brought to the surface would be. The experimental setup involved the cultivation of two species for TERRE and twelve for TalVeg. Cultivation for both projects was carried out in pots, though for TalVeg these were located in the open air while for TERRE they were kept in growth chambers with a controlled environment. For TalVeg the cultivation period lasted two years, for TERRE six months. In order to answer the research questions, aggregate stability tests according to Le Bissonnais (1996 & 1997) were carried out as well as comparative measurements of the respiration rate in samples with the aggregates intact and samples in which the aggregates were destroyed.

The results show that cultivation success is far higher on topsoil than on subsoil and that an effect on the stability of aggregates can only be observed in topsoil and there only between the control treatment with bare soil and the cultivated soil. For TalVeg, aggregate stability is generally highest in pots that have been cultivated with Poaceans, followed by Fabaceans. A significant difference between respiration in samples with intact and destroyed aggregates can only be observed in the aggregate fraction 3-0.2 mm. The increment in respiration rate shows no significant variation in relation to the cultivation treatment applied. In TalVeg, the respiration experiments reveal no significant difference between samples with whole and crushed aggregates in any of the fractions. The increment in respiration rate, however, shows a significant difference between the cultivation with different plant families and species. Here, the Fabacean species exhibit the largest positive influence in the protection of carbon in soil aggregates. The conclusions to be drawn from these results are that the length of the cultivation period has a major influence on the results of both aggregate stability and carbon protection. It appears that for TERRE the period was too short for differentiated effects to entirely emerge. The results for TalVeg demonstrate that Poacean species might in the long run have the largest effect on aggregate stability while Fabaceans have the highest impact on carbon protection.

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List of Abbreviations

AMAP	Unité botanique et modélisation de l'architecture des plantes et des végétations
ANOVA	Analysis of variance
CEFE	Centre d'Ecologie Fonctionnelle et Evolutive
EC	European Commission
EMSINK	EMbankments as a carbon SINK
ESP	Exchangeable Sodium Percentage
INRA	Institut National de la Recherche Agronomique
ITN	Innovative Training Networks
MSCA	Marie Sklodowska-Curie actions
MWD	Mean Weight Diameter
POM	Particulate Organic Matter
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TERRE	Training Engineers and Researchers to Rethink geotechnical Engineering for a low carbon future

Contents

Chapter 1

Introduction

Soil aggregates are an aspect of soil structural properties important to eco-engineering because they have a major influence on the erodibility of soil, its infiltration rates, water retention capacity as well as nutrient cycling. All of these factors carry great importance for issues such as erosion mitigation or the stabilization of slopes, as well as the choice of which species to utilize for various eco-engineering goals (Bronick 2005).The purpose of this Master thesis is to investigate the stability of soil aggregates and how it is influenced by the cultivation of various species on the soil and the microbial activity in the soil. This is an important aspect for practical purposes in eco-engineering - for often in the course of projects the question arises which species should be used to optimally influence soil properties and stability.

Research for this thesis was carried out at the Unité botanique et modélisation de l'architecture des plantes et des végétations (AMAP) of the Institut National de la Recherche Agronomique (INRA) in Montpellier, France, in the framework of two larger projects involving aspects of soil aggregation - TERRE and Talveg. The first of these two projects, TERRE is a Marie Curie research projects involving European researchers of geotechnics and soil sciences and aims to raise awareness of sustainability in geotechnical engineering, with a particular focus on carbon sequestration. The second project, Talveg, is a nation-wide French eco-engineering project in which a tool for optimal development of bio-technosols is currently being evaluated. The contributions of AMAP/INRA Montpellier to these projects as well as the data used for this thesis and the experiments carried out will be described in greater detail further on.

This thesis first gives an outline of the projects in the frame of which the research was conducted to relate the research questions and results to a larger eco-engineering context. Subsequently, the underlying hypotheses will be presented and a comprehensive summary of the theoretical background will be given, exploring the morphology of soil aggregates and their importance, as well as the mechanisms by which they are broken down and the factors influencing the resistance to such a breakdown. Next, the experimental setup and the methods used will be described, followed by a presentation and discussion of the results.

1.1 The Projects

As outlined in the introduction, the research for this Master thesis was conducted at the Institut National de la Recherche Agronomique in Montpellier, France, in the frame of the two large-scale projects - TERRE and Talveg. In this chapter, the two projects will be described in some detail to illustrate the relevance of the research conducted for this thesis to the larger context of eco-engineering, and by extension environmental engineering. Both projects described below deal to some extent with the use of plants, specifically for the greening of and erosion-prevention on embankments.

1.1.1 TERRE

The acronym "TERRE" stands for "Training Engineers and Researchers to Rethink geotechnical Engineering for a low carbon future", which concisely summarizes the project's aim. The goal of the project is to increase the carbon-efficiency of geotechnical infrastructure to improve the competitiveness of the European construction industry. It aims to expand the generally mechanistic view of geotechnics to include more nature-centric aspects by also considering the direct and indirect effects of biological activity and gas-liquid interactions. In order to achieve this, TERRE set up a Europe-wide, interdisciplinary PhD programme with a collaboration of eleven universities and research centres and three SMEs (small and medium-sized enterprises) hosting 15 Early-Stage Research Fellows (terre-etn.com).

TERRE is a Marie Sklodowska-Curie Innovative Training Networks (ITN-ETN) project, which belongs to the Marie Sklodowska-Curie actions (MSCA) (European Commission b). These, in turn, are a part of the work programme for Horizon 2020, a Research and Innovation Programme by the European Union to boost the Union's economy and secure its global competitiveness in the frame of Europe 2020 (European Commission a).

For this thesis, data was gathered from experiments conducted by TERRE PhD student Lorenzo Rossi at AMAP/INRA Montpellier. (The precise name of his project is EMSINK (EMbankments as a carbon SINK), however, as it was generally referred to as "TERRE" during work in the laboratory, this moniker shall be kept throughout this thesis.) The focus of Rossi's research is to compare the use of organic topsoil and mineral subsoil in geotechnical projects and the implications that the use of these two different soils have for eco-engineering measures such as revegetation, as well as for carbon emissions. In order to investigate the differences in carbon fluxes between the two kinds of soil, he cultivated a total of 36 samples of topsoil and subsoil with either *Medicago sativa* or *Lolium perenne* in a controlled environment at Ecotron in Montferrier (http://www.ecotron.cnrs.fr), with a C^{13} enriched atmosphere during six months. The data used for this thesis was gathered during the evaluation phase of this experiment.

The basic premise of Rossi's thesis is that subsoil has a lower initial carbon content compared to topsoil. According to the carbon saturation theory (Six et al. 2002), this would mean that subsoil has a higher potential for short-term carbon sequestration. The reason for this is that it has lower levels of already established organo-mineral complexes on its active surfaces

(aggregate surfaces, clay and silt) and thus a higher potential for establishing stable bonds with carbon. Rossi hypothesises that using subsoil will increase the short-term carbon sequestration. Currently, it is the norm in geotechnics to cover the subsoil that is used to construct e.g. embankments with a layer of fertile topsoil before revegetation. Rossi points out that this is not sustainable because not only is fertile soil a finite resources, which could be used in agriculture instead, but also because of the economic and environmental costs of the transport of the soil. Therefore, Rossi postulates that planting directly on the subsoil used to build the infrastructures would be more economical and sustainable and might increase carbon sequestration.

1.1.2 TalVeg

TalVeg is a decision support system for the selection of plants for bio-technosols. It was developed by the French company Valorhiz, which specializes in bio-technosols, revegetation and soil remediation projects as well as agriculture and green areas. It consists of databases of plants, soils and symbiotic microorganisms, as well as of mathematical models simulating the dynamics of plants and water and, finally, models computing biodiversity and ecosystem functions. The aim of TalVeg is to optimize the vegetation used on geotechnical engineering structures associated with infrastructure and industrial sites and to tailor the plant communities according to the specific needs of each individual case (Tagourdeau et al. 2016, Valorhiz 2017).

Currently, TalVeg is in its evaluation and calibration phase and in collaboration with several institutions, INRA among them, experiments in the field and the laboratory are being conducted to optimize the decision support system.

At INRA, ex-situ experiments are carried out to investigate the effect of plant biodiversity on microbial communities in the soil and on the processes of aggregate stabilisation in soils that need to be remediated. The aim is to establish a link between the properties of roots and the stability of soil aggregates. Overall, 12 species from 5 families of plants that are commonly used to revegetate embankments in southern regions are investigated.

1.2 Hypotheses and Research Questions

This chapter gives a brief outline of the research questions and hypotheses that form the basis of this thesis.

1.2.1 Research Questions

The main research questions of this thesis are the following:

- What are the species with the largest effect on aggregate stability and carbon protection?

- What are the differences regarding aggregate stability between the utilization of topsoil and subsoil for cultivation?

These two questions have a direct practical impact for geotechnical projects and eco-engineering. As mentioned above already, the knowledge which species are most effective in increasing aggregate stability facilitates the choice of vegetation for the greening of, for example, embankments that is targeted at increasing the erosion resistance of recently finished projects. Furthermore, as is outlined in the project description of TERRE, the knowledge of the differences between the use of topsoil vs. subsoil for cultivation is also vital for practical purposes. If the results show that the use of subsoil entails similar values for aggregate stability after cultivation with the same species under the same conditions, the frequency and extent of the use of topsoil in geotechnical projects could be reduced. This in turn would mean an improvement from the point of view of sustainability as the very fertile topsoil could be put to use elsewhere (for example in agriculture) and also it would not have to be transported to the construction site of the geotechnical project.

1.2.2 Hypotheses

There are two main hypotheses that were formulated in response to the main research questions.

- Species that are members of the Fabacean plant family have a higher positive impact on aggregate stability and carbon protection than others.
- Cultivation of plants on subsoil will have a higher relative impact on aggregate stability than cultivation on topsoil.

The first of these hypotheses is based the assumption that Fabacean species can have a larger stabilising influence on soil than other species due to the fact that symbiotic bacteria associated with their roots allow them to fixate Nitrogen from the air, which enhances their growth and gives them a competitive edge in comparison to other species. Not only does this mean that their root growth is expected to be higher and (and thus reaching farther into the soil that is meant to be stabilised), it also entails higher microbial activity in the rhizosphere of these plants. As will be explained below, higher microbial activity - and thus the incidence of secretion products from microorganisms as well as dead and decomposing microorganisms - enhance the formation and stability of aggregates. Also a higher density of roots and thus larger amounts of root exudates etc. have a positive effect on aggregate stability.

The second of these hypotheses relates to the fact that the initial level of organic material in subsoil is far lower than in topsoil. As the explanation given in the subsequent chapter will expostulate, organic material is crucial for the formation and stability of aggregates. Therefore, the initial aggregate stability values in topsoil can be expected to be far higher than in subsoil. The cultivation of plants entails an increase in organic material in the soil - and the import of root exudates into the soil as well as an increase in microbial activity in the rhizosphere.

Because of the lower initial level of organic material in subsoil, it can be hypothesized that the effect of cultivation and the sudden increase in organic material will be much more pronounced in this kind of soil than in topsoil.

Chapter 2

Theoretical Background

In this chapter, the theoretical background of soil aggregation will be described in detail, as it is important to be fully aware of the mechanisms that underly the process investigated in order to successfully carry out the experiments, analyze the data and interpret the results. First, an explanation of why the stability of soil aggregates is an important issue worth being researched is given, followed by a general introduction into the current state of research on the structure and composition of soil aggregates. Subsequently, the various breakdown mechanisms that threaten the stability of soil aggregates will be explained and the factors increasing the stability of aggregates, thus counteracting the breakdown mechanisms, will be elaborated. The descriptions given below are of a general nature and are meant to provide an overview of the subject matter.

2.1 The Importance of Soil Aggregate Stability

The size and distribution of soil aggregates is a vital aspect of soil texture. It has a critical influence on soil functions such as the ease with which water and air can move through the soil, the resistance of soil to erosion, its capacity for nutrient retention and release, soil crusting, root penetration, crop yield and soil biological activity (Bronick & Lal 2005 ,Chen et al. 2017). Bronick and Lal (2005) point out that a favourable soil structure and high aggregate stability are important points for the increase of soil fertility and agronomic productivity, as they enhance porosity and decrease erodibility. All of this means that the ecosystem services provided by soils are influenced heavily by soil structure. Thus, aggregate stability also has a major economic importance. Jónsson, Davíðsdóttir and Nikolaidis (2017) point out the importance of including these soil ecosystem services into valuation of ecosystem services, as the functionality of all terrestrial ecosystems depends on the integrity of the soil on which they are based.

One aspect of the importance of soil aggregates for soil ecosystem services deserves to be underlined in particular in the context of this thesis. As mentioned above, the size, distribution and stability of aggregates can have a major influence on the rate of nutrient retention and

release in any given soil. This includes carbon sequestration and release, a process which is of great importance for global climate change and associated processes. Currently, global Soil Organic Carbon (SOC) stocks are estimated to lie at ca. 100.34 Pg (Yigini, Montanarella, and Panagos 2017), a large and important reservoir for the global carbon cycle (Wei et al. 2016). Considering the important role of aggregates in the retention and release of nutrients, closer research into the nature of aggregate influence on carbon storage is vital.

Scientifically, aggregate stability also carries a particular interest. It can be measured with relative ease - although there are several disputes over the best method to do so (Almajmaie at al. 2017) - and may serve as valuable input data during modelling. Already Barthès and Roose (2002) suggested that aggregate stability may be used as a suitable indicator for soil erodibility during modelling. Soil aggregate stability has been used in erosion prediction equations in order to calculate the soil erodibility factor. Xiao et al. (2017 a) developed and tested two equations for the estimation of rill and interrill erosion, which no longer use erodibility factors but rather an Aggregate Stability Index A_s and have found it reliable.

Thus it can be summarized that the importance of soil aggregate stability lies not only in its direct effect on various soil functions and, by extension, in the greater ecological and economic context of these functions, but also in its scientific value as an indicator for modelling purposes. With their importance now sufficiently established, it is now necessary to look more closely at the morphology and formation of soil aggregates.

2.2 Soil Aggregate Structure and Formation

Soil aggregates are the basic units of soil structure. They are composed of primary particles and the agents binding these together. The primary particles may include mineral particles, soil organic matter or smaller soil aggregates, depending on the type of aggregate. Binding agents may consist of a combination of soil organic carbon, crystalline and amorphous metal oxides and hydroxides, bridges formed by metal ions between mineral and organo-mineral particles (Bronick & Lal 2005). Usually, a combination of binding mechanisms occurs, though depending on the soil type under scrutiny, one or another may be dominant. However, it has to be mentioned that the exact mechanisms of aggregate formation under different circumstances and in different kinds of soil are not yet fully understood and that further research is required.

Usually, soil aggregates are differentiated according to their size and a distinction is made between microaggregates (those with a mean diameter of less than 0.25mm) and macroaggregates (larger than 0.25 mm). However, the differences between microaggregates and macroaggregates extend to more than their relative sizes - they also differ in their components. Microaggregates consist of mineral primary particles and organic debris. Macroaggregates, on the other hand, are made up of microaggregates as well as particulate organic matter (Wang at al. 2017). Furthermore, micro- and macroaggregates also differ in their properties. For example, Li at al. (2017) found that larger soil aggregates are associated with lower inter-aggregate tensile strength, while microaggregates are much less susceptible to external influences.

The process of aggregate formation is a complex one and is still not fully understood (Li at al.2017), with various concepts proposed by different researchers. According to the aggregate hierarchy concept proposed by Tisdall and Oades (1982), microaggregates form first and later develop into macroaggregates. However, this hierarchical concept may not always be valid - it has for example been stipulated that it might not apply in cases where kaolinit is the dominant clay in the soil (Zhao 2017). Also Li at al. (2017) observed a process of primary macroaggregation, followed by secondary microaggregation when they observed changes in aggregate size mass distribution and aggregate carbon content in two years during an eight-year field experiment. This experiment was conducted to determine how to speed up soil development and restoration with different agricultural practices, but observations regarding aggregate formation were also made. It was furthermore found that soil organic matter is the main constituent that binds mineral particles together into larger-sized aggregates.

Bronick and Lal (2005) present a concise overview of the many factors influencing the process of soil aggregation (see Figure 2.1). According to this paper, the complex dynamics of aggregation are the result of the interaction of many factors, including environmental factors, aspects of soil management, the influence of plants and inherent properties of the respective soils (such as mineral composition, ion exchange capacity, the concentration of Soil Organic Carbon (SOC) etc.), as well as microbial activities and water availability. As depicted in Figure 2.1, this palette of factors can be summarized into groups of exogenous factors, pedogenic processes, soil properties and anthropogenic perturbations, all of which, of course, interact with one another.

Bronick and Lal (2005) furthermore state that different bonding mechanisms dominate depending not only on the initial conditions of the soil, but also at different stages of aggregate formation. Microaggregates are formed by organic molecules attached to clay and polyvalent cations and can then conjoin to form macroaggregates. Alternatively, macroaggregates can form around a nucleus of particulate organic matter (POM). When POM decomposes, microorganisms release exudates that can enhance aggregate formation.

As can be seen from this, the factors influencing the development of soil aggregates are varied and complex and need to be studied in detail. However, it is equally important to understand the mechanisms by which aggregates are broken down into smaller pieces, thus threatening the integrity of soil structure.

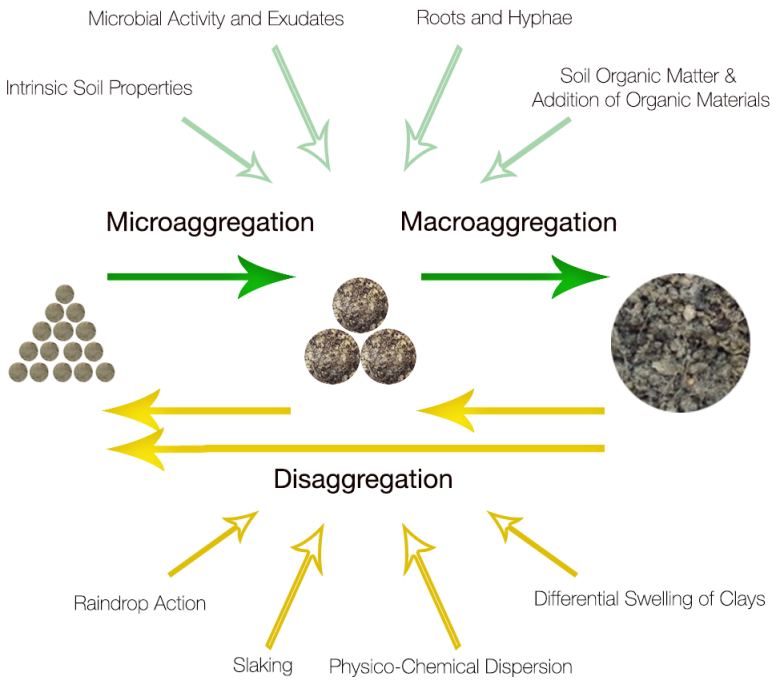

Figure 2.1: A diagram of factors influencing the formation and destruction of soil aggregates

2.3 Breakdown Mechanisms

The breakdown of soil aggregates and thus the deterioration of soil structure is an elemental part of soil erosion. Soil erosion in general can be caused by both wind and water erosion, although the focus of this thesis lies on the latter element - which is why the following descriptions of aggregate breakdown mechanisms will be limited to mechanisms involving water. Generally it can be noted that soil erosion by water on a larger scale can be differentiated into rill and interrill (or sheet) erosion. The former refers to erosion by rivulets of water that form during rainfall that run in discernible channels, the latter to the detachment of soil particles by rain splash and transport by shallow flow (Kutilék and Nielsen 2015, Barthès and Roose 2002). Once the particles are detached by raindrop impact, they can be transported by the surface flow. Interril erosion has been identified as the dominant process (Zhang and Wang 2017).

Concerning the breakdown of soil aggregates, there are various mechanisms by which aggregates can be broken up into smaller pieces. The most important of these are breakdown by raindrop action, by slaking, physiochemical dispersion and differential swelling of clays. These four mechanisms will be described in detail in this chapter, as they also form the basis of various aggregate stability tests that have been developed by researchers, among them also for the method by Le Bissonnais (1996 &1997), which was utilized for this thesis.

2.3.1 Raindrop Action

Soil aggregate breakdown by raindrop action refers to the mechanical dispersion of aggregates because of the kinetic energy of raindrops as they impact onto the soil. As already mentioned above, raindrop impact is the most important driving force for interrill erosion. The severity of the erosion depends on the size of kinetic energy during a rainfall event, which is determined by the number, size and velocity of raindrops (Li at al. 2018). Fu at al. (2017) found a linear correlation between raindrop size and the amount of splash erosion for two Loess soils from the Shanxi Province in China.

Apart from detaching soil materials, it also increases the sediment transport of rill erosion (Zhang and wang 2017). When sediment fragments become available for transport and deposition through the disintegration of aggregates, fine sediment particles and microaggregates can clog the pores still existing in the soil, a process known as surface sealing. As a result, the infiltration capacity is decreased providing a positive feedback loop for surface runoff and erosion (Shi at al. 2017). In an investigation using synchrotron based X-ray micro-computed tomography (SR- micro-CT), Li at al. (2018) furthermore found that this can lead to increased slaking, a process described in more detail below, thus again providing positive feedback for the breakdown of soil aggregates.

2.3.2 Slaking

Slaking is the process in which aggregates are broken down because of the compression by entrapped air during the wetting of soil. When water infiltrates soil through pores, this often happens faster than it is possible for the air, which formerly filled those pores, to escape. As a result, this air is compressed and exerts pressure at the surrounding aggregates, which break down, resulting in an alteration in the distribution of particle sizes.

Therefore, the impact of slaking is determined by the major factors - the nature of soil porosity and the rate at which wetting occurs Li at al. (2017). The faster wetting occurs, the more air is likely to be entrapped. Equally, the harder the configuration of the porous space in the soil makes it for air to escape during wetting, the higher the magnitude of slaking is going to be. Wei at al. (2016) found that in many soils, aggregate breakdown may be induced exclusively by slaking, for example when initially dry aggregates undergo rapid wetting, or when soils consist of elementary particles or microaggregates that are smaller than 100 micrometers. Also Almajmaie (2017) found that the entrapment of air is one of the main driving forces of aggregate breakdown during rapid wetting.

2.3.3 Physiochemical Dispersion

The mechanism of aggregate breakdown by physiochemical dispersion consists in the reduction of bonding forces when soil is wetted. As mentioned above, it is, among other things, ion

bridges that act as an attractive force between the particles of which aggregates are composed. When soil is wetted, these attractive forces are diminished.

According to Le Bissonnais (1996), the rate of aggregate breakdown by physiochemical dispersion depends on the electrolyte concentration of the soil solution, especially on the exchangeable sodium percentage (ESP), the electrolyte concentration of the water and also on parallel disturbance by the other three mechanisms described above. It is important to mention that the products of physiochemical dispersion are largely primary particles, and not, as in the other three processes, both microaggregates and primary particles.

2.3.4 Differential Swelling of Clays

Finally, aggregate breakdown can also be precipitated by the differential swelling and shrinking of clay particles in aggregates during the wetting and drying of soil. When the particles grow in size and subsequently shrink again microcracks may appear in the aggregates, facilitating their breakdown. According to Le Bissonnais (1996), the main factors influencing the rate of breakdown by differential swelling are the same as those which influence slaking - the difference between the two mechanisms being the clay content of the soil in which they occur. The higher the clay content of the soil, the greater the impact of the process of differential swelling.

