Plant litter decomposition in agro-ecosystems: a functional study on the effect of resource history, chemical composition of plant litter and the dynamics of the microbial community.

Master's thesis (7.5 ECTS)

Author: Manon Neilen (st. nr. 3354822), Master student Environmental Biology, Utrecht University
Examiner: Heike Schmitt, Institute for Risk Assessment Sciences (IRAS, Utrecht University)
2nd reviewer: Eiko E. Kuramae, Netherlands Institute of Ecology (NIOO-KNAW)

Prologue

This thesis is part of my master in Environmental Biology at the Graduate School of Life Science, Utrecht University. I chose to write my thesis in the form of a proposal for the AWL Open Call NWO programme. For my thesis I wrote the scientific background and the experimental set up for a study that we like to obtain a grant for. The other, practical, information is to be included later and is not part of my thesis. The paragraph numbers in this thesis are consistent with the numbers of the official proposal form. I hope you enjoy reading the application.

Table of contents

3a. Scientific summary (max. 250 words)

Please provide the scientific summary (similar as on the fact sheet). Please note that this summary will be used to invite reviewers to assess your proposal, it should therefore have sufficient scientific content. Word count: 250

Microbial decomposition of plant litter is of vital importance to agricultural ecosystems because it facilitates nutrient cycling in soils. The functional dynamics of the microbial community and their interaction with plant substrate chemistry are poorly understood. In the majority of global carbon models, soil communities are assumed to be functionally similar with regard to decomposition. The underlying idea is that a high degree of functional redundancy is present in microbial systems due to high species richness, rapid adaptation and physiological flexibility. However, this idea has recently been challenged. It is now considered likely that microbial communities are functionally dissimilar. However, the mechanisms by which these differences arise are not yet clear. Among the possibilities are local adaptation of communities or differences in overall ability, which implies that some soils are simply more active than others. This insight calls for research to clarify the interaction between soil microbial community and plant litter composition to provide better estimates for carbon models. Research questions that will be addressed are:

1) Are soils functionally dissimilar?

2) Is home field advantage or ability the driving mechanism for differences in litter degradation between temperate soils?

3) At what time scale does local adaptation occur?

The project is innovative as the interaction between the microbial community and plant litter chemistry in artificial soils has not yet been studied. Moreover, the use of state of the art methods like shotgun – mRNA metatranscriptomics and pyrolysis-molecular beam mass spectrometry will provide a high resolution insight in plant litter decomposition dynamics.

3b. Summary for the broad scientific committee (approximately 250 words)

Please provide a summary aimed at the division-wide committee (Earth and Life sciences). This summary may focus more on background and motivation than the scientific summary. Word count: 250

Decomposition of plant litter in soils is mediated by a multi trophic assembly of species of which the functional traits and interactions are only starting to be understood. During decomposition plant organic material is reduced to its basic chemical constituents, like ammonium, water, phosphate and carbon dioxide. These components then become available to the community again. The process of decomposition is of vital importance to natural as well as agricultural ecosystems because it facilitates nutrient cycling in soils. Microbial species, like fungi and bacteria, are the main players involved in this process. In the majority of global carbon models, microbial soil communities are assumed to be functionally similar with regard to decomposition. However, recent developments suggest that different soil communities may have different abilities to degrade plant organic matter.

This insight calls for research to clarify the interaction between soil microbial community and plant litter composition to provide better estimates for modelling carbon dynamics. In the proposed project we aim to study the dynamics of the community involved in of plant litter decomposition. We will set up experiments to find out whether soils are functionally different. Also, the mechanisms driving the possible differences will be explored.

The project is innovative because it is the first to simultaneously study the interaction between microbial decomposer community and plant litter chemistry in an artificial as well as natural agricultural soils. Moreover, the use of state of the art molecular and chemical methods will provide a high resolution insight in plant litter decomposition dynamics.

3c. Summary for the general public (max. 100 words)
Please provide in a title and summary for the general public, preferably in Dutch. Word count: 103

Titel: “Afbraak van plantaardig materiaal in landbouw bodems: een functionele analyse.”
Afbraak van plantaardig materiaal in landbouwbodems is een belangrijk onderdeel van de koolstofkringloop. Bacteriën en schimmels spelen een grote rol in het afbraakproces. In klimaatmodellen wordt vaak de aanname gedaan dat alle bodems op dezelfde manier plantaardig materiaal afbreken. Recent onderzoek wijst uit dat de activiteit en afbraakcapaciteit per bodem verschilt. Het doel van deze studie is om de relatie tussen de activiteit van bodemorganismen en de chemische samenstelling van het plantaardig materiaal te onderzoeken. Een beter begrip van de afbraakdynamiek kan bijdragen aan een betere inschatting van koolstofemissie uit bodems in klimaatmodellen.