2.4 Factors influencing Soil Aggregate Formation and Stability

After the description of the various mechanisms of soil aggregate breakdown in the preceding section, this section is dedicated to the exposition of the factors positively influencing the stability of these aggregates. As stated in the introduction to this chapter , the description of these factors given here is a very general one and closer details will be provided in the discussion section of this thesis. In general it must be noted that the factors described below vary in their importance both with respect to their stabilizing influence in micro- and macroaggregates and in different soils and conditions. For example, Tisdall and Oades (1982) note that while the stability of macroaggregates is influenced strongly by roots and fungal hyphae - and can thus be impacted heavily by the management of the soil - microaggregates are more dependent on persistent organic binding agents, which are often characteristic of the soil and thus more independent of management practices. Furthermore, as already mentioned, the exact mechanisms of soil aggregation in different conditions and soils are not yet exactly know, meaning that more research is needed to understand the relative importance of the factors described below.

2.4.1 Soil Properties and Soil Type

As described above, the type of soil under investigation as well as the original properties of said soil have a major influence on the stability of the aggregates formed. Depending on the elements to be found in the primary particles, the formation of ionic bridges is more or less likely. Furthermore, the level of organic matter content in the soil as well as conditions regarding the water regime in the soil influence the formation of organo-mineral complexes. That is the reason why the mechanisms and dynamics of the formation and break-down of soil aggregates vary considerably between the soil types and why studies conducted on the Loess Plateau of China might not be applicable, for example, in more clayey European soils. One factor that has been under close consideration as having a notable influence on the stability of soil aggregates is the level of carbonates in the soil. Soils formed on calcerous geologies behave differently with regard to aggregation than other kinds of soil do (Rillig at al. 2003).

2.4.2 Microbial Activity and Exudates

Another factor that has been shown to have an important influence on the process of aggregation is the activity of soil microbes and the substances they secrete into their environment. In recent years, environmental microbiology and microbial ecology have emphatically underlined the importance of soil microbes for a multitude of soil processes. For the formation of soil aggregates, soil microbes are important in as far as they exude certain substances, many of them rich in organic carbon, into their immediate environments - be it to create the microconditions neccessary for the formation of a biofilm or to aid them in the breakdown of nutrients. These substances, however, can also be conductive towards the formation of aggregates by functioning as binding agents holding primary particles in place. Furthermore, dead microorganisms can also function as binding agents in the process of their decomposition. Therefore, a higher microbial activity in the soil can be associated with higher rates of aggregation and increased aggregate stability (Bernard et al. 2007, Blankenship et al. 2017, Tang et al. 2011).

2.4.3 Roots and Hyphae

As already mentioned above, roots and the hyphae of fungi have a major stabilizing influence, predominantly on macroaggregates. The ways in which they exert that influence are varied.

To begin with, they bind soil particles together physically. Hyphae, for example, have been observed to enmesh soil particles by forming a sort of net, even if not all soil particles are in contact with the hyphae. Another mechanism is hyphae cross-linking, though this has been noted to be likely limited to coarse sandy soils and macro-aggregates - limitations enforced by the tensile forces on the hyphae through the weight of particles. It has to be noted that this physical stabilizing influence is exerted not only by the living roots of plants or the hyphae of living fungi. Dead roots and fungi provide excellent preconditions for the growth of saprophytic hyphae, which also have a positive effect on aggregate stability (Degens 1997).

Furthermore, roots and hyphae also positively affect aggregation through the substances they exudate in order to facilitate their own nutrient uptake or to ease their growth process through the soil. Reid and Goss (1981) for example attributed the differences in the effect on aggregate stability between the cultivation of different species to the different amounts in labile organic carbon input by the roots of the different species.

There are different factors that affect the magnitude of the influence of roots and hyphae on the formation of aggregates. First of all, there is the location of hyphae and roots in the soil to consider. Secondly, their persistence - especially in the case of fungal hyphae - has a considerable influence on the way in which they stabilize aggregates. For example, aggregates formed by some hyphae were found to be stable after the death and composition of the host plant roots. The same was not found to be true for saprophytic hyphae. Thirdly, also the length of hyphae and roots in the soil can be considered of major importance to the magnitude of the positive influence on aggregation. The denser the network of roots and hyphae in the soil, the higher the positive effect on aggregation is. This is valid for both the physical binding mechanisms and the chemical ones through the root exudates. As Shen (2016) notes, external hyphae can represent up to 15% of the soil organic carbon of a given soil sample.

2.4.4 Soil Organic Matter (SOM) and Addition of Organic Materials

Another factor influencing the stability of soil aggregates that has to be mentioned in more detail is Soil Organic Matter, or SOM. SOM is composed of the remains of dead plants and animals as well as microorganisms and exudates from roots and secretions from microbial activity. Thus, soil organic matter has a close relation to the roots and hyphae as discussed in the previous section. Soil organic carbon (SOC) is one of the principal components of SOM, although the exact ratio of nutrients can vary considerably according to the source of the SOM. SOM and its components can form stable organo-mineral complexes that enhance aggregation (Zhao at al. 2017).

SOM has been studied extensively in relation to its effect on aggregate stability, as it is a factor that can be easily influenced through human activity such as the addition of manure. Due to the important nature of aggregate stability for soil structure and soil health - and thus the fertility of soil - it has been studied closely in relation to agriculture. For example, Wang at al. (2017) describe a long-term study of manure treatment of soil over 23 years. It was found that the treatment affected mostly macroaggregates. In manured soil, a greater proportion of macroaggregates and a generally higher SOC content, microbial biomass and enzyme activity was found. Also Zhao at al. (2017) conducted a comparative study on the stability of aggregates and the distribution of aggregate sizes in red soils under different land uses. This study reached the conclusion that the effects of land use on soil aggregates are driven principally by the different input of SOM into the soil through the different land uses.

A major point that has to be considered with a view to the magnitude of the effect of SOM on aggregate stability is not only its concentration in the soil, but also the dynamics of its decomposition. Shi at al. (2017) emphasize the importance of SOM as a key factor determining aggregate stability with regard to the effectiveness of agricultural amendments

such as the addition of wheat straw to the soil or the use of green manure from intercrops. This study points out that the effectiveness of the aforementioned amendments is determined, in turn, by factors determining decomposition, such as the C:N ratio and glucose, cellulose and lignin properties. After testing the effect of organic residues on disaggregation during heavy rainfall events, it reaches the conclusion that the amendments were effective in enhancing soil aggregate stability. Also Abiven (2007 a), investigating the effects of different aggregate binding agents, showed that the intrinsic decomposability of organic substances influences the dynamics of aggregation and the stability of aggregates. This study concludes that easily decomposed substances were influenced more heavily by the content of microbial exudates while recalcitrant materials were influenced largely by fungal hyphae.

With regard to the direct relationship between aggregates and SOM in general and SOC in particular, it has to be noted that aggregates are not only aided in their formation by these materials, they also protect them from metabolisation by microorganisms. Considering that it reduces the respiration in the soil and thus the release of CO_2 into the air, the protection of SOC by aggregates can be seen as an important ecosystem service. However, not all kinds aggregates offer the same level of protection. As Wang at al. (2017) note, the availability of soil organic matter varies between microaggregates and macroaggregates. The SOC in microaggregates is usually less accessible for decomposition, while macroaggregates occlude more particulate organic C and have a higher saturation of SOC. Chen at al. (2017) observed that macroaggregates have a greater storage capacity for SOC, while SOC in microaggregates is older than in macroaggregates, which implies that the storage in these kinds of aggregates is more permanent.

Chapter 3

Methods and Materials

In this chapter a summary of the methods and materials used in gathering the data for this thesis is provided. First, an overview of the setup for the experiments carried out for the projects TERRE and TalVeg will be be provided, followed by a description of the sampling process and, finally, an exposition of the methodology of the tests that were performed.

3.1 Experimental Setup

This section provides a short overview of the setup of experiments of both the TERRE and the TalVeg project as well as a more detailed description of the treatment of the samples in preparation for the tests carried out, which are described in Chapter 3.3.

3.1.1 TERRE

For the TERRE project, experiments were carried out by PhD student Lorenzo Rossi. The aim was to compare the use of topsoil versus subsoil for eco-engineering purposes in geotechnics with respect to the effects on the carbon cycle. In particular, the input of carbon, soil respiration, microbiological activity, aggregate formation and carbon protection as well as the transfer of carbon and its protection in different soil fractions were to be investigated.

To this end, topsoil and subsoil samples were taken from one soil profile in Pisciotta (Salerno), Italy. The reason for the choice of this particular soil lay in its high clay content (to facilitate organomineral complexation) and the absence of carbonates. Topsoil was collected from a depth of 0-30 cm while subsoil was taken at a depth of 1.1 - 1.4 m. Then, in two replicates, 18 topsoil and subsoil samples each were placed in 20 by 20 x 20 cm plastic pots and either planted with six individuals of *Medicago sativa* or *Lolium perenne*, or left bare (six replicates each). Thus, a total of 36 pots of subsoil and topsoil samples planted with *Medicago sativa*, *Lolium perenne*, or left bare was prepared (see Figure 3.3). These pots were

then placed in three growth chambers at Ecotron in Montferrier, France, and labelled with C^{13} for six months from September 2017 to March 2018. The chambers (shown in Figures 3.1 and 3.2) also allowed for an exact control of environmental conditions - the plants were grown at a temperature of 21°C and an air humidity of 80%, experienced a daily photoperiod of 12 hours and the soils was kept at a level of 45 - 45% of their water holding capacity to avoid leaching and loss of carbon via lisciviation. After six months, the pots were removed from the chambers and the soil was prepared for analysis.

Figure 3.1: Incubation chambers for the TERRE experiments at Ecotron in Montferrier

Figure 3.2: TERRE experimental pots in the incubation chamber at Ecotron in Montferrier

Figure 3.3: TERRE experimental pots during removal from Ecotron to be transported to AMAP for testing

3.1.2 TalVeg

Experiments for the TalVeg project at INRA Montpellier have been ongoing for two years. The first year, species typically used for planting of bare earth were cultivated in monoculture in disturbed soil in order to analyze the connection between plant parameters and aggregate stability. The cultivation was carried out at the Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), in 78 pots of 70 cm x 70 cm x 25 cm, tilted at an angle of 20°C to facilitate runoff (see Figure 3.4). The plants were cultivated from seeds, which were inserted in diagonal rows with 5 cm between them. As the experimental site is out in the open, the plants were subject to typical mediterranean weather conditions, but they were also irrigated regularly and cut.

The soil used for cultivation was taken from a shallow depth from the Cevennes, a mountain range in the South of France. The reason it was taken from there was that soil from this region contains a very low level of carbonates, which could otherwise influence analyses.

The species chosen for cultivation are listed in Table 3.1 They were selected because they are species that are typically used for the greening of embankments in the South of France. The selection was made in cooperation with the French company Valorhiz, which co-finances the TalVeg project to investigate which species can be used in seed mixtures to optimize ecosystem services on embankments that are greened.

Figure 3.4: Experimental site of TalVeg at the Centre d'Ecologie Fonctionnelle et Evolutive in Montpellier

Table 3.1: Legend of abbreviations for plant species used for cultivation

Abbreviation	Species	Plant Family
BS	Bare Soil	
Be	Bromus erectus	Poacea
Dg	Dactylis glomerata	Poacea
Lp	Lolium perenne	Poacea
Lc	Lotus corniculatus	Fabaceae
Ms	Medicago sativa	Fabaceae
Ov	Onobrychis viciifolia	Fabaceae
Pl	Plantago lanceolata	Plantaginaceae
Pp	Poa pratense	Poacea
Ps	Sanguisorba minor	Rosaceae
Tp	Trifolium pratense	Fabaceae
Tr	Trifolium repens	Fabaceae

In the first year, root traits both physical and chemical were investigated and DNA extracted from the soil was sequenced. Furthermore, carbon fractioning was carried out and aggregate stability tests performed. Moreover, rainfall experiments were carried out to investigate the erosion stability of the soil cultivated with the different species. Furthermore, secondary parameters such as aggregate stability, root density etc. were investigated. For the second year, eighteen new treatments, this time with species mixes were installed alongside the monospecific pots. These mixes were based on the results of the monospecific pots from the first year and are supposed to maximise erosion resistance. In spring 2018, rainfall experiments were carried out for these polyspecific pots as well.

3.2 Sampling Methodology

This section provides a short summary of the way in which the samples analyzed in later tests were taken from the experimental setup described above. This may be relevant for the interpretation of data gained in the tests that will be outlined in the following section.

3.2.1 TERRE

For the TERRE experiment, the entire pot with all the earth and plants it contained was sampled. First, a line was drawn on the surface of the pot, dividing it in two halves with three plant specimens each. One half was used for the analysis of soil properties, the other for the analysis of all the factors pertaining to the biomass. On the soil half, the biomass was cut, the litter was collected and both were bagged and frozen for later reference. Then, the dimensions of the sample were measured and noted to later calculate its volume, and the sample was cut in half using a large knife (see Figure 3.6). The soil half of the sample was removed manually and left to air-dry for five to seven days in small aluminium containers at the laboratory. Special care was taken to separate the top third of the soil from the lower two thirds, as it was reasoned that the effects of the plants would be seen most clearly in the soil sections most densely penetrated by their roots. Therefore, all subsequent tests were performed on the top third of the samples.

The biomass half of the sample was subjected to manual root washing. Once the plants were fully isolated, the aboveground and belowground biomass was separated and subsamples of leaves and stems and roots in different positions were taken and scans of roots and leaves were performed - this data, however, is not directly relevant to this thesis.

After air-drying, approximately 500 g of the top third of both the topsoil and the subsoil samples were taken and sieved manually with 5mm, 3 mm, 0.2 mm and 0.02 mm sieves. Furthermore, a subsample for the analysis of microbial communities was taken and samples of aggregates adhering to roots were taken for a nano-SIM analysis. Of the fractions resulting from the sieving, 40 g each was taken for aggregate mineralization tests and 20 g of the 5-3mm fraction was taken for aggregate stability tests. Furthermore, 150-200 g of the top third of the sample were taken and sieved with a 200 micrometer sieve. Of the resulting fraction smaller than 200 micrometers, 60 g was taken for carbon fractioning tests and 20 g for substrate induced respiration tests. A visual representation of the sampling process can be found in Figure 3.5 (though it has to be noted that the exact quantities taken for each experiment changed slightly).

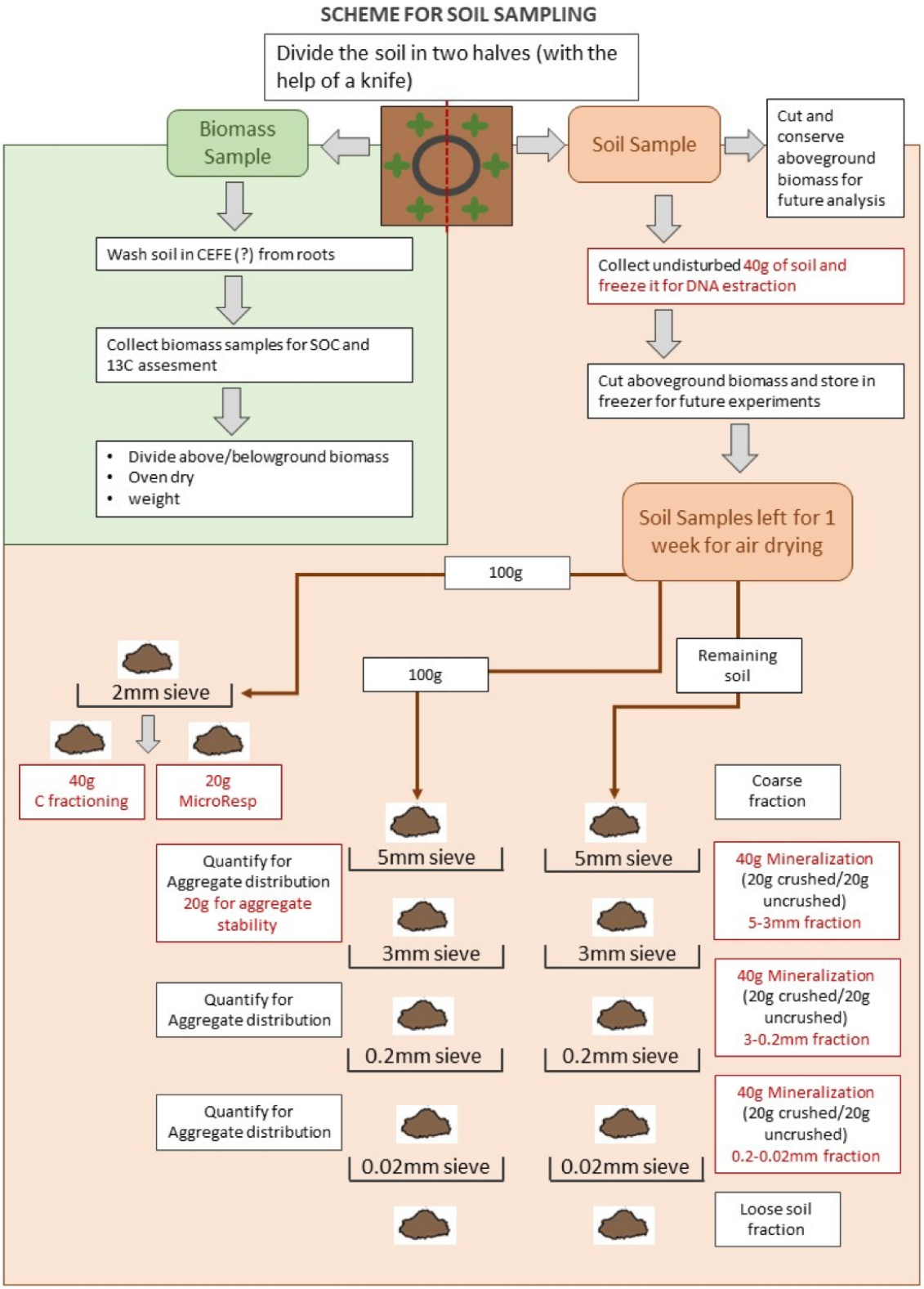

Figure 3.5: Scheme for soil sampling for the TERRE project ©Lorenzo Rossi

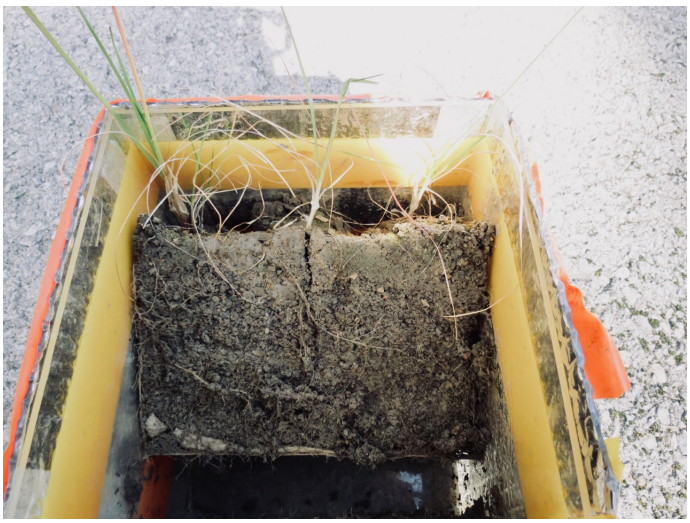

Figure 3.6: Example of the extraction of a sample from the TERRE pots, with roots visible

3.2.2 TalVeg

The soil samples from the TalVeg project were taken using core samplers (length: 20 cm, diameter: 5 cm), which were manually driven into the experimental pots using a hammer (see Figure 3.7). The sampling instrument was lined with a piece of plastic foil to facilitate the removal and storage of the soil cores (see Figure 3.8). For each pot, three cores were taken for treatment, each centered on an individual plant in order to also capture a representative root sample.

The first of these cores was taken for the analysis of the root system. The aboveground biomass was removed, separated into living and dead biomass, and then dried. The core was then separated into two subsamples - one of the top 10 cm of the sample and of the bottom 10 cm. Root washing was performed on each subsample separately.

The second was taken for induced respiration tests to be carried out with a microcatharometer at the Centre d'Écologie Fonctionnelle et Évolutive (CEFE).

The third core was also separated into top 10 cm and bottom 10 cm and consequently air-dried and used for aggregate stability tests.

Figure 3.7: Removal of the soil core from the experimental pots using a hammer and a piece of piping inlaid with plastic foil

Figure 3.8: Extraction of the soil core from the piping

3.3 Tests Performed

After the general introduction into the experimental setup for both TERRE and TalVeg, this chapter now offers a description of the tests that were performed in order to obtain the results for this thesis - namely the aggregate stability and respiration tests.

3.3.1 Aggregate Stability Tests

In this section, aggregate stability will be introduced. First a general introduction into the aggregate stability tests developed by Yves Le Bissonnais (1996 & 1997) will be given, followed by an exposition of the exact protocol used for this thesis.

General Description of Method according to Le Bissonnais 1996 & 1997

The core part of this thesis is an evaluation of the effect of the different treatments - the planting of different species of different plant families, the use of topsoil versus subsoil - on the stability of soil aggregates. Therefore, the test used to determine the stability of said aggregates is of critical importance.

Currently, the number of available aggregate stability tests used in recent publication is large. Almajmaie at al. (2017) offers an evaluation of different methods, but notes that there are no guidelines for the selection of procedures. Theoretically, the selection should follow the purpose of the analysis, the soil type and the predominant kind of breakdown that aggregates would experience under field conditions. In practice, aggregate stability is often determined by wet sieving. If this is the case, slaking is predominantly responsible for aggregate breakdown, while other breakdown mechanisms are underrepresented.

For this thesis, a part of the aggregate stability tests developed by Le Bissonnais (1996) was used. In two papers, published in 1996 and 1997 Le Bissonnais proposed a unified framework of aggregate stability tests. These take into account all of the breakdown mechanisms described in Chapter 2.3 through a combination of three treatments and an additional dispersion test. The treatments described in Le Bissonnais (1996 & 1997), carried out on aggregates of 3-5mm obtained by sieving from air-dried samples, are as follows:

- Fast Wetting - Aggregates are immersed in water in order to simulate their response to rapid wetting, as would occur in the field during heavy rain. This treatment offers a straightforward, comparatively simple way to compare the basic stability of a variety of soils and is also integrated in a variety of other methodologies to test aggregate stability.
- Slow wetting - This test is supposed to simulate the field situation of wetting under gentle rain by placing the aggregates on a filter paper on a tension table with a matrix potential of -0.5 kPa and allowing them to saturate by capillary force. Less destructive force is applied in this method and it is particularly apt for a comparison of soils of lower stabilities.
- Mechanical breakdown by shaking after pre-wetting - This test aims to eliminate the influence of slaking on the breakdown of aggregates to better observe the other mechanisms. In order to do so, the air that is normally entrapped in aggregates during wetting and causes slaking, is removed by pre-wetting. This is achieved either in vacuum by rewetting or by wetting with a nonpolar liquid like ethanol. Subsequently, the pre-wetted aggregates are immersed in water and agitated mechanically.

The distribution of fragment sizes after all three treatments is determined by sieving them in a 50 micrometer sieve immersed in ethanol. Subsequently, the fraction larger than 50 micrometers is collected, oven-dried and dry-sieved by hand. Using the mass percentage of each size fraction, the mean weight diameter (MWD) is calculated. Ethanol is used to prevent further breakdown and stabilize what remains of the aggregates after the treatments.

Le Bissonnais (1996) offers the following classification of soils with regard to the stability of their aggregates and their tendency to form crusts as a result of aggregate breakdown, according to the MWD obtained in the treatments described above:

Table 3.2: Classification of soils according to aggregate stability by Le Bissonnais (1996)

Class	**MWD [value/mm]**	**Stability**	**Crustability**
1	<0.4	Very unstable	Systematic crust formation
2	0.4-0.8	Unstable	Crusting frequent
3	0.8-1.3	Medium	Crusting moderate
4	1.3-2.0	Stable	Crusting rare
5	>2.0	Very stable	No crusting

An additional treatment that is proposed by Le Bissonnais is to measure the clay dispersion in one of the suspensions resulting from treatments 1-3 above in order to also take into account the process of physico-chemical dispersion. This is appropriate if a large part of the sample falls under the fraction smaller than 50 micrometers after treatments 1-3 and the fragment size distributions obtained from these treatments are similar, as this indicates that the soil tested is affected by physico-chemical dispersion. The methods suggested to carry out these measurements are the pipette method and turbidimetry.

Almajmaie at al. (2017) notes that the unified framework proposed by Le Bissonnais is not used widely because it is a complex, time-consuming method. It is also criticized that the effect of breakdown by raindrop impact is not considered in any of the treatments. However, the method has seen successful recent use , e.g. by Xiao at al. (2017 a) and Shi at al. (2017).

Aggregate Stability Test Protocol used for this Thesis

For this thesis, only the fast wetting method according to Le Bissonnais (1996) was used. The reason for this is that it was the situation of heavy rainfall, which this treatment simulates, is the one that the experiments were aiming to recreate.

The soil aggregates of sizes between 3-5 mm that were obtained in the preparatory procedures described above were oven-dried for a minimum of 24 hours to eliminate antecedent moisture. Then, approximately 5 g of aggregates were taken and immersed in 50 ml of deionized water for 10 minutes in a 250 ml beaker.

Subsequently, the water was removed using a 25 ml pipette without disturbing the wetted

Figure 3.9: Fast wetting test carried out at AMAP

sample. The wet sample was then transferred to a 50 micrometer sieve submerged in Ethanol and the fractions larger than and smaller than 50 micrometers were separated (see Figure 3.9).

The fraction larger than 50 micrometers was transferred to a petri dish and oven-dried for a minimum of 48 hours before being sieved manually to separate the fractions in a sieving column of 2 mm - 1 mm - 0.5 mm - 0.2 mm -0.1 mm - 0.05 mm (see Figures 3.10 and 3.11). After weighing the fraction larger than 2 mm was washed to isolate the small stones in the soil to separate them from the actual aggregates of that size. The stones were dried, weighed separately and their weight subtracted from the initial mass and the mass of the fraction larger than 2 mm.

Finally, the mean weight diameter was calculated according to the formula:

MWD=$\sum_{i=1}^{n}\ w_{\mathrm{i}} * x_{\mathrm{i}}$

x_{i}= mean of mesh size of adjacent sieves

w_{i}= weight of the fraction of aggregates of a certain size as a proportion of the sample weight

Aggregate stability tests were carried out in three replicates for all of the samples taken from the experimental pots for both TERRE and TalVeg

Figure 3.10: Weighing of the aggregate fractions after fast wetting test and drying using a manual sieving column

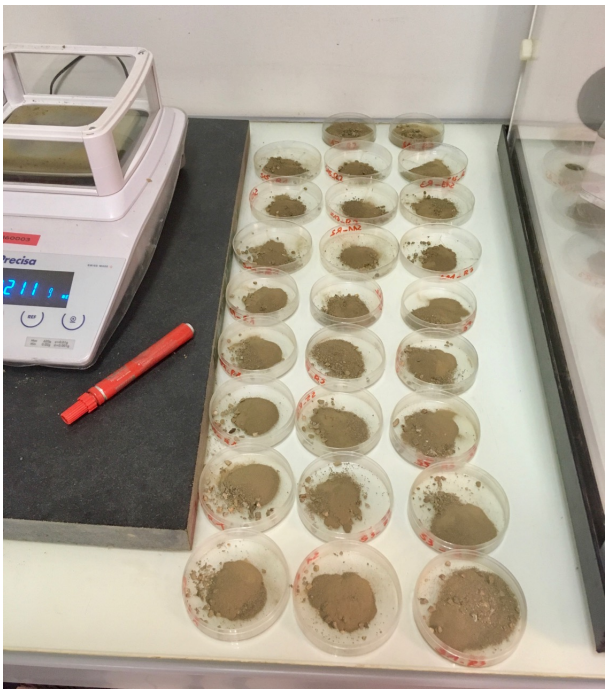

Figure 3.11: Final aggregate fractions after fast wetting test

3.3.2 Respiration Tests

The respiration tests were carried out in order to gain an idea of the level of protection of Soil Organic Carbon by soil aggregates. In order to do so, half of the sample destined for the experiment was ground in a mortar, thus destroying the aggregates. The basic idea is that since SOC that was formerly protected by aggregates becomes available for metabolization by microorganisms after crushing, the respiration rate in the crushed samples would be higher than in the uncrushed ones. Thus, the level of protection of SOC in soil aggregates can be estimated by looking at the difference in respiration between crushed and uncrushed samples.

After drying and sieving, approximately 20 g of each fraction from every sample from the TERRE and TalVeg projects were taken. Half of this amount was then crushed manually in an agate mortar to destroy aggregates and leave the Soil Organic Carbon vulnerable to metabolization by soil organisms. The samples were placed in 250 ml flasks (see Figure 3.12) and wetted with water using a syringe. The amount of water used was calculated to represent approximately 80% of the previously measured field capacity. For TERRE the amount of water used lay at 2.6 ml, for TalVeg at 2.5 ml. However, to achieve even wetting of the smallest fraction of aggregates (0.2-0.02 mm) and the crushed aggregates in TERRE, some additional water had to be added to account for the larger surface area of the aggregates. For these samples, the amount of water lay at approximately 3.4 ml (the exact amount was noted for later calculations in an Excel sheet).

The wetting of the soil was meant to allow soil microorganisms that had lain dormant during the time the soil had been dried to reactivate their metabolisms and to begin to respire the available Soil Organic Carbon. The rate of respiration was measured using a microcatharometer (CP-4900 Varian Inc., Palo Alto, USA) at the Centre d'Écologie Fonctionnelle et Évolutive at Montpellier. Straight after wetting, the bottles containing the samples were sealed air-tight using rubber corks. Then, the CO2 concentration in the bottles was measured by taking air samples through a syringe thrust through the cork. Measurements were carried out at the start of the analysis (t0), at 72 hours (t2) and at 7 days (t3). These time-steps were chosen due to a certain priming effect (Birch effect) that the sudden addition of water to the air-dried samples has in the first few days of the incubation. Further details on this effect can be found in the next chapter. Calibration samples of plain air were taken before analyses at all three times. Inbetween the analysis, the sealed bottles were incubated at 25C.

The principle of the microcatharometer is depicted in Figure 3.13, the actual instrument is shown in Figure 3.14. The machine consists of two distinct parts: the chromatograph and the detector. The chromatograph aims to separate the various gases present in the air taken from the samples. It consists of a mobile phase (the carrier gas used is helium) and a stationary phase, in this case a porous solid material (adsorption chromatography). Every air sample is subjected to a retention force due to the stationary phase and a force moving it forward in the form of the carrier gas. The higher the affinity of the molecules of the gas to the stationary phase of the chromatograph, the longer they will be delayed. This depends on the size and the weight of the molecules as well as on their polarity and charge. The detector, located at the end of the chromatographic column (colonne analytique) is a Thermal Conductivity Detector. It identifies the gases through their thermal conductivity (calibrated to that of the carrier gas)

Figure 3.12: Samples prepared for the respiration tests

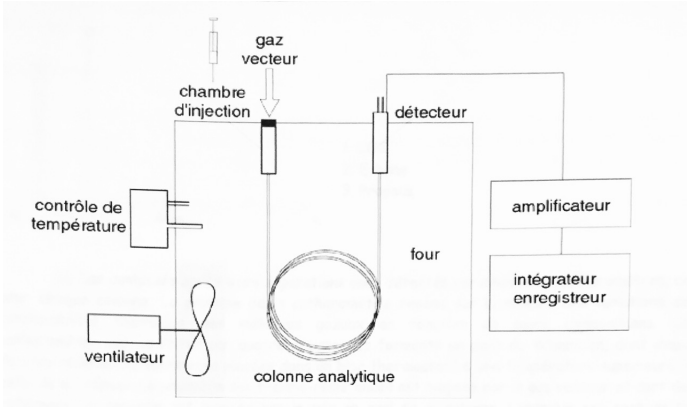

Figure 3.13: Scheme of the microcatharometer (c) CEFE

by measuring their thermo-resistance, which is higher for larger, heavier gases and lower for smaller ones. An analysis of the data was carried out in the software SOPRANE. An example of a chromatogram resulting from one such analysis is shown in Figure 3.15 (CEFE 2017).

Generally, this measurement of respiration follows the protocol developed by Anderson & Domsch (1978). However, conventional respiration experiments furnish the microorganisms in the sample with an unlimited carbon source (Beare at al. 1991), as the focus lies mostly on the highest potential activity of soil microbes and not on the amount of carbon in the soil. The experiment conducted in the course of this thesis deviated from these conventional substrate-induced respiration tests (SIR) by the lack of addition of any nutrients.

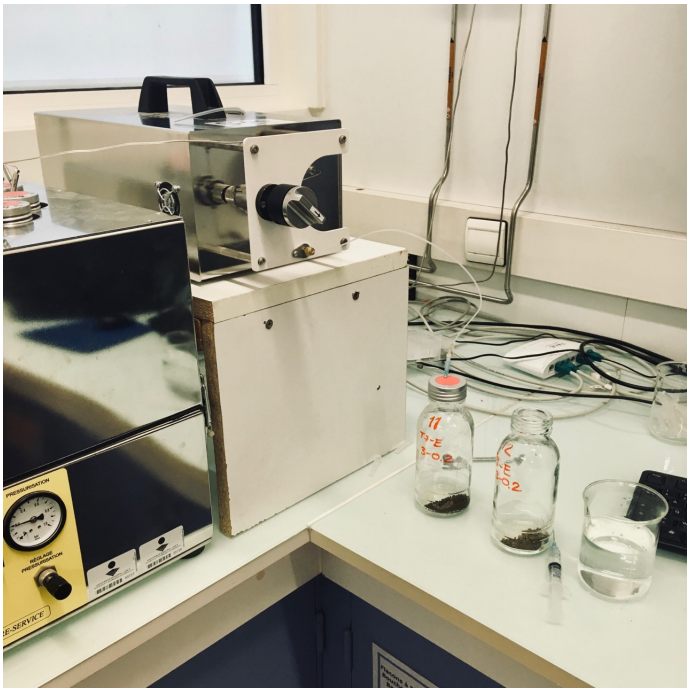

Figure 3.14: Setup for the respiration tests at CEFE

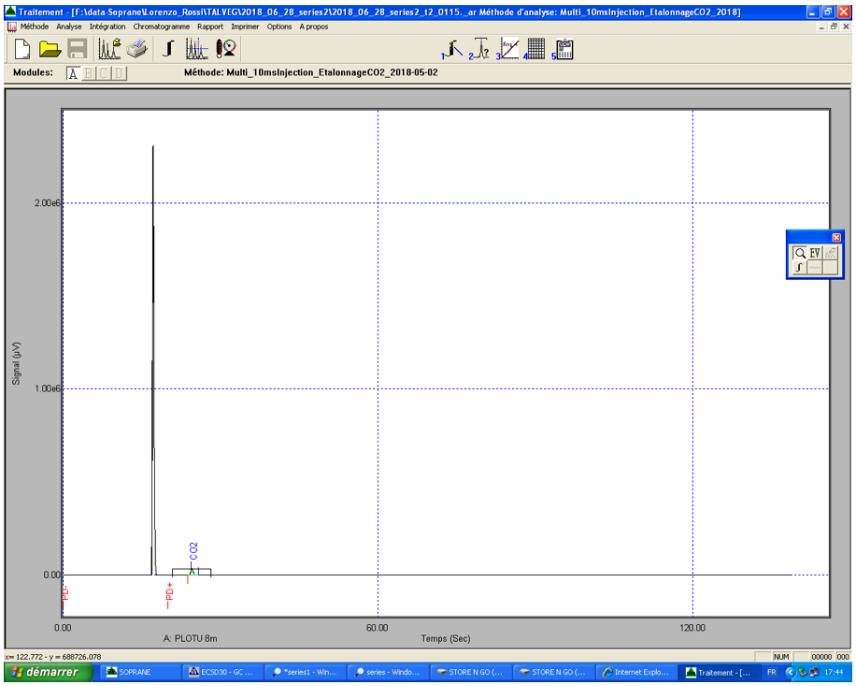

Figure 3.15: Screenshot from the program SOPRANE showing a typical graph of the gas distribution in a sample

Chapter 4

Data Analysis

This chapter provides a brief outline of the statistical analyses that were carried out on the data used for this thesis. Results will be presented in the next chapter, and a discussion of said results in the following chapter.

4.1 Aggregate Stability

The first step in the analysis of the aggregate stability data after the completion of the experiments was to check the completeness and plausibility of the data obtained. Subsequently the data was exported as .csv file and imported into R. Subsequently, it was determined whether the data met the assumption for an ANOVA, namely:

- independence of cases
- normality (using Shapiro-Wilk test)
- homoscedasticity (equality of variances, using Levene's test)

If the assumptions were met, a one-way ANOVA (analysis of variance) was calculated and the results visualized.

If the data did not meet these assumptions, one of the following transformations was applied:

- Square Root Transformation
- Log Transformation
- Tukey's Transformation (Tukey's ladder of power, which uses an iteration of the Shapiro-Wilk test to find the lambda value to maximize the W statistic)

- Box-Cox Transformation

If the assumptions were met, an ANOVA was performed, if not, a non-parametric alternative was such as the one-way test or the Kruskal Wallis Test was chosen.

In the case of the aggregate stability data for TERRE, the assumption of normality was met, but the assumption of homoscedasticity could not be met, even with the transformations listed above. Therefore, a one-way test (which assumes normality but not homoscedasticity) was performed. Furthermore, after examining the initial results of this test, the data was divided into two subsets - one comprising the subsoil, the other the topsoil samples.

Furthermore, also for the data from the TalVeg experiments, the conditions for the ANOVA could not be met for the largest part. Therefore, the non-parametric Kruskal Wallis Test was performed to determine whether the there was significant variation in the data. If this was the case, the pair-wise Wilcox rank sum test was calculated in order to determine the exact differences between the treatments.

4.2 Respiration Experiments - Microchatharometer

For the statistical analysis of the data from the respiration experiments in the microchatharometer, the respiration rate per gram soil sample and minute was calculated. The data retrieved from the program SOPRANE comprised the CO_2 concentrations of the samples in ppm and the time at which the measurements were taken. First, the time difference in minutes between the second and third measurement was calculated. Secondly, the CO_2 concentration was converted into micromoles and the difference in concentration was calculated for each sample between the second and third measurement was calculated. Finally, the difference in concentration was divided by the time difference and the initial sample weight to calculate the respiration rate per g sample and minute.

The reason why the time and concentration differences between the second measurement (taken at 72 h after rewetting of samples) and the third measurement (taken at 7 d after rewetting) was used, lies in the fact that it has been observed that the increase in respiration rate just after rewetting is significantly higher than it is once it has reached a steady state. This is known as the "Birch Effect", as it was first described by H.F. Birch (1958). In order to avoid this effect, the more representative respiration between the second and third measurement was taken to calculate the respiration rate.

For both the TERRE and the TalVeg samples, first a comparison was made between ground and not ground samples in all three sizes of aggregate fractions used (5-3 mm, 3-0.2 mm or 0.2-0.02 mm) and, in the case of TERRE, also for the two different types of soil (subsoil vs. topsoil) to see where significant variations in the respiration rate occurred. Subsequently, the increment of the respiration rate was calculated (respiration in the ground samples - respiration in the not ground samples) and compared according to the species the pots had been planted

with. This was to see the effect of the individual treatments on the variation of respiration between the samples in a ground and not ground state.

The core principle was to compare the respiration rate in the ground and not-ground samples to draw conclusions regarding the level of protection of Soil Organic Carbon in aggregates. Since carbon that was formerly protected by aggregates becomes available after crushing, the respiration rate in the crushed samples is expected to be higher. In order to reduce the increase in respiration rate between the crushed and uncrushed samples to a single variable, the increment in respiration rate was calculated by deducting the respiration in the uncrushed sample from that in the crushed sample. Therefore, the higher the increment between crushed and uncrushed is, the more formerly protected carbon becomes available. The bottom line of this thought process is that a higher increment in respiration rate points towards a higher level of protection of SOC in aggregates.

Chapter 5

Results

This chapter offers an outline of the results obtained from the experiments described above. A discussion of these results will follow in the next chapter. For the sake of brevity in the main text, only the graphs showing significant results as well as a few figures giving a general overview of results were left in the main text. The remaining graphs can be found in a separate appendix.

5.1 TERRE

In this section, the results of both the aggregate stability tests and the respiration tests for the project TERRE will be presented.

5.1.1 Aggregate Stability

The results for the aggregate stability tests for TERRE are displayed in figures 5.1-5.3. The analysis focussed on the differences in aggregate stability depending on the type of soil and the species used for cultivation.

As can be seen in Figure 5.1 there is a highly significant difference between the aggregate stability in topsoil and subsoil ($p=1.06*10^{-15}$), with the topsoil samples generally displaying a much larger aggregate stability than their counterparts originating from subsoil.

Figures 5.2 and 5.3 show the differences in aggregate stability depending on the species used for cultivation for both topsoil and subsoil. These results show clearly that there is a significant difference between the control treatment with bare soil and the cultivation with *Lolium perenne* and *Medicago sativa* in the topsoil ($p=0.000242$). However, this difference could not be observed in the subsoil. Furthermore, there was no significant difference - neither in topsoil nor in subsoil - between the cultivation of *Lolium perenne*, and *Medicago sativa*.

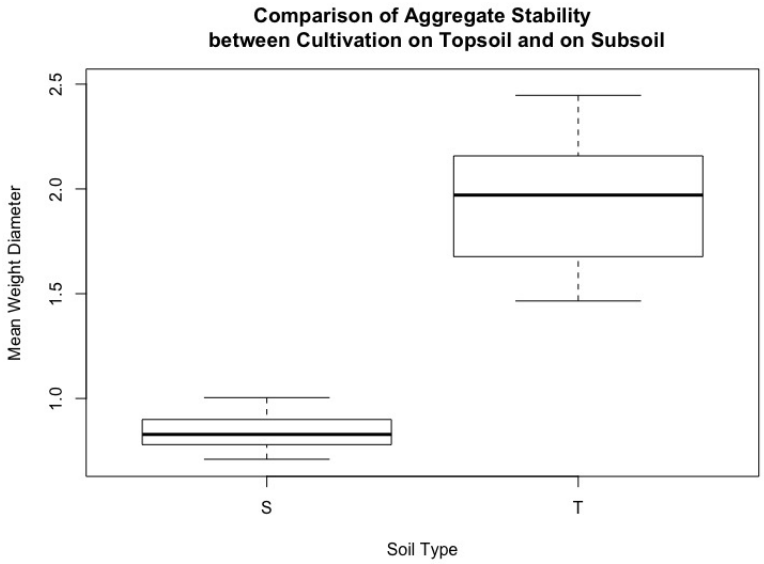

Figure 5.1: Comparison of aggregate stability between topsoil [T] and subsoil [S] samples

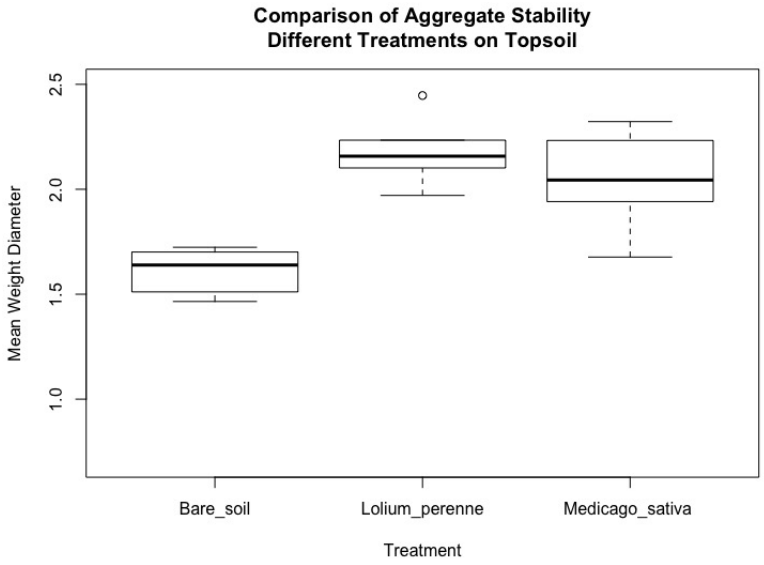

Figure 5.2: Results of the comparison of the variation of aggregate stability according to different treatments for the TERRE topsoil subsample

5.1.2 Respiration Measurements

For TERRE, the respiration rate was put in relation with the soil type, the state of the samples (ground or not ground), the treatment (species) and the aggregate fraction used (5-3 mm, 3-0.2 mm or 0.2-0.02 mm). Furthermore, the increment in respiration between the ground and not ground sample was calculated, put in relation to the species with which the pots were planted and compared across aggregate fractions and soil types.

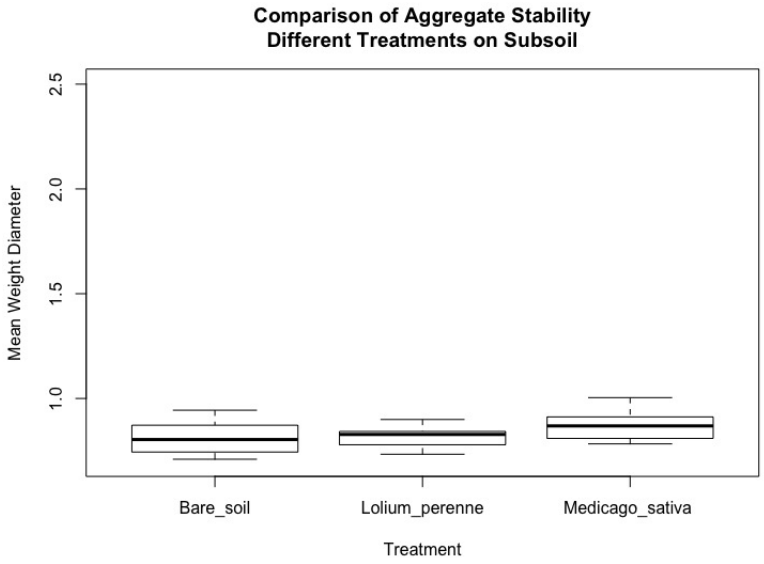

Figure 5.3: Results of the comparison of the variation of aggregate stability according to different treatments for the TERRE subsoil subsample

Regarding the variation of respiration between ground and not ground samples, there was a significant difference only in the topsoil in the aggregate fraction of 3-0.2 mm (p=0.03798, Figure 5.4). Here, the respiration was lower in the samples that had not been ground. In the fractions 5-3 mm and 0.2-0.02 mm, no such significant difference was detectable - neither in the topsoil, nor in the subsoil samples. In the comparison of ground and not ground samples between topsoil and subsoil samples, it becomes apparent that in topsoil, there is a significant difference, with a higher respiration in the ground samples (p=0.01907, see Figures 5.5- 5.6), while in subsoil no significant difference exists.