4. Description of the proposed research

Max. 4 pages (and max. 3600 words); including figures, excluding literature references.
Include details of objectives, innovative aspects, scientific approach, preliminary data, impact, and literature references (include full bibliographical details). Word count: 3610

Introduction

Decomposition in soils is mediated by a multi trophic assembly of species of which the functional traits and interactions are only starting to be understood (Scharroba et al. 2012). However, it is clear that microbes play an important role. In many soils, microbes make up more than 90% of the biomass and they are now recognized as an important determinant of ecosystem process rates (Baldrian et al. 2012; Dilly et al. 2004; Hättenschwiler et al. 2011; Ng et al. 2014).The process of decomposition is of vital importance to natural as well as agricultural ecosystems because it facilitates nutrient cycling in soils (St. John et al. 2011; Baumann et al. 2009). Generally, two factors were considered to drive decomposition rates, being climate and litter quality (Freschet et al. 2012). Climate affects decomposition because temperature and moisture have direct effects on the activity of the microbial community (St. John et al. 2011). The recalcitrance of plant litter is thought to affect decomposition rates as well, with litter types that have more lignin decomposing at a slower rate compared to litter rich in labile carbon (Ng et al. 2014). Traditionally, microbial communities have been assumed to be functionally similar with regard to decomposition (Keiser et al. 2011). The underlying idea is that given the high species richness, rapid adaptation and physiological flexibility of microbial communities a high degree of functional redundancy is present in microbial systems. This way, any spatial or temporal change will only induce a negligible change in ecosystem processes (Strickland et al. 2009). This assumption of 'functional equivalence' of soil communities is the basis of the majority of terrestrial carbon models (Allison & Martiny 2008). In these models it is assumed that any soil community carries out decomposition at similar rates.

Although intuitively tempting, the idea of 'functional equivalence' has recently been challenged (Balser & Firestone 2005). That can be problematic if terrestrial carbon models based on static parameters will not represent true estimates of carbon loss from soils (Keiser et al. 2011). In recent studies, the functional make up of the decomposer community is starting to be considered a determining factor for plant litter decomposition. It is now clear that microbial communities display bio geographical patterns like the ones described in animals and plants (Hanson et al. 2012). So, if concepts like drift, selection, dispersal and mutation apply to microbial community assembly, it is unlikely that all communities are functionally the same. Although in recent years some efforts have been made, the dynamics of soil communities during decomposition of plant matter are still only poorly understood. In a recent litter transplant study we found dissimilar patterns of CO_2 production when applying maize litter to soils of different origin (Neilen et al. unpublished data). Several other studies confirm this result, suggesting that microbial decomposer communities are functionally dissimilar (Strickland et al. 2009; Keiser et al. 2014; Kagata & Ohgushi 2012; Cleveland et al. 2014)

One selection mechanism that can explain functional dissimilarity between decomposer communities is local adaptation of a microbial community towards a substrate. The chemical composition of a substrate puts selective pressure on the community, thereby favouring the species that are able to perform the degradation processes required. The process described here is thought to induce a 'home field advantage' (Gholz et al. 2000). That is, if a community encounters a substrate that it was

previously exposed to, the community will be able to maintain higher decomposition rates, as compared to a similar community that was not exposed to that particular litter type before.

It was proposed that local adaptation especially applies to the decomposition of recalcitrant compounds, like lignin and cellulose. These recalcitrant compounds may require specific enzymes or reactions that are only performed sufficiently by specialist taxa (Wallenstein et al. 2013). Another mechanism that may attribute to functional difference among soil communities is ability (Keiser et al. 2014). The idea is that, regardless of home field advantage, some communities may have a larger ability to degrade organic matter compared to others. These communities do not only maintain high respiration rates when exposed to their 'home' substrate, but are able to degrade any substrate at high rate (Keiser et al. 2014). The differences in ability among soils are suggested to arise from differences in substrate history, were communities exposed to more recalcitrant litter in the past maintain a higher ability compared to communities that arise in nutrient rich environments with labile substrates (van der Heijden et al. 2008). Both the home field advantage and the ability theory suggest that substrate history is a determining factor in shaping microbial communities in soils. So, in agricultural systems, where crops provide the main substrate input, the chemical nature of the crop may be of importance in shaping the microbial community. However, it is unclear at what time scale these effects occur.