In relating the differences in the measured respiration rate between ground and not ground samples stemming from different soil types - topsoil or subsoil - it became apparent that there is a significant difference both in all the aggregate fractions ($p=4.88*10^{-7}$ for 5-3mm - Figure 5.7, $p=2.00*10^{-7}$ for 3-0.2 mm - Figure 5.8, and $p=5.77*10^{-7}$ for 0.2-0.02 mm - Figure 5.9). In all cases, respiration was significantly higher in topsoil than in subsoil samples.

When looking at the varying respiration rates between samples that had been subjected to different treatments - cultivation with *Medicago sativa* or *Lolium perenne* or the control treatment with soil that was left bare - it was revealed that there were significant variations in all fractions in types of soil (in topsoil: p=0.00021 for ground samples and p=0.118 for not ground samples, in subsoil: p=0.00030 for ground samples and 0.0111 for not ground samples, Figures 5.10 - 5.13). In topsoil, the respiration of samples cultivated with either *Medicago sativa* or *Lolium perenne* were significantly higher than those in the control treatment. In subsoil samples, the cultivation with *Lolium perenne* seems to have had a negative impact on respiration in the samples that had been ground. As can be seen in Figure 5.11, respiration rates in samples that had been cultivated with *Lolium perenne* and subsequently ground had a lower respiration rate than those in the control treatment. *Medicago sativa* seems to have had a similarly positive impact on respiration as in topsoil, as the samples that had been cultivated

with this plant showed a higher respiration rate than both the control treatment and *Lolium perenne*.

Looking solely at the increments in respiration rate in relation to the species used for cultivation, no significant differences were found in any of the three aggregate fractions or in the two soil types when relating the increment to the cultivation treatment. However, this lack of significance seems to have been due mostly to the great variability in the data, as some differences can be observed on a purely visual basis - as can be seen in Figures 5.14 and 5.15 as well as figures 9.1-9.8 in Appedix A.

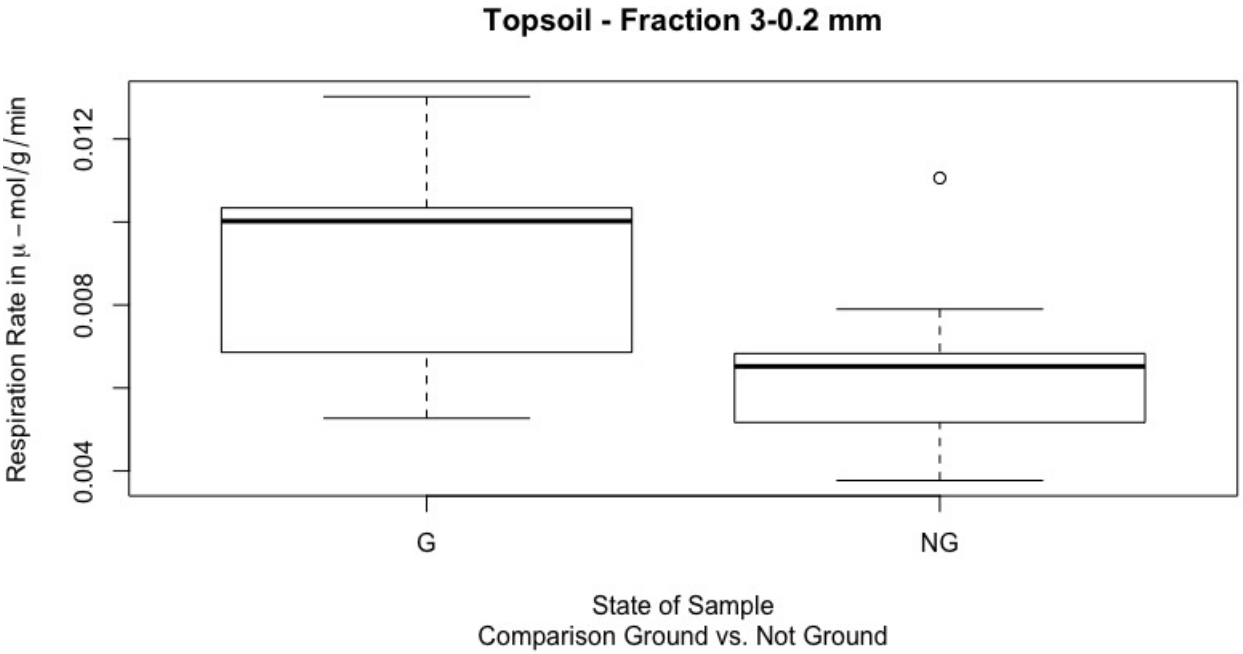

Figure 5.4: Comparison respiration rates between ground and not ground samples - topsoil fraction 3-0.2 mm

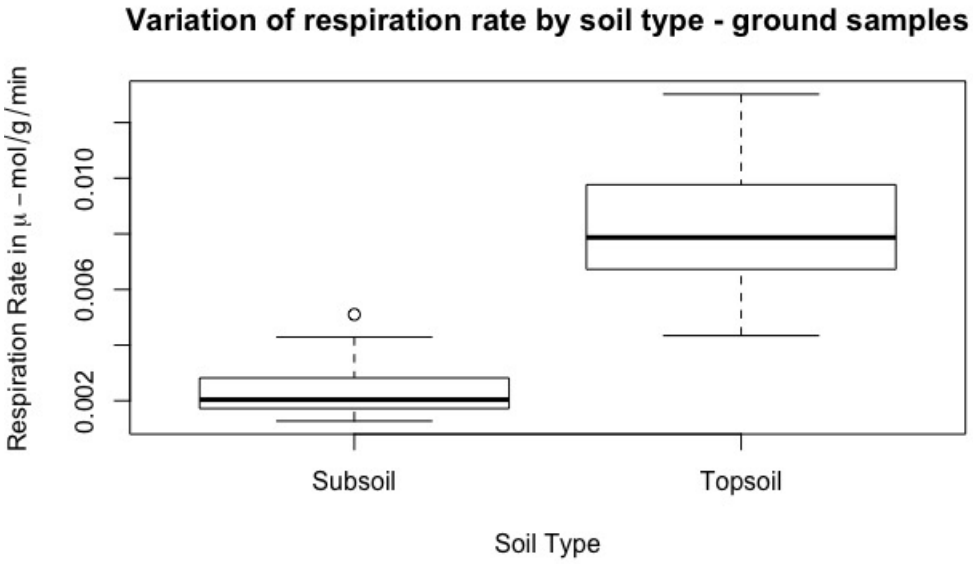

Figure 5.5: Comparison respiration rates between topsoil and subsoil samples - ground samples

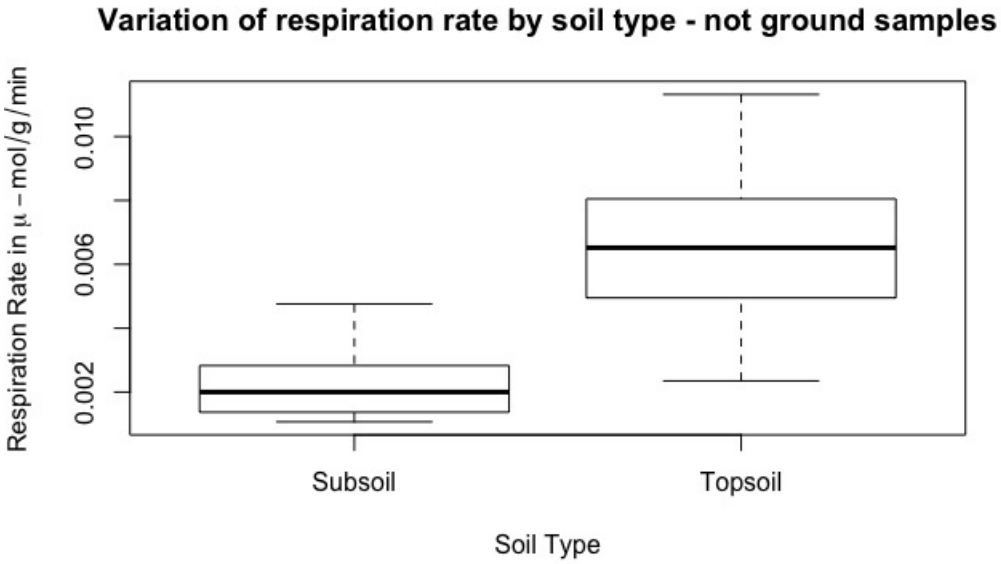

Figure 5.6: Comparison respiration rates between topsoil and subsoil samples - not ground samples

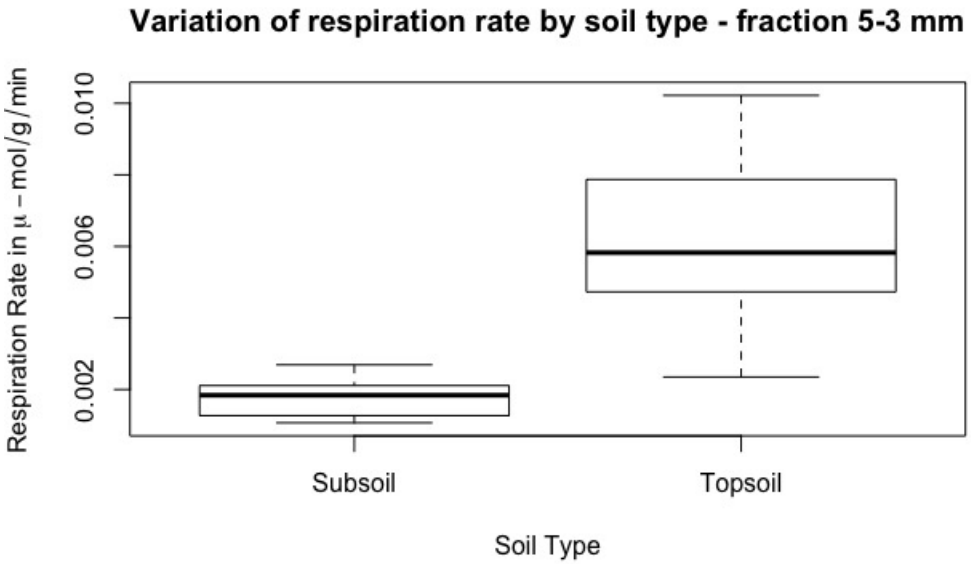

Figure 5.7: Comparison respiration rates between topsoil and subsoil samples - fraction 5-3 mm

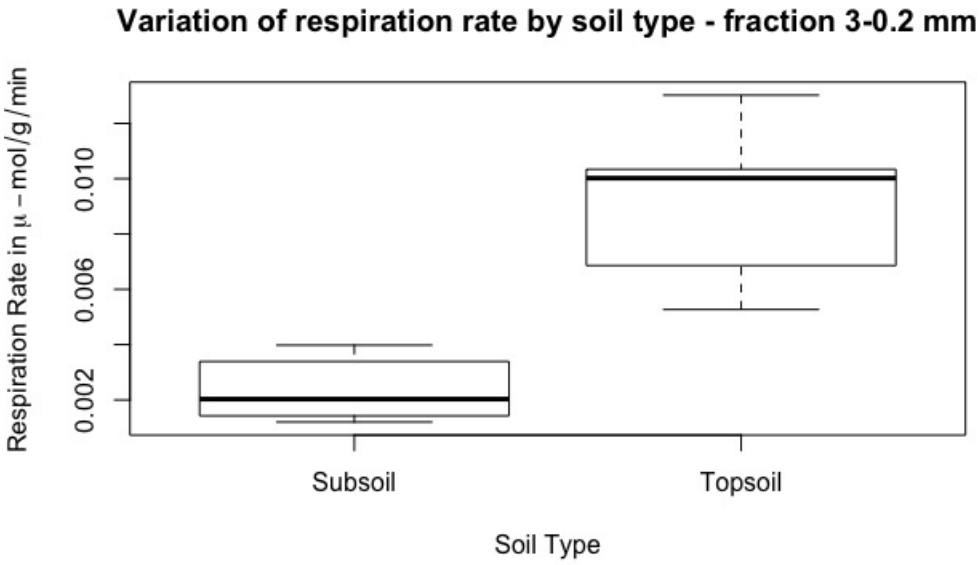

Figure 5.8: Comparison respiration rates between topsoil and subsoil samples - fraction 3-0.2 mm

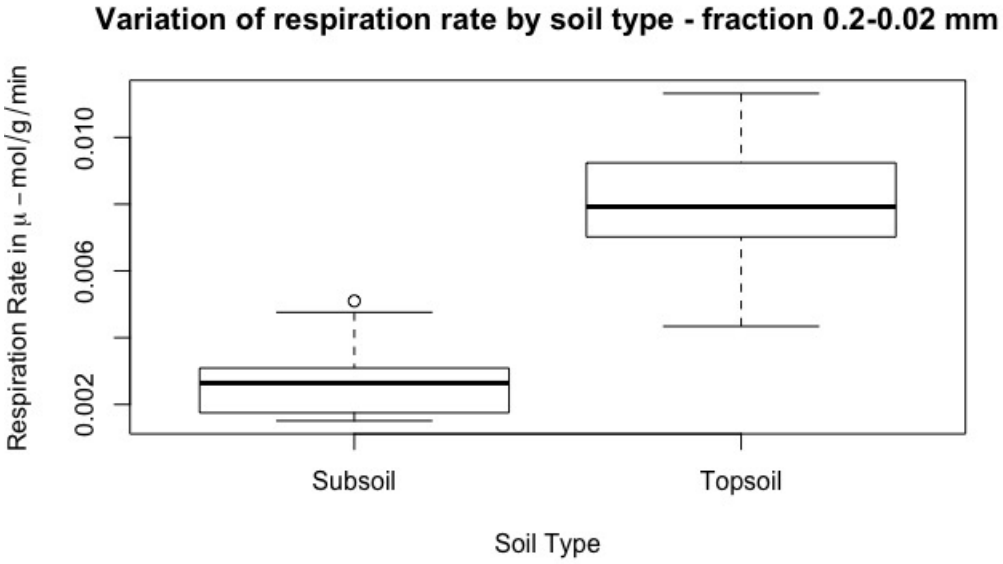

Figure 5.9: Comparison respiration rates between topsoil and subsoil samples - fraction 0.2-0.02 mm

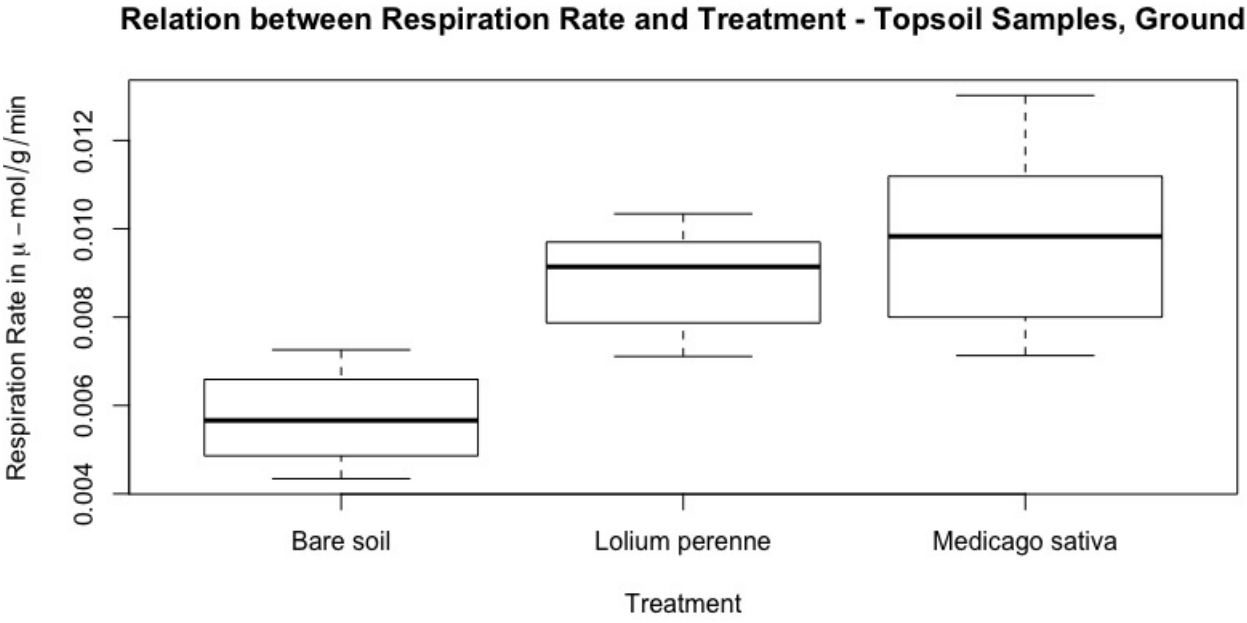

Figure 5.10: Comparison respiration rates between treatments - topsoil, ground samples

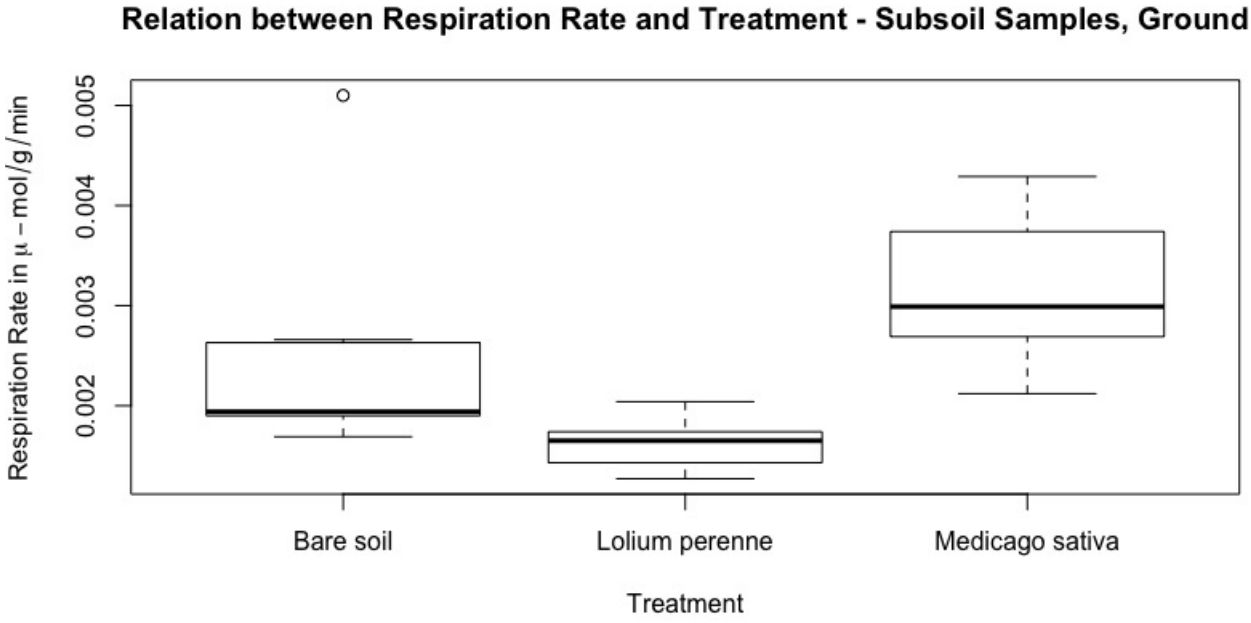

Figure 5.11: Comparison respiration rates between treatments - subsoil, ground samples

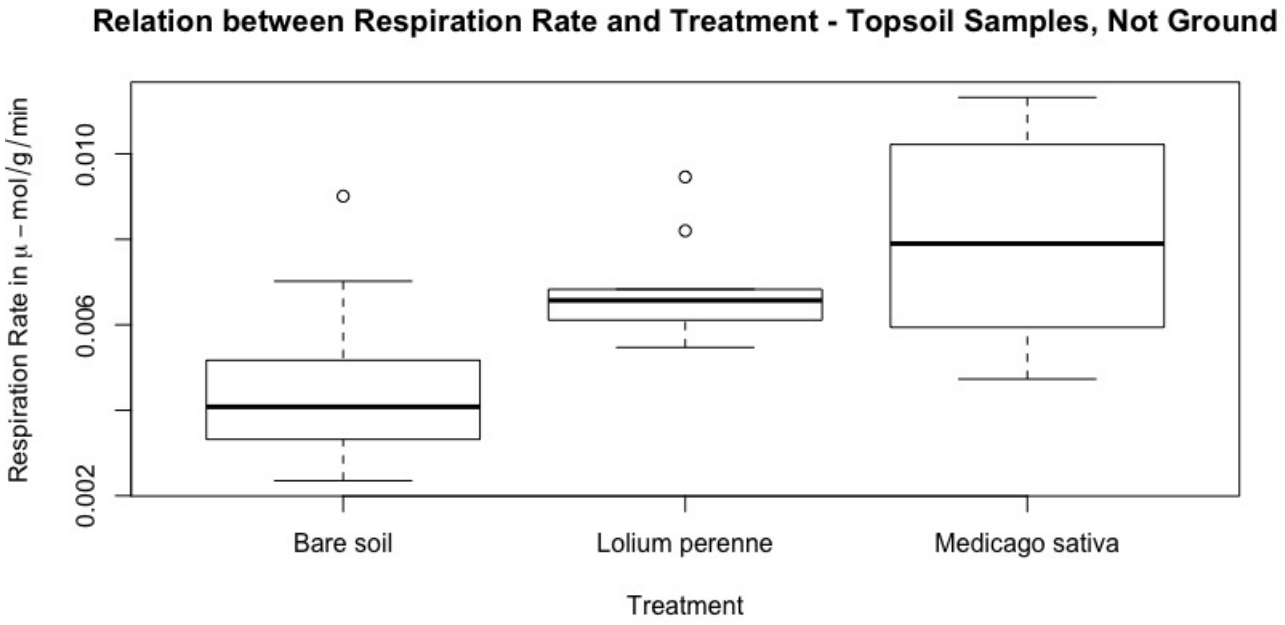

Figure 5.12: Comparison respiration rates between treatments - topsoil, not ground samples

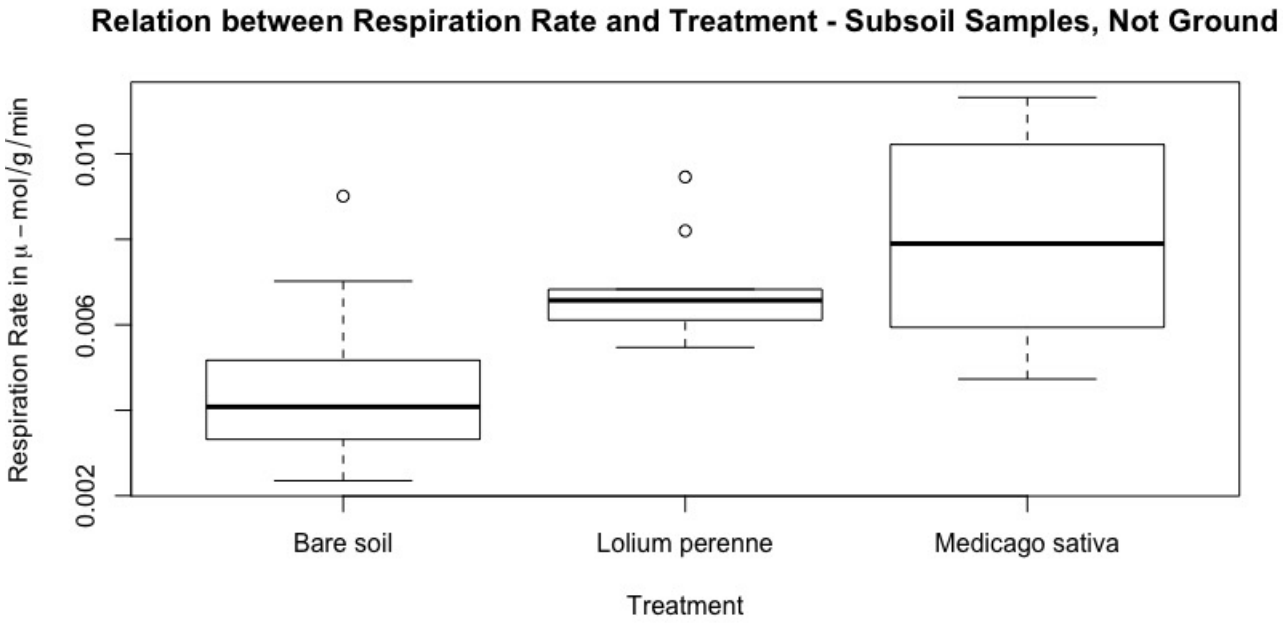

Figure 5.13: Comparison respiration rates between treatments - subsoil, not ground samples

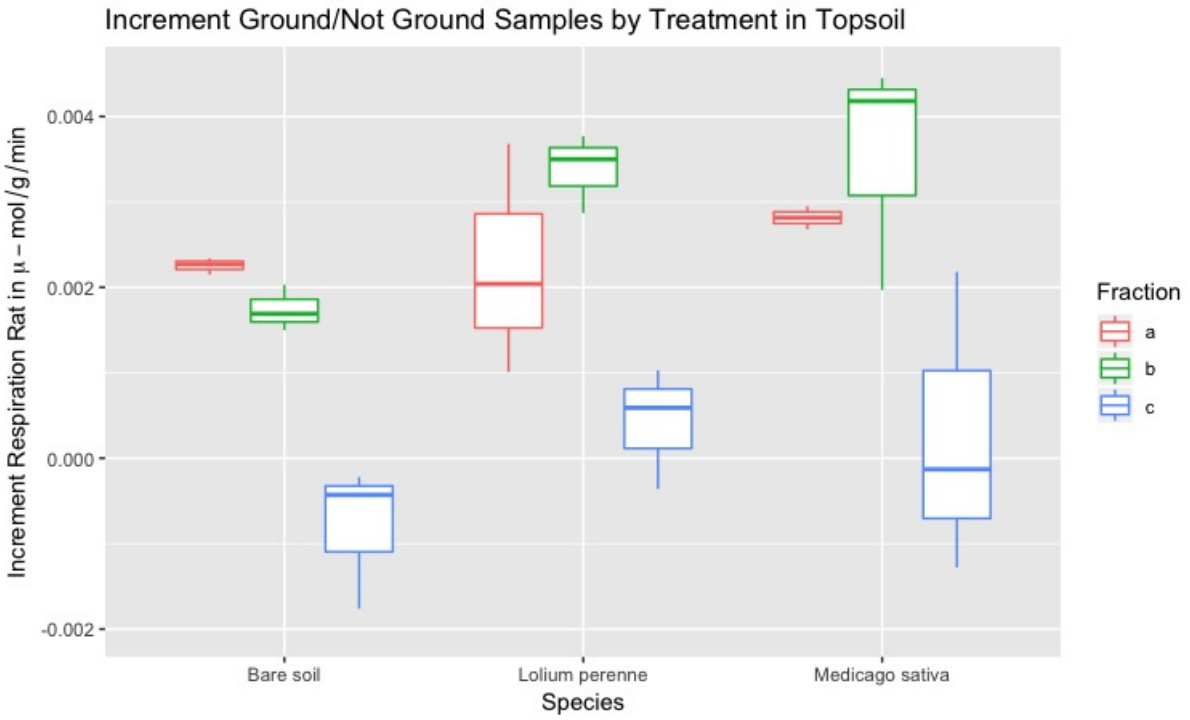

Figure 5.14: Increment of respiration rate by treatment in topsoil (a = 5-3 mm, b=3-0.2 mm, c=0.2-0.02 mm)

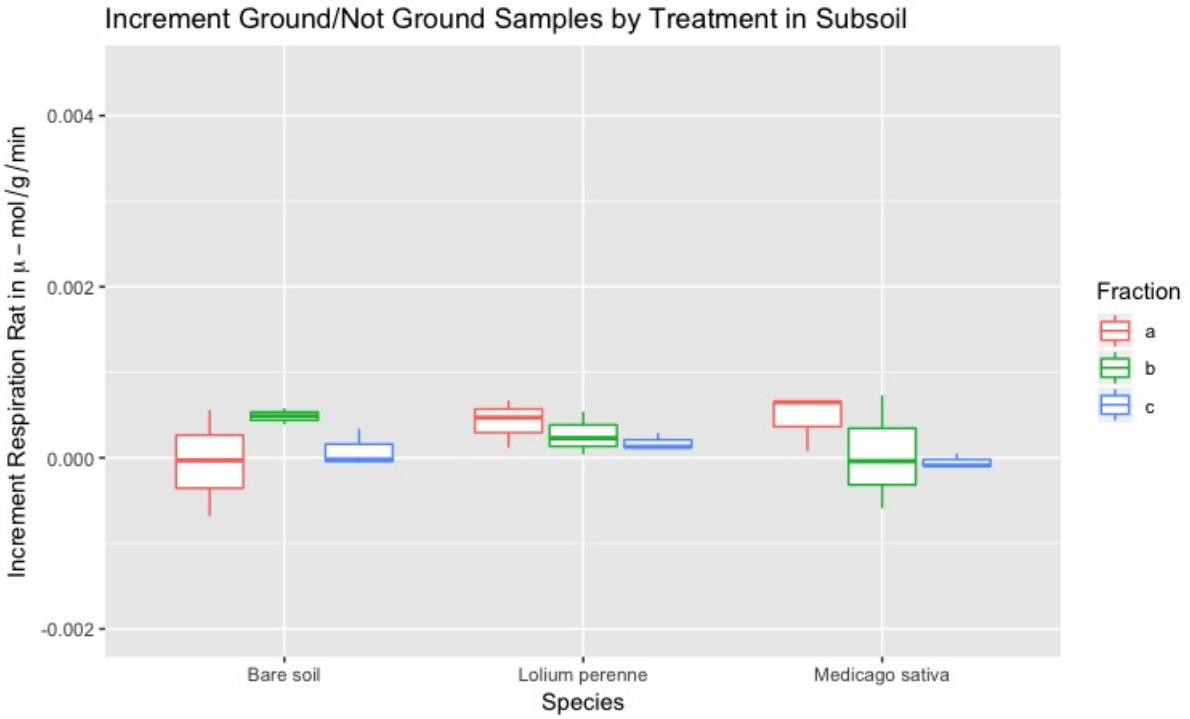

Figure 5.15: Increment of respiration rate by treatment in subsoil (a = 5-3 mm, b=3-0.2 mm, c=0.2-0.02 mm)

5.2 TalVeg

In this section, the results of both the aggregate stability tests and the respiration tests for the project TalVeg will be presented.

5.2.1 Aggregate Stability Tests

The results for the aggregate stability test for the TalVeg samples are shown below in Figures 5.16-5.18. The analysis focussed on the variation of aggregate stability with the depth at which the sample was taken (depth A: 0-10 and depth B: 10-20 cm) and the variation depending on which species was used for calculation.

As can be seen from Figure 5.16, the samples taken at a depth of 0-10 cm display a significantly higher aggregate stability than those taken at a lower depth ($p=1.93*10^{-11}$).

Furthermore, there was a significant difference between the aggregate stability in dependance on the species which had been used for cultivation in the soil at 10-20 cm depth ($p=0.05$, Figure 5.18), but not in the soil at a depth of 0-10 cm (Figure 5.17). Looking at the plant family that had been used for cultivation there was no significant difference in the effect on aggregate stability, although there seemed to be an observable trend ($p= 0.077$) to be found in the samples taken at the lower depth of 10-20 cm, with Poaceans showing a slightly higher effect on aggregate stability. In the samples taken at a depth of 0-10 cm, there was no significant variation and no discernible trend in the stability of aggregates depending on which plant family had been used for cultivation.

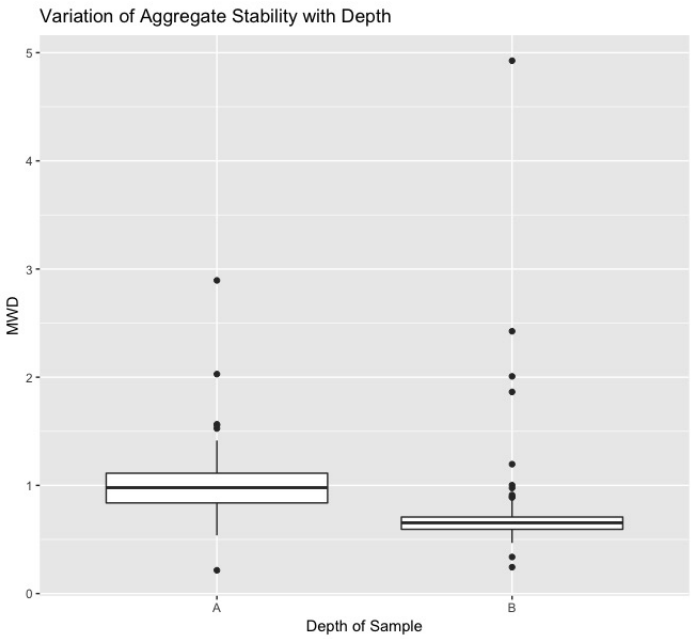

Figure 5.16: Variation of Aggregate Stability with Depth (Depth A = 0-10, Depth B = 10-20 cm)

Table 5.1: Legend of abbreviations for plant species used for cultivation

Abbreviation	Species	Plant Family
Bs	Bare Soil	
Be	Bromus erectus	Poacea
Dg	Dactylis glomerata	Poacea
Lp	Lolium perenne	Poacea
Lc	Lotus corniculatus	Fabaceae
Ms	Medicago sativa	Fabaceae
Ov	Onobrychis viciifolia	Fabaceae
Pl	Plantago lanceolata	Plantaginaceae
Pp	Poa pratense	Poacea
Ps	Sanguisorba minor	Rosaceae
Tp	Trifolium pratense	Fabaceae
Tr	Trifolium repens	Fabaceae

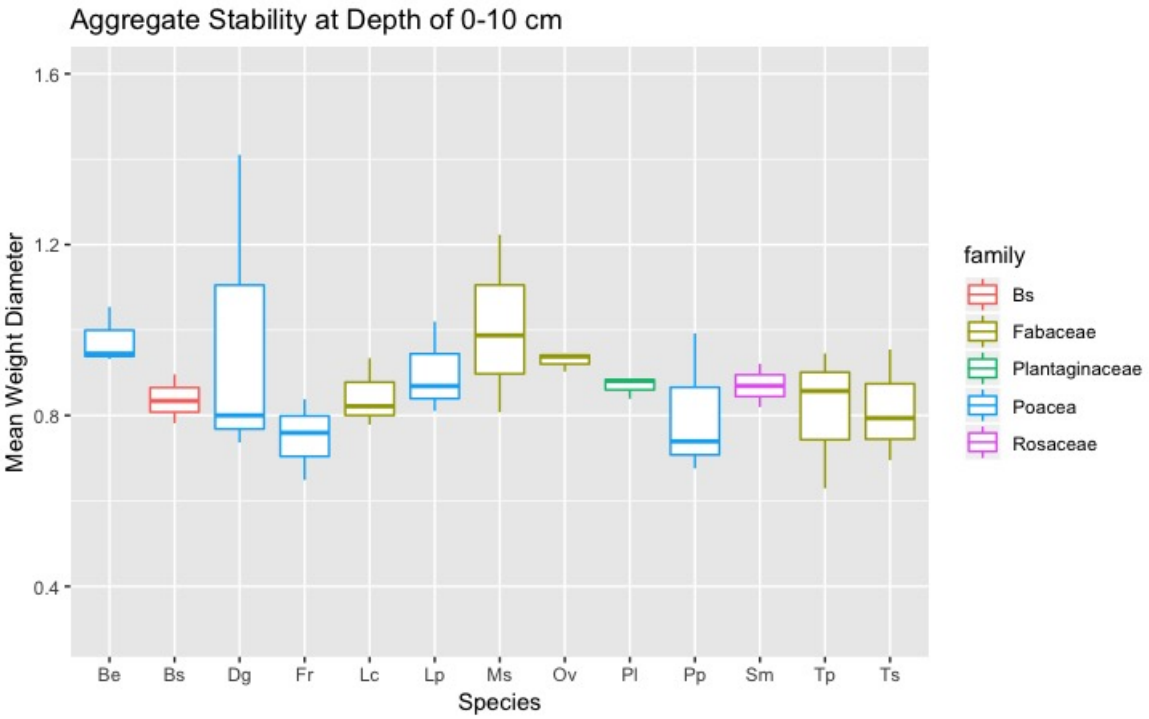

Figure 5.17: Variation of Aggregate Stability with Species at a Depth of 0-10 cm

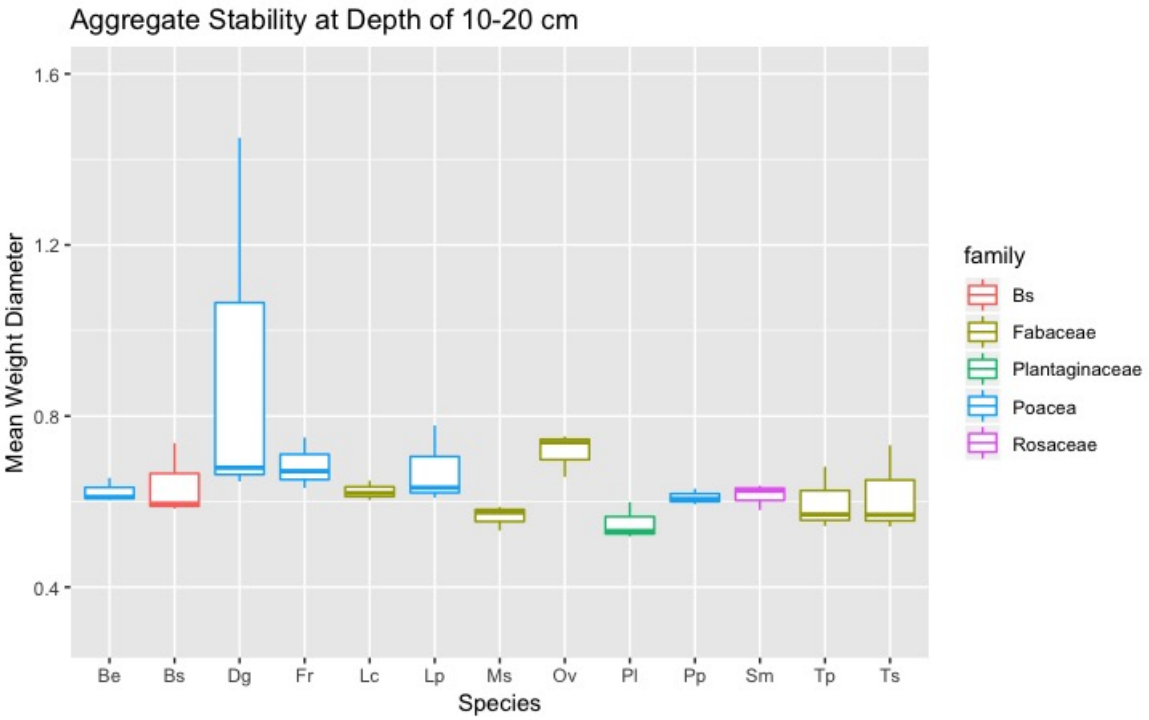

Figure 5.18: Variation of Aggregate Stability with Species at a Depth of 10-20 cm

5.2.2 Respiration Measurements

For TalVeg, the respiration rate was put in relation with the plant species and plant family, and the state of the sample (ground vs. not ground). Furthermore, the increment in respiration rate was calculated and put in relation to the species used for cultivation in each of the three fractions. As mentioned in the exposition of the methodology, due to time limitations, respiration measurements could only be performed for the samples taken at a depth of 0-10 cm. That is why there is no consideration of the influence of sampling depth on any of these results.

In the comparison between the samples that had been ground and those that hadn't, analysis revealed that no significant difference was visible in any of the fractions. However, this can possibly be attributed to the great variation in respiration rates between the species.

Looking at the differences in respiration between the samples depending on which family of plant they had been cultivated with, it was found that significant differences occurred in all three fractions for both ground and not ground samples. In all of these cases, the samples that had been cultivated with species that belong to the family of Fabaceans exhibited higher respiration rates than the others. As for the differences between the respiration rates in dependence on which species of plant the sample had been cultivated with, significant differences could also be found in almost all of the aggregate fractions. The only exception is in the fraction 5-3 mm, where the p value for the relation between respiration rate and species is not significant for the samples that had not been ground. Especially *Onobrychis viciifolia*, *Trifolium pratense* and *Trifolium repens* tended to exhibit higher respiration rates than the other species. The p values for these analyses can be found in Table 5.2, a visual representation can be found in Figures 5.19 - 5.24.

Table 5.2: P values for the relation between respiration rate and species and plant family used for cultivation in TalVeg

Fraction	**State**	**Significance: RR ~ Family**	**Significance: RR ~ Species**
5-3 mm	Ground	0.00093	0.0064
	Not Ground	0.01466	0.0710
3-0.2 mm	Ground	$2.73*10^{-6}$	0.0011
	Not Ground	$1.68*10^{-5}$	0.0019
0.2-0.02 mm	Ground	0.00024	0.0123
	Not Ground	0.00051	0.0286

The analysis of the increment in respiration between ground and not ground samples in dependence on the species used for cultivation, revealed that significant differences occur in the fraction 3-0.2mm (p=0.00147) and the fraction 0.2-0.02mm (p=0.004271). The same is true when looking at the influence of the plant family used for cultivation - a significant influence on the respiration rate can be found in the fraction 3-0.2 mm ($p=1.88*10^{-5}$) and the fraction 0.2-0.02 mm ($p=3.66*10^{-5}$). As can be clearly seen in Figures 5.25-5.27, it is the family of Fabaceans that displays the highest increment in respiration rate, in particular *Onobrychis viciifolia*, *Trifolium pratense* and *Medicago sativa*.

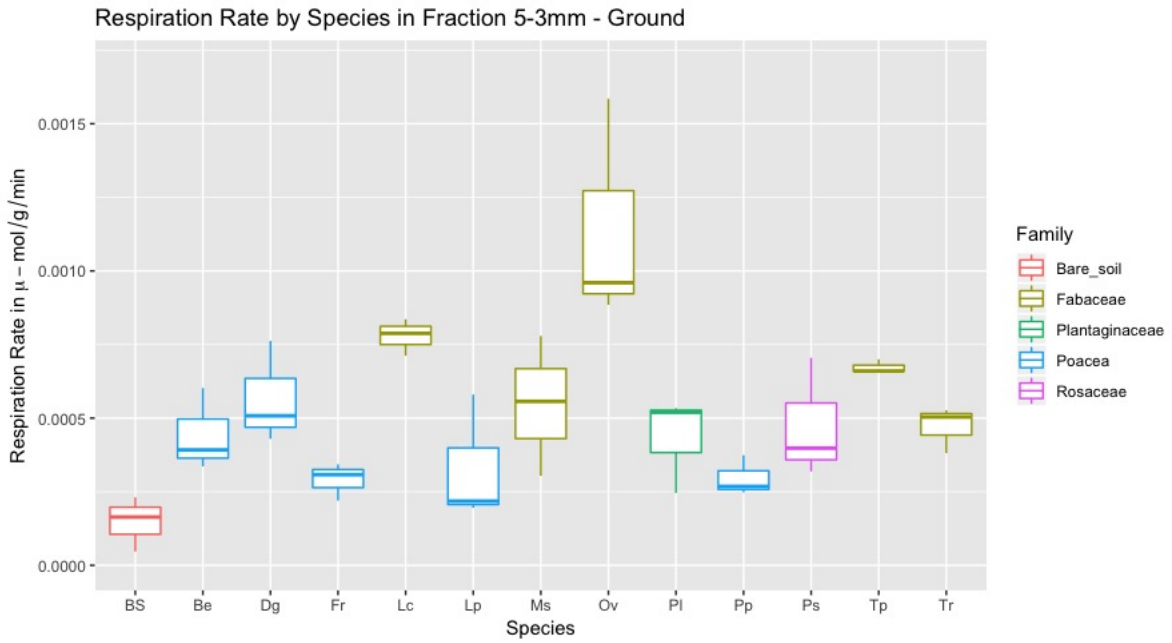

Figure 5.19: Comparison respiration rates between species and families in fraction 5-3 mm, ground samples

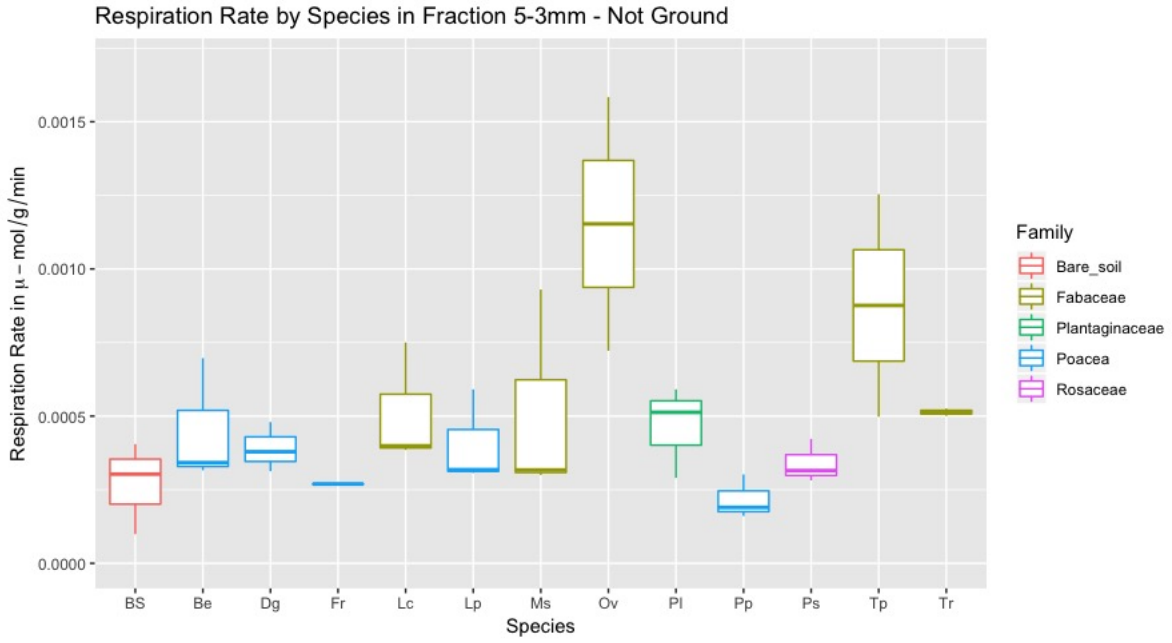

Figure 5.20: Comparison respiration rates between species and families in fraction 5-3 mm, not ground samples

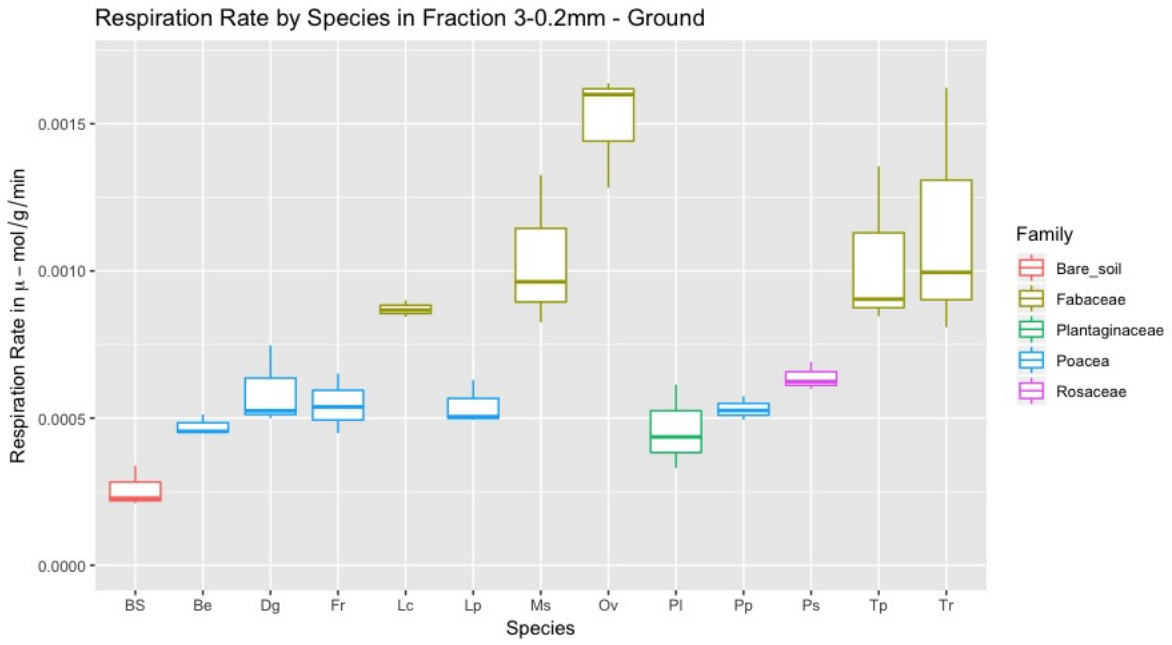

Figure 5.21: Comparison respiration rates between species and families in fraction 3-0.2 mm, ground samples

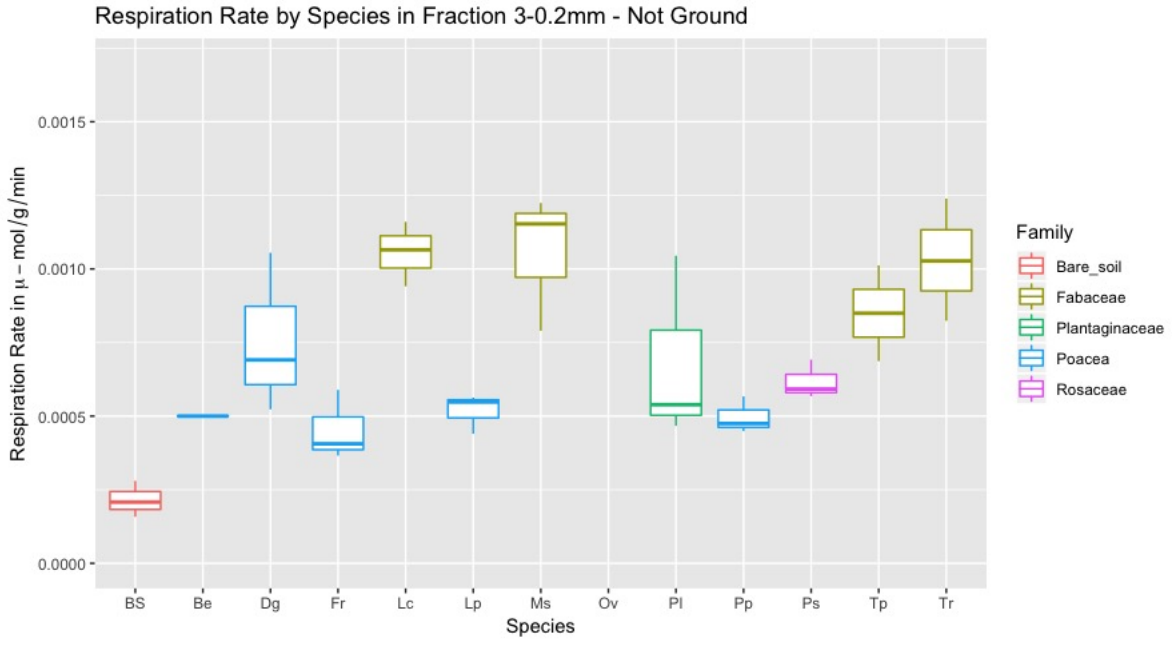

Figure 5.22: Comparison respiration rates between species and families in fraction 3-0.2 mm, not ground samples

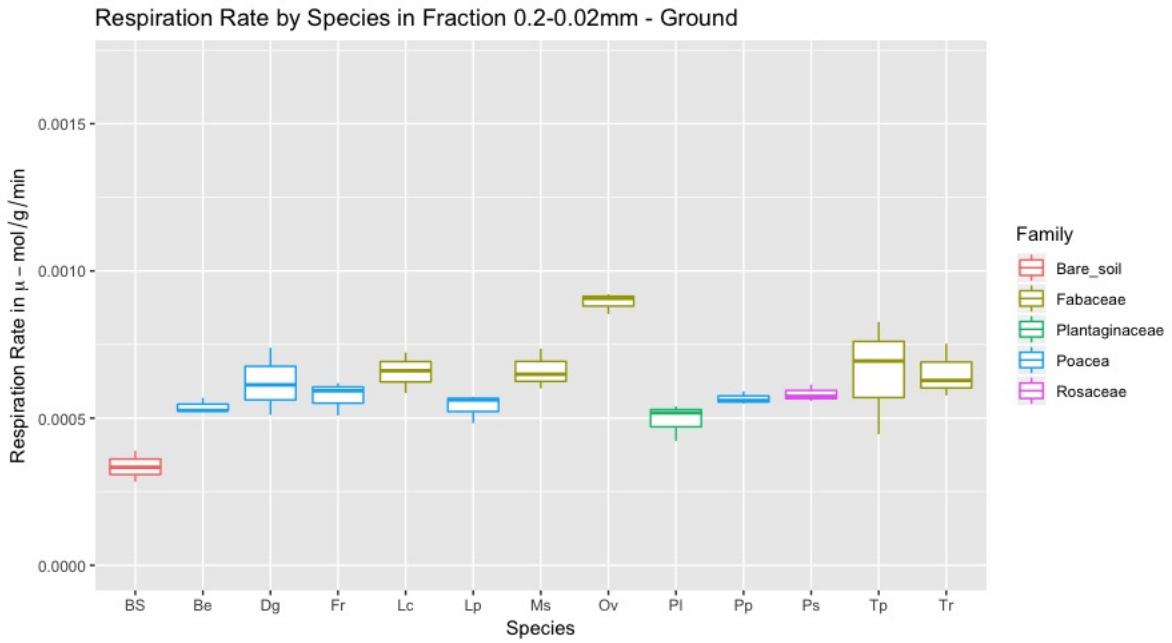

Figure 5.23: Comparison respiration rates between species and families in fraction 0.2-0.02 mm, ground samples

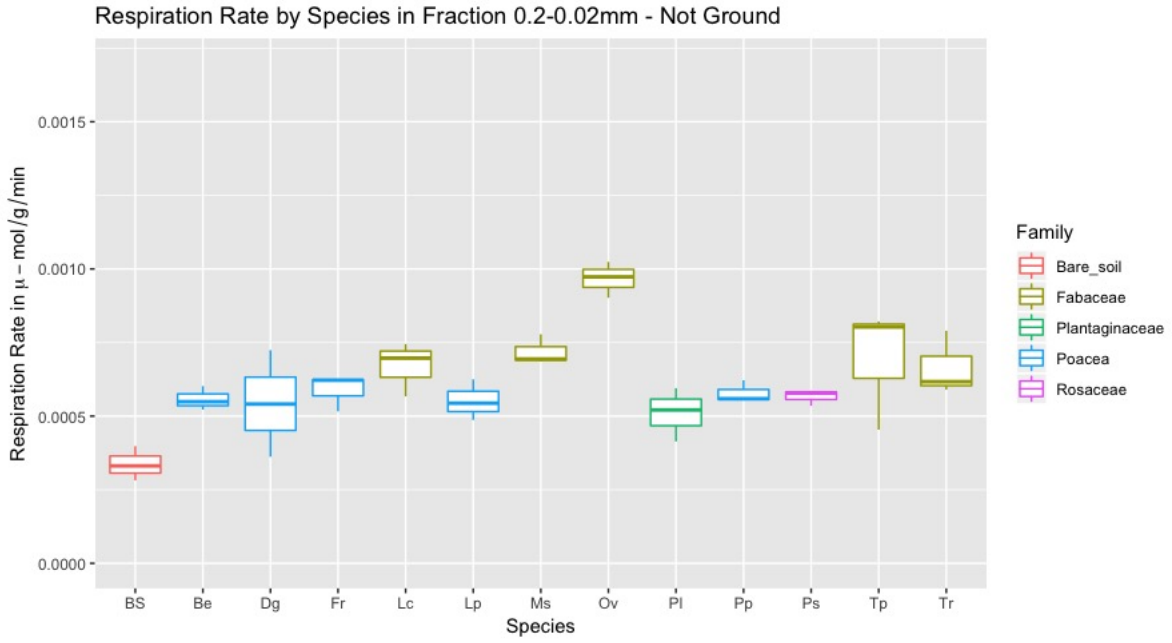

Figure 5.24: Comparison respiration rates between species and families in fraction 0.2-0.02 mm, not ground samples

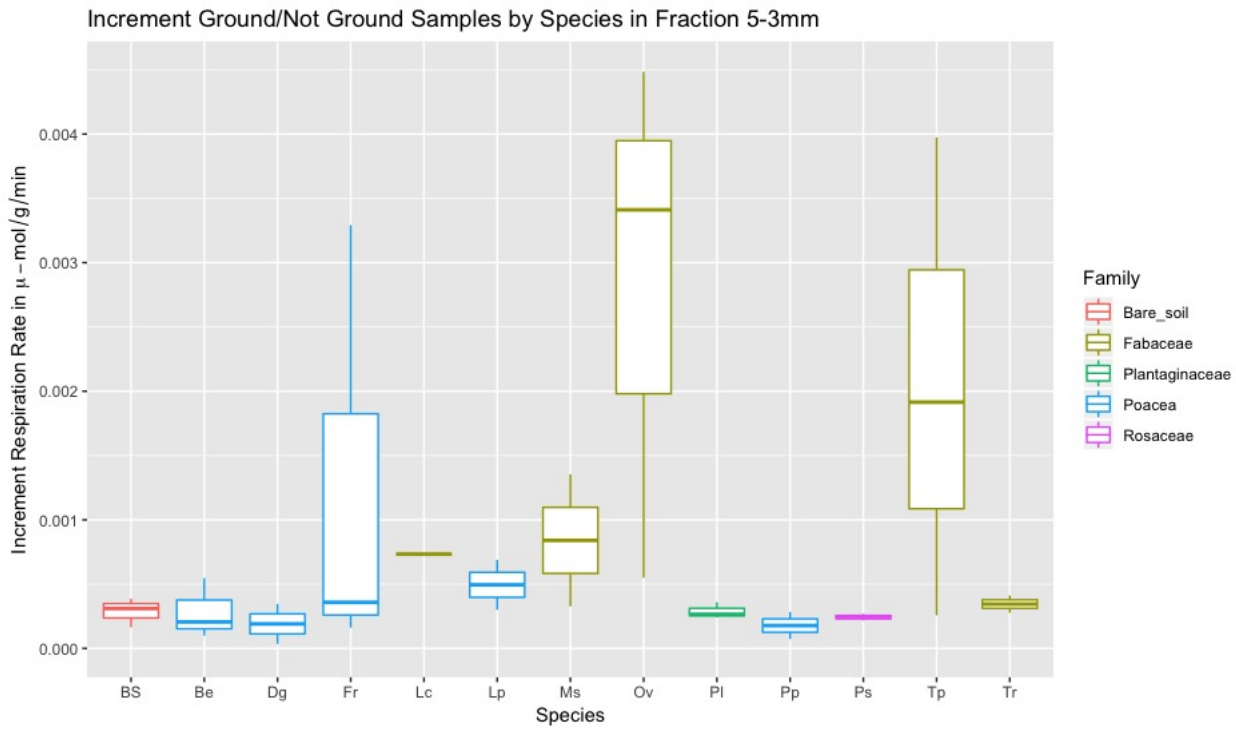

Figure 5.25: Comparison increment of respiration rate in dependence on plant species - fraction 5-3 mm

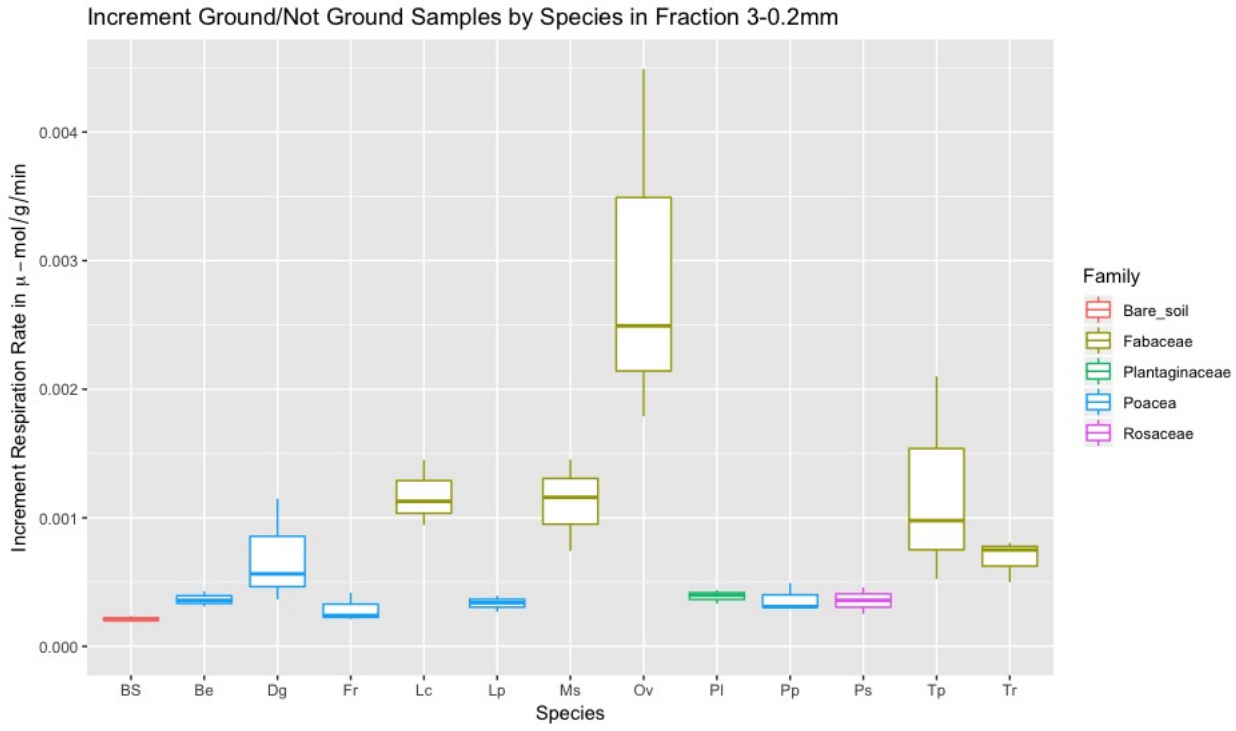

Figure 5.26: Comparison increment of respiration rate in dependence on plant species - fraction 3-0.2 mm

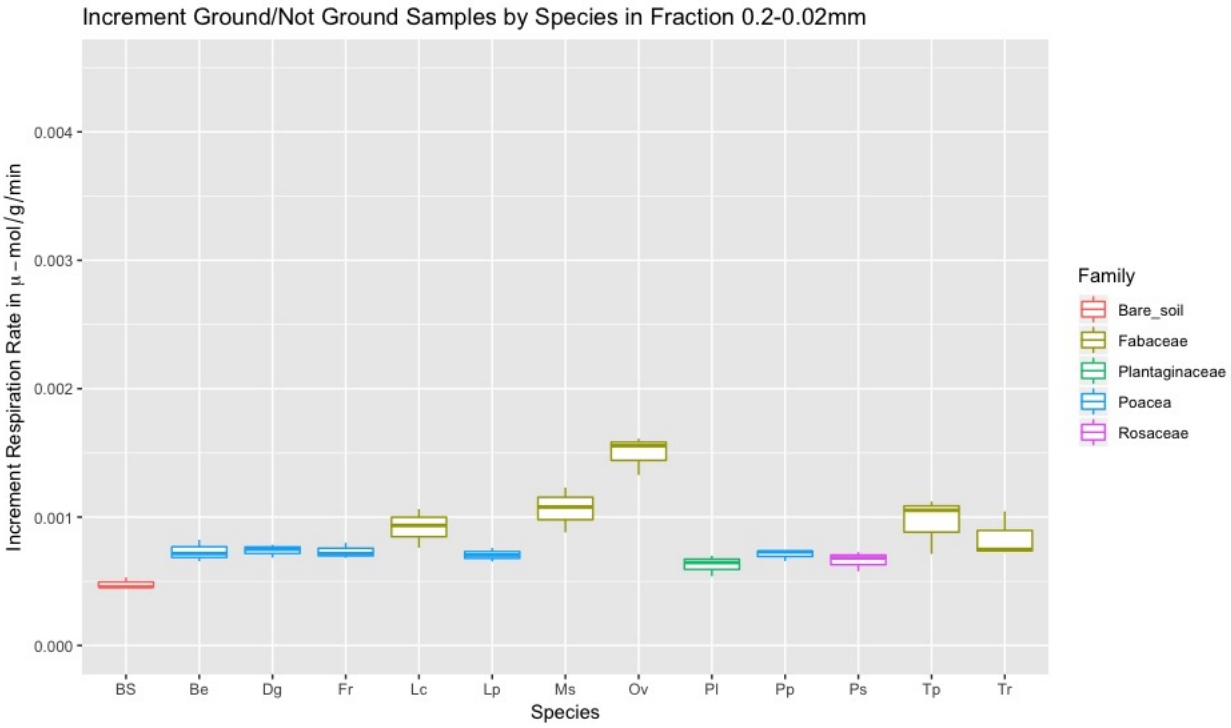

Figure 5.27: Comparison increment of respiration rate in dependence on plant species - fraction 0.2-0.02 mm

Chapter 6

Discussion

This chapter offers a discussion of the results presented above. Unlike the previous chapters, this present one does not discuss the two projects separately. Rather, it discusses the results of the same experiments in the same section, pointing out parallels where possible.

6.1 Aggregate Stability

6.1.1 TERRE

The most salient results that can be summarised from the experimental results concerning aggregate stability in the TERRE experiment are that:

- the stability of aggregates is generally higher in topsoil

  and

- that significant differences between the stability of aggregates in dependence on the cultivation treatment could only be observed in topsoil. Even here, the difference was only significant when comparing the non-cultivated control treatment of bare soil with the cultivated soil. There was no significant difference between the effect of the species used for cultivation.

The first of these results - that aggregate stability is higher in topsoil than in subsoil - is in accord with literature values (Zhao et al. 2014). The reason for the generally higher stability of aggregates in topsoil is that here, the stabilizing influences are generally more preponderant - from the higher content of SOM, which facilitates the formation of organo-mineral complexes, to the higher density of roots, which contribute to both physical and chemical binding mechanisms. Going back to the introduction on the general nature and mechanisms of aggregate formation,

the lower initial content of Soil Organic Carbon in subsoil can also be seen as a hindrance to the formation of microaggregates - and thus macroaggregates (Poirier et al. 2014).

However, while this aspect of the results was to be expected, several others were not. As mentioned above, a difference in aggregate stability could be seen between the cultivated pots and the pots left bare as a control treatment in the topsoil. Now, the question poses itself, why there was no such difference to be observed in the subsoil and why no difference between the species used for cultivation was visible.

Going back to the original hypotheses put forth for this thesis - that the relative effect of cultivation on aggregate stability would be higher in the subsoil than the topsoil and that Fabacean species such as *Medicago sativa* would have a higher impact on aggregate stability - the results outlined in the previous chapter would mean that both of them would have to be rejected. While it has been shown that direct carbon and nitrogen enrichment has had higher effects on subsoil than on topsoil (Keidel et al. 2018, Poirier et al. 2014), this is seemingly not entirely the case for the indirect enrichment of the soil with these elements through plant root exudates.

One possible explanation for this is that the cultivation conditions were not ideal during the experiment. As outlined in the introduction, the main focus of the experiment as devised (by the TERRE PhD student at AMAP, Lorenzo Rossi) was to trace carbon fluxes in the soil. It was for this reason that the plants in the experimental pots were grown not in the open air but in cultivation chambers at Ecotron, a facility at Montferrier just outside Montpellier, where the atmosphere could be controlled exactly and enriched with C^{13}. Even though the cultivation parameters were meant to offer conditions for ideal growth with respect to photoperiod, moisture etc., the plants did not prosper ideally in the chambers. As can be seen in Figure 6.1, especially plants grown on subsoil languished rather than flourished. This was to be expected in as far as subsoil offers fewer readily available nutrients to the plants and thus growth was bound to proceed more slowly (Skrindo and Halvorsen 2008).

The last aspect mentioned - the fact that growth on subsoil was expected to proceed more slowly than on topsoil - could also been seen in a more general manner. The experiment for TERRE only offered the plants a growth period of six months. It could very well be that this time span was too short for them to unfold their full effects on aggregate stability, especially under less than ideal cultivation conditions. This becomes especially salient when the results of TERRE are compared to those of TalVeg - an experiment that lasted two years with open air cultivation. In TalVeg, as will be explained more closely in the subsequent section, clear differences between the effects of different species on aggregate stability, could be discerned.

To summarize, it can be said that the effect of cultivation is visible, even after a relatively short growth period under less than ideal conditions, on topsoil. It would probably take longer or require a better cultivation environment for similar effects to become visible on subsoil as well, and for distinctions between the effect of the cultivation of different species to become entirely apparent. In the way of an outlook, it might be interesting to factor the biomass development - especially the root biomass - into the analysis of results. A closer look at the root density in the pots in the different treatment might provide a particular improvement in the understanding of the influence of the cultivation of different plants on aggregate stability.

Figure 6.1: Comparison of cultivation success on subsoil (left) and topsoil (right) - *Lolium perenne*

6.1.2 TalVeg

To summarise the most striking results for the aggregate stability tests in TalVeg as outlined in the previous chapter, they are as follows:

- Aggregate stability is higher at a sampling depth of 0-10 cm than at a sampling depth of 10-20 cm
- There is a significant difference between the cultivation with different species at both depths, but higher at a depth of 10-20 cm.
- Trends regarding the difference between the effect of the cultivation of different plant families on the stability of aggregates could be observed only at a depth of 10-20 cm. However, these results show a great variability, for example in the species of *Dactylis glomerata*. In general, Poaceans show the greatest effect, with Fabacean species in second place.
- There is a generally high variation in all of the results for this experiment.

The first of these results - the observation that aggregate stability is higher the closer the sample was taken to the surface - is in accord with results found in literature (Zhao et al. 2014). This is a parallel to the results of TERRE, described above, but it has to be noted that the samples referred to as subsoil in TERRE stem from far greater depths and experienced different cultivation conditions. TERRE's subsoil samples were taken at depths of over one meter and

thus have far lower concentrations of SOM, truly belonging to the mineral subsoil. Furthermore, the subsoil samples from TERRE were brought to the surface and cultivated directly, while the "subsoil" samples for TalVeg were simply taken from a slightly deeper part of the soil core that was sampled in the cultivation boxes. The samples that were taken in TalVeg at a depth of 10-20 cm still belong largely to the organic topsoil. Nonetheless, it is nonetheless interesting that the comparatively negligible difference in depth still yields significantly different results in relation to aggregate stability. This, most likely, can be traced back to lower root densities and thus lower concentrations of SOM at this slightly greater depth in the soil.

One of the most interesting questions arising from the results highlighted above is why the effect of cultivation is higher at the greater sampling depth of 10-20 cm. It is possible that the stabilizing effects were more pronounced at a lower depth because roots or SOM in general accumulated at these depths. Considering the longer cultivation period in the TalVeg project and the better cultivation conditions in the open air when compared to TERRE, it is possible that the roots of the plants developed well and would have penetrated the soil even further if it had been possible in terms of the depth of the pots. In this case again, data on root biomass and root density would be helpful in furthering our understanding of the matter. It could also be that due to the better cultivation conditions and longer cultivation time, the greater relative influence of the carbon-enrichment on aggregates in subsoil in comparison to topsoil that was observed by Keidel et al. 2018, Poirier et al. 2014, already mentioned above, could unfold in TalVeg.

Regarding the initial hypothesis applicable to this experiment - namely that Fabaceans would have a higher effect on aggregate stability (Haynes and Beare 1997) - it has to be rejected. As outlined above, it was actually the Poacean species that have the most pronounced effect on aggregate stability. The reason for this is probably that the longer cultivation period of two years has allowed the Poacean species to form an extensive root network. The original hypothesis was based on the fact that due to the symbiotic bacteria associated with their roots, Fabacean species would have an advantage with regard to growing speed and root biomass development and thus SOM enrichment in the soil. However, while this is visible to some extent and Fabacean species still exhibit a higher effect on aggregate stability than, for example, the Rosacean species, considering the longer cultivation period, Poaceans have probably had time to compensate for the Fabacean advantage.

The final point outlined above - namely that the variation is generally very high among these samples - may be traced back to either the nature of the soil sample or the protocol of the test.

With regard to the soil sample, it must be emphasised that the soil used for TalVeg displayed an abundance of small stones. For this reason, 10 g were used for the aggregate stability test, instead of the 5 g used for TERRE. This is because the last step of the aggregate stability tests - after immersion first in water an then in ethanol and subsequent drying and sieving - involves the washing of the largest resultant aggregate fraction. This is meant to separate actual large remaining aggregates from stones that had just been lightly coated with earth. However, even with the comparatively high amount of soil sample used for this test, the final step showed that about 80% of the initial weight of the sample consisted of stones. Consequently, the results could have been influenced greatly by even a small increase or decrease in the number of stones

in the soil subsample.

With regard to the second aspect - the nature of the protocol of the aggregate stability test, it must be pointed out that this test was not conducted by the author of this paper alone. Due to the large number of samples, magnified by three by the number of replicates, a laboratory assistant at AMAP conducted half of the tests. The protocol specifies an immersion of the aggregates in water for precisely ten minutes. After that, the water is gently removed from the beaker in which the aggregates have been immersed and the aggregates are gently moved to a sieve immersed in ethanol. Then, the sieve is gently shaken to separate the fraction smaller than 50 micrometers and with the help of a spray flask filled with ethanol, the remaining aggregates are gently transferred to a petri dish in which they are subsequently dried in the oven. It now has to be understood that the gentleness and precision with which any of these actions are carried out may vary considerably between two persons and that part of the variation in the results may also be attributed to this.

6.2 Respiration Tests

6.2.1 TERRE

To summarise the most striking results from the respiration tests for TERRE, they are the following:

- Generally, respiration is higher in topsoil samples.
- A significant difference between the ground and not ground sample can be observed only in the aggregate fraction 3-0.2 in the topsoil subsample.
- Species have a significant effect on the respiration rate in all of the fractions in the topsoil and the subsoil subsample.
  - In topsoil, cultivation with *Medicago sativa* and *Lolium perenne* led to higher respiration rates in both ground and not ground samples.
  - In subsoil, cultivation with *Medicago sativa* always resulted in higher respiration rates, but cultivation with *Lolium perenne* led to lower respiration rates in the samples that had been ground - even compared to the bare soil treatment.
- There was no significant difference in the increment of respiration between ground and not ground samples to be observed between the different treatments in any of the fractions.

To begin with the generally higher respiration in the topsoil samples, this is entirely within the realm of expectation. The reason for this higher respiration, referring back to the introductory chapter of this thesis, is the higher content of SOM in the topsoil samples. Since more

organic matter, and in particular, organic carbon is available, more metabolic activity and thus respiration, occurs.

Concerning the reason why it is only in the topsoil subsample in the aggregate fraction of 3-0.2 mm that a significant difference in respiration rate between ground and not ground samples can be observed, it can be theorised that it is due to both the dynamics of aggregation and the nature of aggregates at this size.

Regarding the first of these points, one must refer back to the results of the aggregate stability tests outlined above. As they have shown, no significant aggregation occurs in the subsoil. Therefore, it matters less if the few aggregates that have formed are destroyed through grinding as the amount of carbon that becomes available for respiration is negligible. Therefore, even though aggregation in subsoil was limited, it matters little in relation to carbon protection because there is only a very low level of carbon to protect in the first place.

As for the second point - the nature of aggregates at this size - one must refer back to the introductory chapter on aggregate formation. The aggregates in the larger fraction of 5-3 mm are actually clogs, consisting of macroaggregates as well as organic matter etc. It may be theorised that due to the greater spatial variability of organic matter in these kinds of aggregates, and the greater amount of organic matter already readily available without grinding, the amount of carbon that does become available after aggregates have been crushed, is in fact negligible. Moreover, the pores in these kinds of aggregates are relatively big, allowing microorganisms to move rather easily, granting access to SOC. If one pursues this train of though further, the aggregates in the smaller fraction of 0.2-0.02 mm may be protecting an amount of carbon so small that it is in itself negligible. Therefore, the significant difference in the fraction of 3-0.2 mm could be explained by the fact that it is only in this fraction that no larger amounts of organic matter are available for metabolisation before crushing, but that at the same time, the amount of organic matter that becomes available through crushing is still large enough to effect a significant difference in the results of respiration tests. In the literature, evidence regarding the connection between aggregate size classes and SOC respiration is mixed. In an experiment comparing the SOC values of topsoil and deep organic soil in relation to aggregate stability in subtrotpical China, Fang et a. (2015) found that smaller aggregates generally had a higher concentration of organic carbon than larger ones, which might also go towards explaining the variation observed here. However, it has to be noted that in this study, not only were the aggregate size classes different than the ones used for this experiment (they classified "small" aggregates as having a diameter of under 1 mm, while everything above that was classified as "large" - thus the category 3-0.2 mm used in this thesis was basically split), but also the subtropical soil investigated by Fang et al. differed fundamentally in its basic properties from the Mediterranean soil used in the TERRE and TalVeg experiments. On the other hand, Fernandez et al. (2010) and Nollemeyer et al. (2008) found that aggregates in size classes between 1 and 4 mm exhibited higher respiration rates than those smaller than 1 mm.

Another striking aspect of the results outlined above is that cultivation with *Lolium perenne* lowers the respiration rate in ground subsoil samples. The only feasible explanation for this seems to be that a factor that had not been considered during sampling interfered - namely the moss that grew on the bare soil of the control treatment. There was a sparse growth of moss on this bare soil - the spores probably airborne even in the tightly controlled environment of the

Ecotron chambers. This moss was mostly removed during sampling but it was not explicitly considered in the sampling methodology. It would have been necessary to discard the top few centimetres of the sample to completely eliminate this factor - but this was only determined after the samples for the respiration had already been taken. It is therefore quite possible that some of the soil contained accidentally crushed moss and that that led to the higher respiration values in that particular sample of bare soil, thus making it seem like the *Lolium perenne* sample had a lower respiration rate than the bare soil.

In general, it was *Medicago sativa*, the Fabacean species, that had the highest respiration rates in all of the topsoil samples, both ground and not ground. This is in accord with the first hypothesis outlined at the beginning of the thesis. It can most likely be traced back to the high activity of the nitrogen-fixing symbiotic root bacteria associated with Fabaceans. Luo et al. (2016) found similar results in their study investigating the impacts of nitrogen-fixing and non-nitrogen-fixing tree species on soil respiration in China. However, in this study it is further specified that there is a disconnect between soil respiration, on which the nitrogen-fixing species had the highest impact, and the SOC content of the soil, which was not influenced in significantly different ways by the different species.

One last thing that has to be briefly mentioned is the fact that the increment in respiration rate between ground and not ground samples - which is supposed to be a measure for the protection of SOC by aggregates - is not significantly influenced by any of the treatments. This could possibly be attributed to the fact that the cultivation period of the TERRE experiment was too short for the full effects of aggregation to unfold. While some differences in respiration rate have been observed depending on which species the soil had been cultivated with or whether it had been left bare, it might have been too soon to see if the process of aggregation would also significantly impact the protection of carbon by aggregates in the soil.

6.2.2 TalVeg

The most salient results outlined for the respiration tests in the TalVeg experiment in the previous chapter are the following:

- There is no significant difference between the respiration rates in the ground and not ground samples in any of the fractions.
- There are significant differences with regard to the respiration rates in dependance on the plant family in all of the fractions and in dependance on the species used for cultivation in all of the fractions except for the not ground samples of the fraction 5-3 mm. Generally, samples that had been cultivated with Fabaceans exhibited the highest respiration rate.
- The increment in the respiration rate between ground and not ground samples, though not significant in itself, varies significantly in relation to both the plant species and family in the fraction 3-0.2 mm and 0.2-0.02 mm. Here too, Fabaceans showed the highest values.

One of the most striking points in these results is the lack of significant differences between

ground and not ground samples. A possible explanation may lie in the nature of the soil sample. As mentioned already in the discussion of the aggregate stability results for TalVeg, the soil used in the experimental setup was extremely stoney. As a result, the samples, especially those in the larger aggregate fractions, could not be crushed entirely as it would have taken too much time and energy to smash the many small rocks in this fraction. As a result, the samples were crushed for several minutes until it appeared that most of the aggregates that had been mixed among the stones had been destroyed. Another possible reason for the lack of significant difference may lie in the fact that for TalVeg a total of 12 different species was under investigation and that the variability between species was rather high.

In order to underline this, one may look at the significant results concerning the increment in respiration rate. Calculated by deducting the respiration rate in not ground samples from the respiration rate in ground samples, the increment is a measure for the carbon that becomes available when the protection by aggregates is destroyed. Consequently it can be deduced that not only do the various species and families have a significant effect on carbon protection in the fractions 3-0.2 mm and 0.2-0.02 mm, but also that the family with the highest effect is the Fabaceans. The reason why this is not the case in the fraction 5-3 mm may lie in the great spatial variability of organic matter already discussed in the previous section. In general though, the results are in accord with the hypothesis that Fabaceans have a higher effect on carbon protection through aggregates due to the activity of the symbiotic bacteria in their roots.

Chapter 7

Summary and Outlook

This MSc thesis presented an investigation of the effect of the cultivation of soil with different species on the stability of soil aggregates and their function in protecting Soil Organic Carbon from metabolisation by microorganisms. This research was conducted in the framework of two larger projects - TERRE and TalVeg at the Institute National de la Recherche Agronomique in Montpellier, France.

Both projects involved the cultivation of species from different plant families on soil. For TERRE this cultivation was conducted on topsoil and subsoil with *Medicago sativa* and *Lolium perenne*. It lasted six months and took place not in the open air, but in a tightly controlled environment in growth chambers at Ecotron because part of the aim of the overall experiment was to trace the movement of C^{13} through the soil. The cultivation for TalVeg took place over a period of two years in the open air at Montpellier with a total of 12 different species from four different plant families. Both experiments also included a control treatment with bare soil.

The main research questions were which species and families were most effective in increasing the aggregate stability and carbon protection by aggregates and, for TERRE, what the differences were between the cultivation of the same species on topsoil and subsoil. In order to answer these research questions, soil samples were taken from both experiments, dried and sieved. Aggregate stability tests according to the methodology developed by Le Bissonnais (1996 1997) were conducted. Furthermore, respiration experiments were carried out which involved the addition of water to dried samples - both crushed and uncrushed - at the three aggregate fractions of 5-3 mm, 3-0.2 mm and 0.2-0.02 mm and the subsequent measurement of the respiration rate between days three and seven of the test. This latter procedure was meant to gauge the effect of carbon protection by aggregates by comparing the respiration rates in samples with intact aggregates and samples in which the formerly protected carbon had become available through the destruction of aggregates.

The results showed that for the aggregate stability tests, the aggregate stability was far higher in topsoil in TERRE and at a more shallow sampling depth of 0-10 cm in TalVeg. This is in accord with literature values. It was furthermore found that in TERRE in the topsoil samples, a significant difference between the cultivation with *Medicago sativa* and *Lolium perenne* and the

control treatment with bare soil was discernible. However, no difference between the cultivation of these two species with regard to aggregate stability could be observed. Furthermore, there was no significant difference between any of the treatments in the subsoil samples. These results can probably be explained by the fact that the growth conditions in the Ecotron chambers were not ideal for the plants and that it would have taken a cultivation period of more than six months for them to fully unfold their effects on soil aggregates. For TalVeg it was furthermore found that the effect of cultivation was more pronounced at the lower sampling depth of 10-20 cm, even though aggregate stability was overall higher at a more shallow depth, and that Poaceans had the highest stabilising effect on aggregates, followed by Fabaceans. An explanation for these results can probably be found in the longer cultivation period in the TalVeg experiment. Over the span of two years, the plants have had time to fully develop and Poaceans have had the opportunity to compensate for the initial competitive advantage given to Fabaceans by their symbiotic root bacteria. Furthermore there is the consideration that the root system might have developed so extensively as to result in a accumulation of roots at a lower depth because they could penetrate no further. Another observation for TalVeg was that the individual variability of the samples was very high, which might be traced back to the large proportion of small stones in the samples.

The results for the respiration experiments were varied. For TERRE there was a significant difference between ground and not ground samples - and thus a significant effect of aggregates for carbon protection - solely in the aggregate fraction of 3-0.2 mm. The increments showed no significant relation to any of the treatments in any of the fractions - which might have been due to the short cultivation period, which did not allow the plants to reach their peak effect on the protection of carbon in the soil aggregates. However, the respiration rates varied significantly between the soil aggregates taken from the three treatments, with *Medicago sativa* generally having a higher respiration rate than the samples that had been planted with Lolium perenne or left bare. The only exception to this was one subsoil sample that had been ground in which it appeared that *Lolium perenne* had a lower respiration rate than either *Medicago sativa* or the bare soil. This, however, was probably due to moss residue in the bare soil sample, which made its respiration rate appear higher than it actually was. For TalVeg the results showed no significant differences between crushed and uncrushed samples in any of there fractions - though this is probably due either to the high individual variation already alluded to above or the high variability between the twelve cultivation treatments. In the case of TalVeg clear significant differences exist in the increment of respiration in relation to both the family and the species used for cultivation in all three fractions. In this case, the Fabacean species have by far the highest positive effect on the protection of carbon in soil aggregates, most likely due to their symbiotic root bacteria.

Finally, one must reflect on the practical implications that can be derived from these results. For TERRE, it can be noted that in the short term, cultivation on topsoil achieves higher values of aggregate stability and, at least in the fraction 3-0.2 mm, a measurable effect on the protection of carbon by aggregates, than cultivation on subsoil. Furthermore, even though the cultivation for TERRE was not carried out on-site, it was clearly visible that cultivation success was far higher on topsoil than on subsoil. With a view to possible future research it would be extremely interesting to have the same cultivation experiments not in pots in growth chambers but in the open air and to have a longer cultivation period to see if the two different species unfold their individual effects later on. As can be derived from the observations in the TalVeg experiment -

in which the cultivation period lasted for two years - the effects of various species shift over time. It would be extremely interesting to see what form this shift takes comparatively on topsoil and subsoil. For TalVeg it can be surmised that in the long run Poaceans and Fabaceans have the highest positive impact on aggregate formation - though coupled with the high level of carbon protection by Fabacean species, these might be a preferable choice for cultivation if carbon fluxes are taken into consideration. For both TERRE and TalVeg it would also be extremely interesting to factor the biomass data both for the above ground biomass and the root biomass in these soil samples into the considerations. Lastly, it would be fascinating to compare the results obtained from these experiments with results from parallel experiments in which the subsoil used for cultivation is fertilized in order to achieve a similarly high biomass as by cultivation on topsoil.

Overall, it can be concluded that even though the two projects discussed in this thesis have different experimental setups and overall different focuses, common conclusions regarding the stability of aggregates and protection of carbon can still be drawn from the results obtained. These results, as discussed above, have some implication for geotechnical and eco-engineering applications, though further research and a more extensive analysis of data than is feasible in the framework of this thesis is still needed to fully understand all the implications of the cultivation of these species on topsoil and subsoil for the stability of aggregates and the protection of SOC.

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ThesisFinal

Appendix

TERRE

TERRE Respiration Experiments

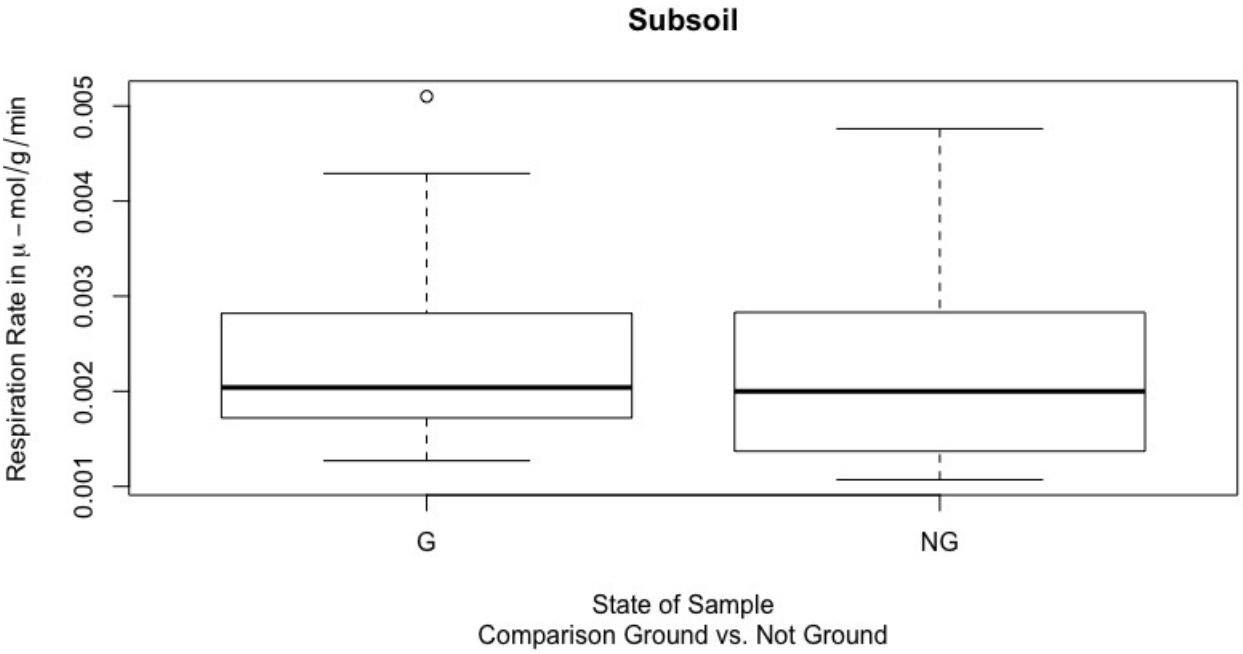

Figure 7.1: Comparison respiration rates between ground and not ground samples - subsoil

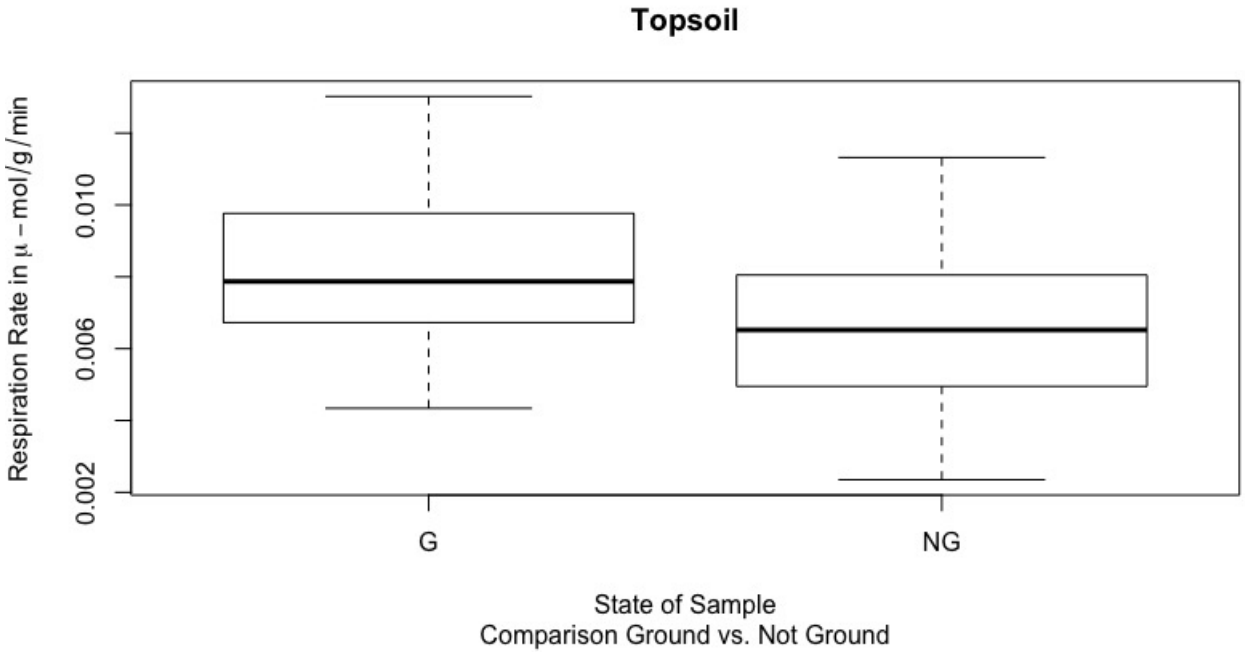

Figure 7.2: Comparison respiration rates between ground and not ground samples - topsoil

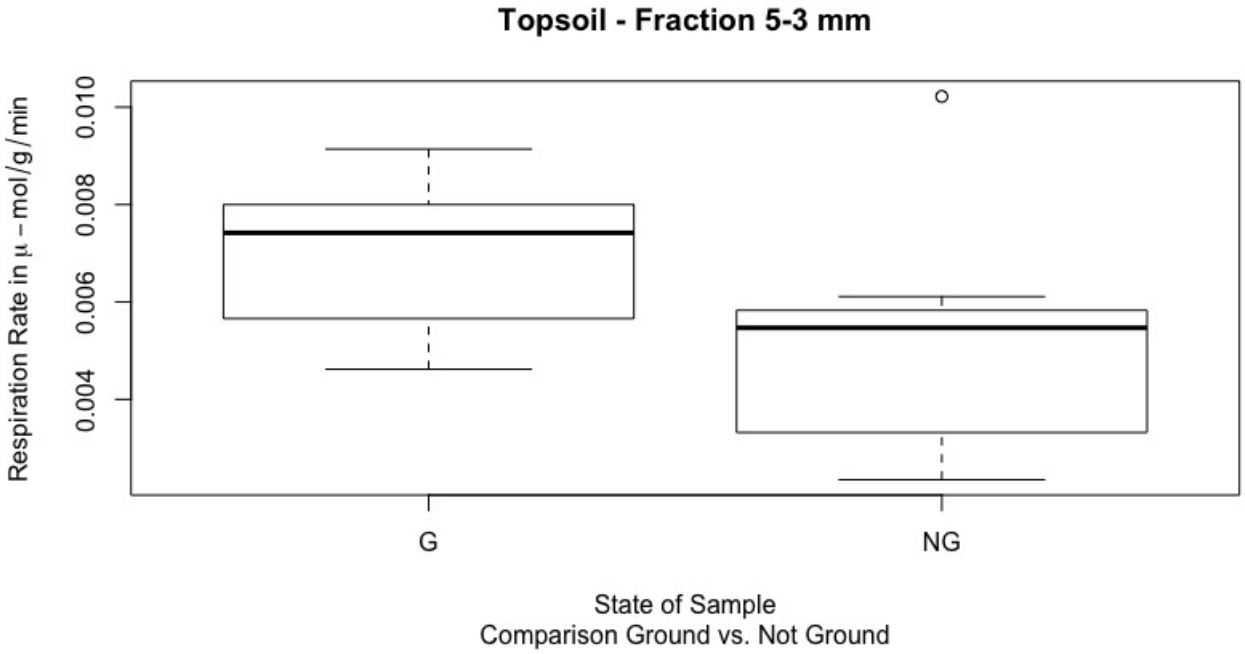

Figure 7.3: Comparison respiration rates between ground and not ground samples - topsoil fraction 5-3 mm

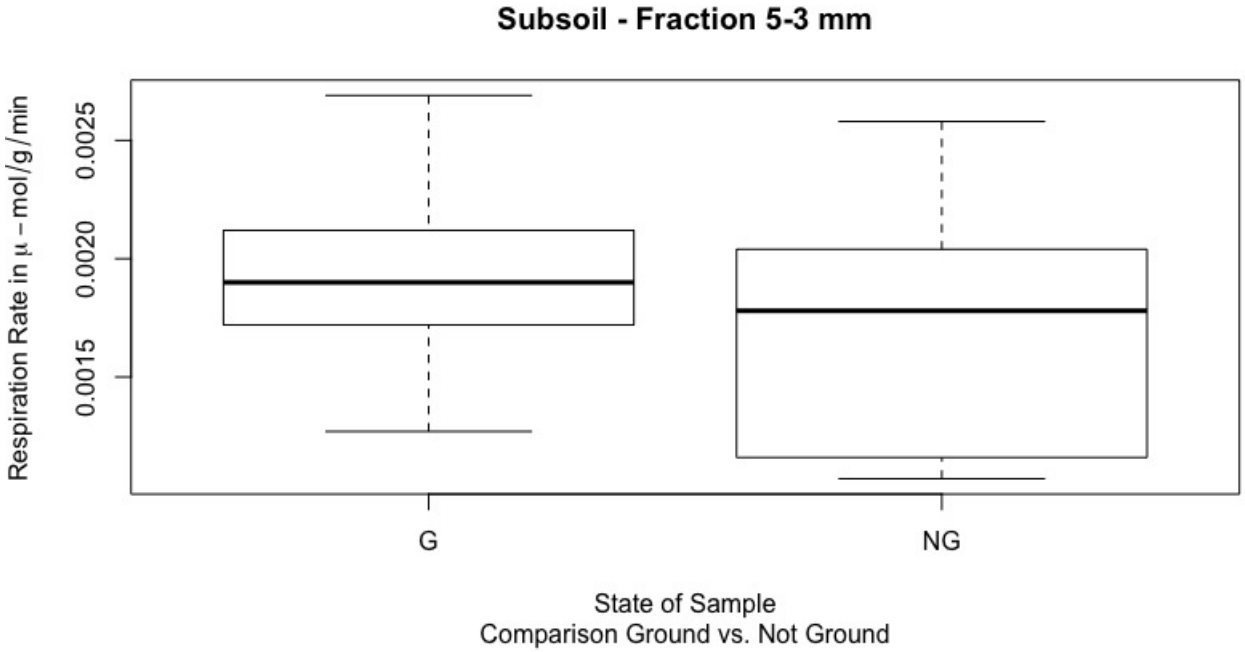

Figure 7.4: Comparison respiration rates between ground and not ground samples - subsoil fraction 5-3 mm

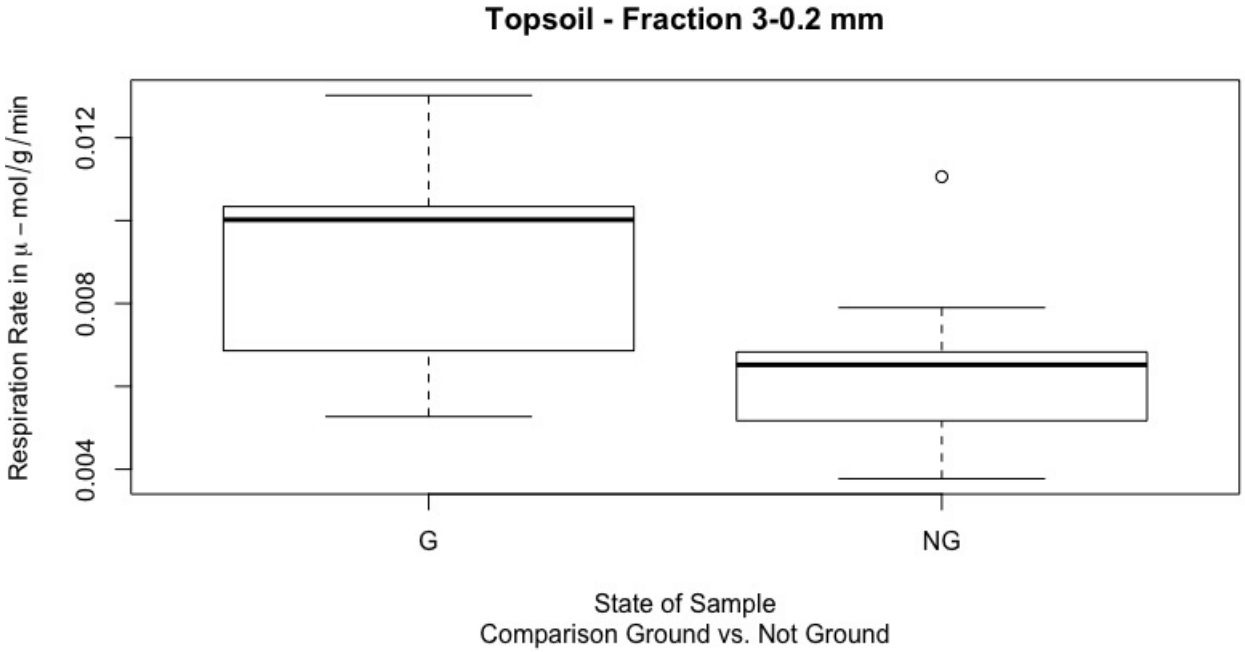

Figure 7.5: Comparison respiration rates between ground and not ground samples - topsoil fraction 3-0.2 mm

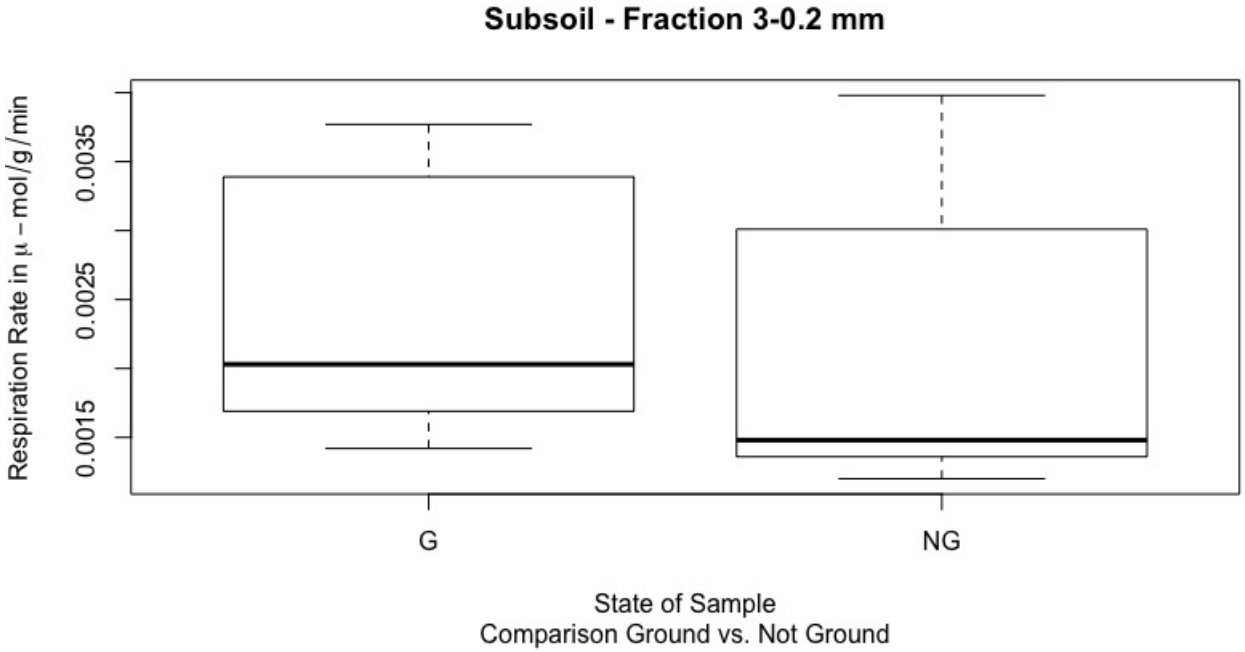

Figure 7.6: Comparison respiration rates between ground and not ground samples - subsoil fraction 3-0.2 mm

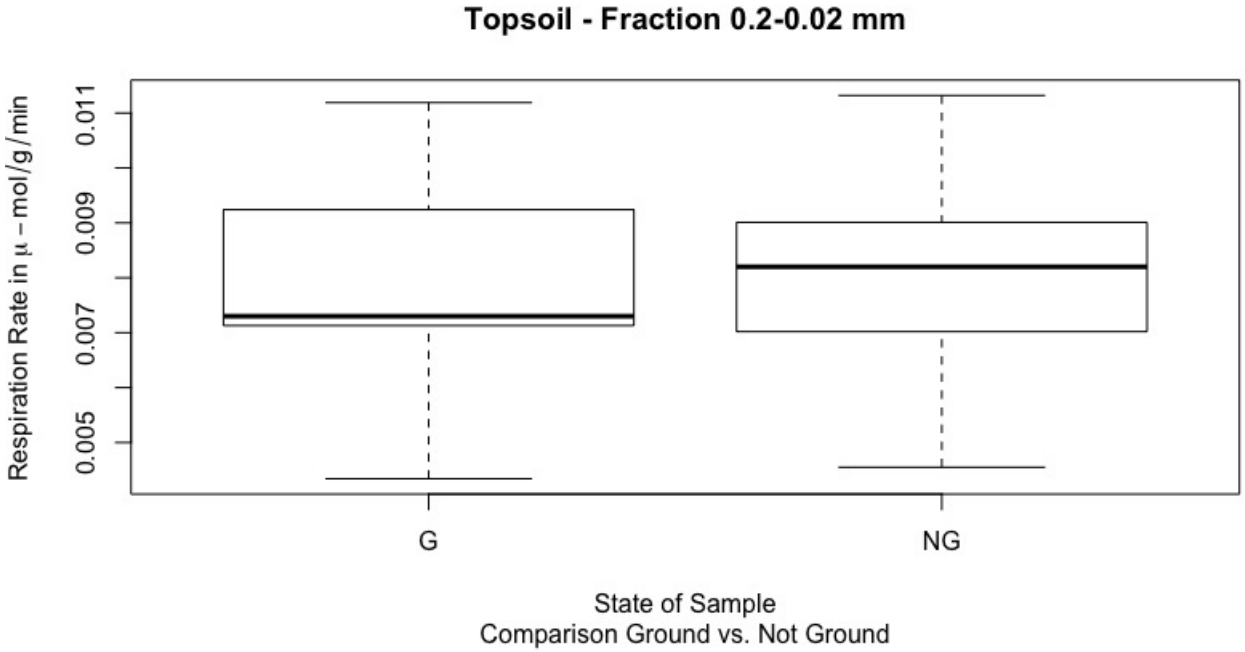

Figure 7.7: Comparison respiration rates between ground and not ground samples - topsoil fraction 0.2-0.02 mm

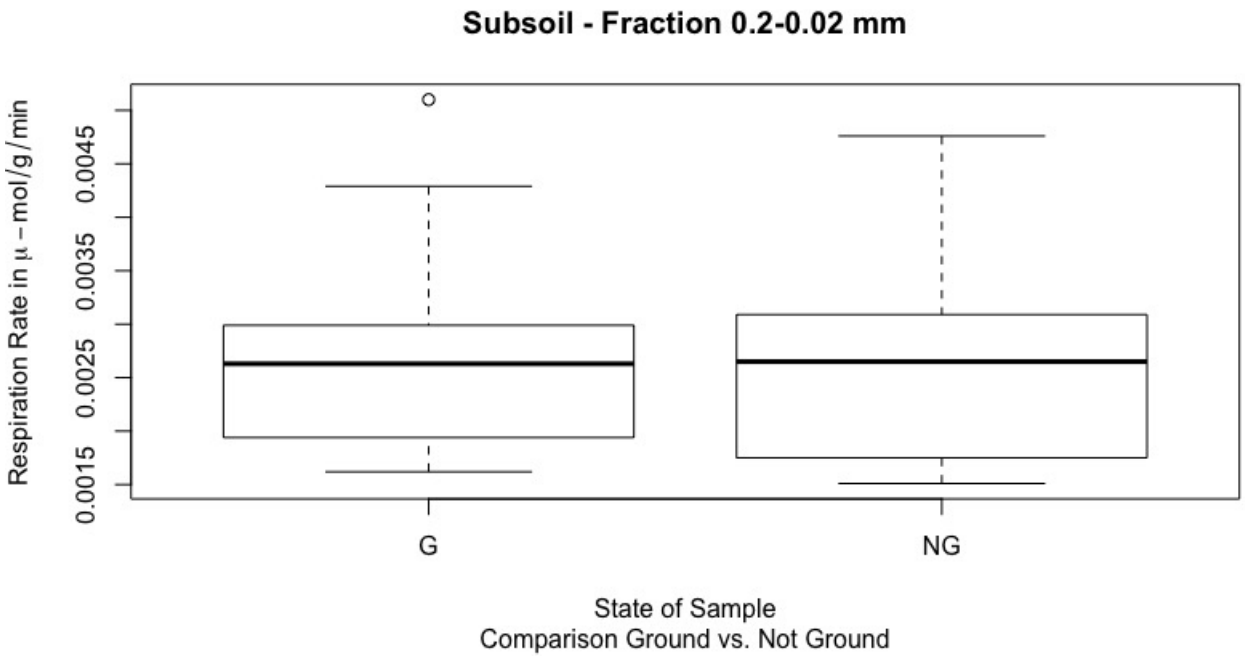

Figure 7.8: Comparison respiration rates between ground and not ground samples - subsoil fraction 0.2-0.02 mm

TalVeg

TalVeg Respiration Experiments

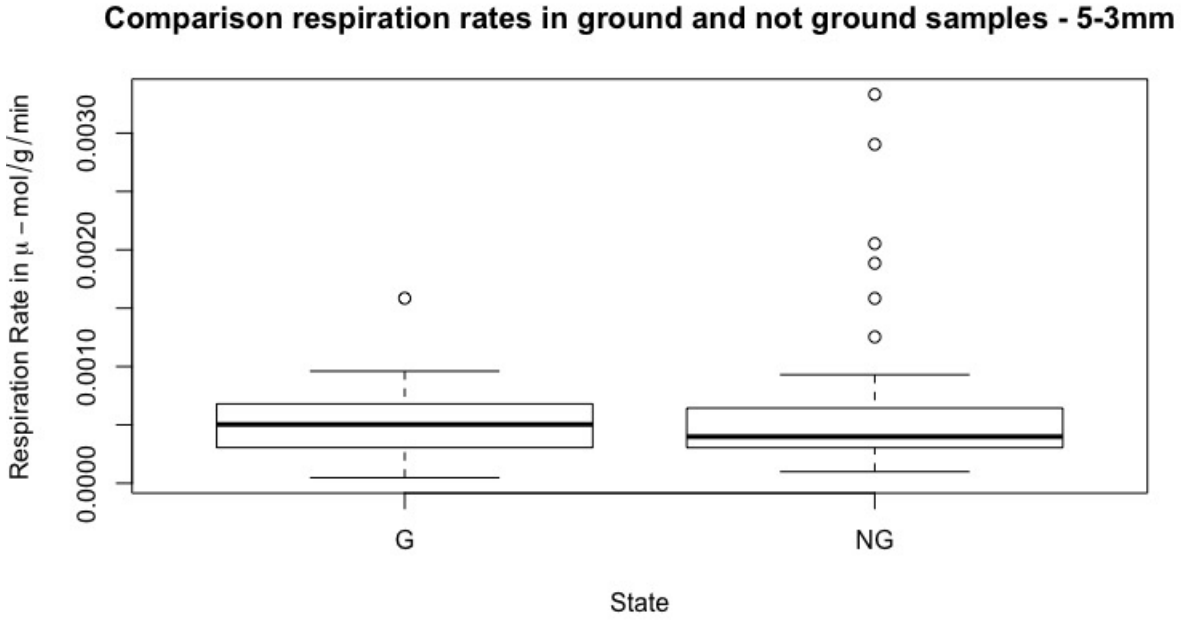

Figure 7.9: Comparison respiration rates between ground and not ground samples - fraction 5-3 mm

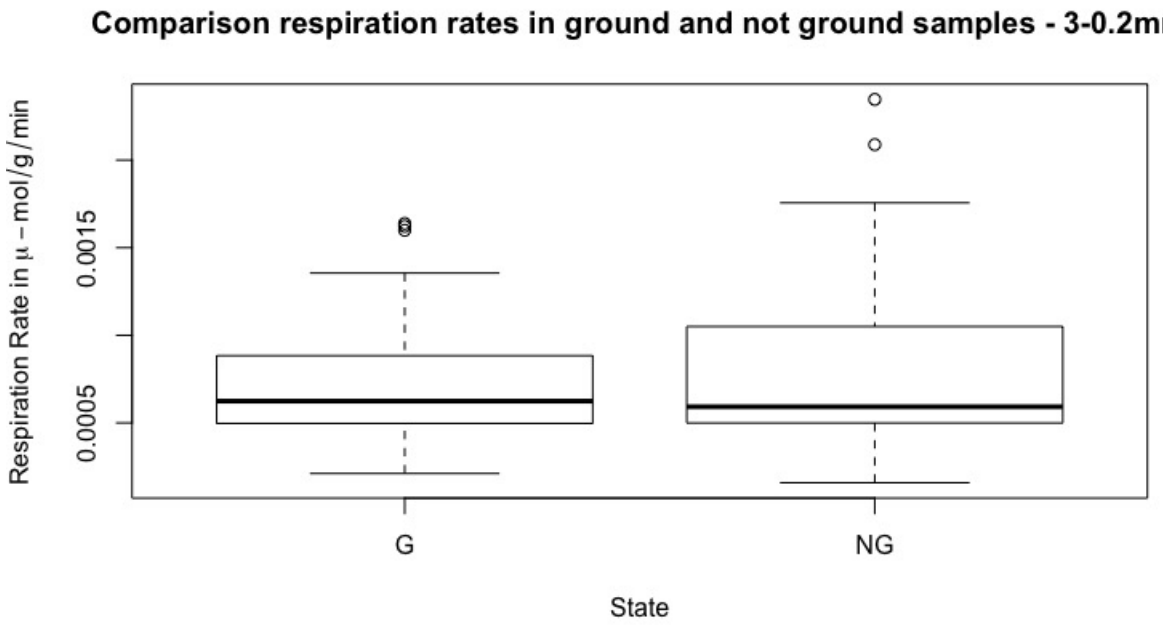

Figure 7.10: Comparison respiration rates between ground and not ground samples - fraction 3-0.2 mm

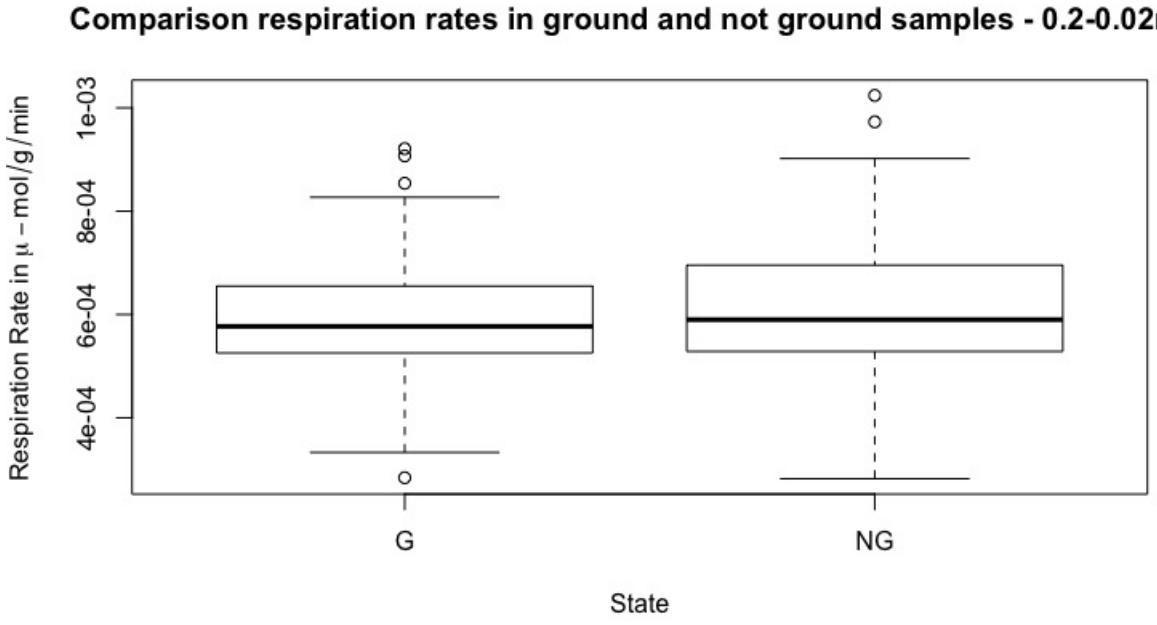

Figure 7.11: Comparison respiration rates between ground and not ground samples - fraction 0.2-0.02 mm