In practice, the existence of functional dissimilarity turns out to be quite challenging to prove. Also, it is hard to distinguish between the possible underlying processes that cause communities to be dissimilar. Most studies use decomposition rates as a measure for adaptation or ability, thereby leaving out the species involved in the process (Gießelmann et al. 2011). Moreover, evidence for dissimilarity appears to be strongly context dependent. Studies that do find an interaction between soil community and substrate often involve transplants of highly distinct litter quality and ecosystems (Freschet et al. 2012). When more similar substrates and ecosystems are studied, often no significant interaction was found (St. John et al. 2011). Studies of microbial diversity and functionality in terrestrial decomposer communities are further impaired by their large diversity and abundance. Also, microbial distribution in soils is known to be highly heterogeneous, which makes it even harder to predict community response to plant litter addition (Bastida et al. 2009).

In this study we aim to gain a deeper understanding of the functional dynamics of microbial plant litter decomposition in agricultural soils. By complementarily studying 'true' agricultural soils and an artificial soil with known community composition we will provide a fundamental insight into the relevant interactions between soil and substrate, without losing the context of real world, true soils. We will use new molecular tools, like mRNA meta-transcriptomics, to link microbial diversity to function, thereby revealing the dynamics behind observed dissimilar decomposition rates in agricultural soils. Moreover, we will use Py-MBMS to perform an in dept survey of the change of the chemical composition of plant litter over time (Wallenstein et al. 2013). By combining these two methods, this study is the first to track the interaction between microbial decomposer community and plant litter chemistry at high resolution and from a functional perspective.

The research questions that will be addressed in this study are:

1) Are soils functionally dissimilar?

2) Is home field advantage or ability the driving mechanism for differences in litter degradation between temperate soils?

3) At what time scale does local adaptation occur?

Innovative aspects

Decomposition in soils is of major importance for terrestrial nutrient cycling and soil fertility. The interaction between the soil microbial decomposer community and plant litter chemistry has been the topic of many studies. Recent studies have shown that plant residue composition influences respiration rates (Neilen et al. unpublished data) and microbial community structure (Baumann et al. 2009). Nevertheless, little is known about the functional response of soil microbial communities to the chemical composition of plant litter. Activity of the decomposer communities is often studied by indirect measures and diversity of the decomposer community is rarely taken in to account. Moreover, the results are not consistent among studies. Studies that do find an interaction between soil community and substrate often involve transplants of highly distinct litter quality and ecosystems (Freschet et al. 2012). When more similar substrates and ecosystems are studied, often no significant interaction was found (St. John et al. 2011).

Understanding the functional dynamics of microbial decomposers will allow for more accurate estimates of soil nutrient cycling and global carbon dynamics. Insight in decomposition dynamics will provide more accurate parameters for models that are used to monitor global carbon cycle dynamics (Keiser et al. 2011).

In the project proposed here we aim to simultaneously study plant litter decomposition in a model system and in a range of natural agricultural soils over time. With the advent of new sequencing techniques it is now possible to study soil communities at much higher resolution. Shotgun mRNA meta-transcriptomics allows for studying the full mRNA profile of complex substrates, like soils. This way, the active functional genes of the entire community can be extracted and linked to the diversity of the soil community. The use of a model system with known species composition has huge advantages since the chemical properties can be optimised for mRNA extraction. Due to the simple nature of the community the functional response of the known microbial species will be relatively easy to track. Complementing the model study with the study of true agricultural soils will put the results in the perspective of the true field situation.

In addition, the chemical composition of the substrate residue will be monitored by means of pyrolysis Molecular Beam Mass Spectrometry (py-MBSM) so that differences in decomposition dynamics will become visible. Py-MBMS is a powerful method for high resolution evaluation of chemical composition of substrates. Previously it was successfully used in decomposition experiments to measure soil organic matter composition and carbon content (Wallenstein et al. 2013; Hoover et al. 2002). By combining these two methods we will obtain a comprehensive, high resolution picture of the complex interaction between soil community and plant substrate. Not only will this project provide insight into dynamics on species level, but also the metabolic capacity of the community will be revealed.

Scientific approach

In the proposed project three experiments will be performed. The first experiment is a long term experiment in which a range of soils will be exposed to different substrates in the lab. After a certain time, the substrates will be changed to see whether adaptation occurs. Experiment 1 will serve to study functional equivalence and adaptation in 'true soils'. Since true soils differ in a range of properties and are highly diverse, a second study which deals with these variables is set up. In experiment 2 an artificial soil will be designed and inoculated with a known mixture of microbial species. Again, this soil will be exposed to several substrates. The combination of experiment 1 and 2 allows for studying plant litter decomposition in a simplified model system, without losing the bigger picture of the true ecosystem variables. The third experiment aims to identify the effect of plant litter chemistry on the dynamics of the microbial community during decomposition. In the next paragraphs the experiments will be discussed in further detail:

Experiment 1): in the first experiment we will study the fundamental assumption of functional equivalence of soils that is underneath most carbon cycling models. We will examine microbial activity of several soils when we expose them to the same substrate. Substrate will be added to soils in meshbags so that the mass loss and change in chemical composition can be measured after incubation. Overall activity will be measured by means of respiration. Special focus will be on the functional make–up of the decomposer community. We will perform shotgun meta-transcriptomics to analyse functional genes (mRNA) involved in degradation as well as 16S and 18S rRNA genes. Simultaneously, the chemical composition of plant litter over time will be monitored by means of py-MBMS to see whether the composition of the residue after a period of decomposition is comparable among soils. This method will allow us to compare activities among soils and provide the first fundamental insight into the assumption of functional equivalence versus functional dissimilarity of soils. Advantages of these methods are: the possibility of linking diversity to function, high resolution data complementary study of microbial community and chemical composition of plant litter residue.

Experiment 2): in the second long term experiment an artificial soil will be created and inoculated with an community of bacterial and fungal decomposers with known species composition and ratio according to (Guenet et al. 2011). This way we rule out all variables (pH, clay content, C:N ratio) other then the microbial community that may affect plant litter decomposition. Simultaneously with the first experiment, we will expose the soil to different substrates and measure respiration and activity/identity of active community by shotgun sequencing of mRNA genes. Later on, some of the soils will be exposed to another substrate types to see whether the community activity changes. Activity of these soils can be compared to the soils that did not change substrate to see whether the ability to degrade a substrate changes. This experiment will allow for studying adaptation under controlled conditions (all environmental variables are kept constant). Advantages of these methods are: the artificial communitys provides a simplified model system for the study of plant litter decomposition. The highly controlled conditions allow for the study of the causal link between substrate composition and community functioning without taking into account environmental variables. Moreover, thanks to the low diversity and high resolution of the mRNA shotgun sequencing, new genes that function in breakdown of organic components may be identified.

Experiment 3): Examination of the effect of substrate composition on microbial degradation. This set-up was designed to distinguish between the two leading hypotheses about the mechanisms that may

cause functional dissimilarity in soils. During this experiment we will expose soils to their 'home substrate', some substrates with other chemical composition and to the individual components of the home substrate. If home field advantage (HFA) causes the difference in decomposition rate then the rate of CO_2 production and the diversity of functional genes is expected to be higher when decomposing 'home substrate' compared to the single components. If the mechanism of functional breadth causes functional dissimilarity then the soils from recalcitrant sites are expected to have higher decomposition rates and functional diversity under all substrates compared to soils from sites with labile substrate types. Advantages of these methods are: high resolution examination of functional response of the microbial community to very specific compounds.

Experimental set up (experiment 1)

A long term decomposition experiment will be set up to examine the microbial community involved in decomposition. For this study three types of soils with differential plant litter history (potato, aspen, maize) will be collected and brought to the lab. Additionally, the matching substrates will be collected. The chemical nature of the substrates ranges from labile (potato, lignin: 0.9% of biomass , C:N ratio: 12.1) to recalcitrant (maize, lignin 3.2% of biomass, C:N ratio: 35.9) (Flores et al. 2005). A litter transplant experiment will be performed in a way that all combinations of soil and substrate are present. The experiment will last for 300 days with new substrate added at 100 day intervals. Every seventh day soil samples and litter samples are taken to obtain a mRNA shotgun transcriptome and chemical analysis of the residue substrate (see methods below) (Myrold et al. 2014)(Wallenstein et al. 2013; Hoover et al. 2002). Complementary to the soil study the CO_2 production over time will be monitored by means of a respiration monitor (BiometricSystems, Darmstadt).

Experimental set up (experiment 2)

Along with experiment 1, another long term experiment will be performed using artificial soil with a known microbial community. The soil will be created according to the protocol proposed by (Guenet et al. 2011)**.** During each 100 day interval plant litter is applied to the soil, in a way that the plant litter type is the same during the first and the last interval, but a different substrate is applied during the second interval. Functional response of the community is monitored in the same way as in experiment 1.

Experimental set up (experiment 3)

A third, shorter term, experiment will set up to study the effect of chemical composition of plant litter on the microbial community. The experimental set-up for this experiment is similar to the one of experiment 1. The difference is that it will only run for 100 days. The soils will be exposed to individual compounds that occur in plant litter, like lignin, glucose and cellulose. Again, microbial activity will be monitored by means of respiration and mRNA-meta-transcriptomics and compared to the soil and home substrate treatment of experiment 1.

Methods and bioinformatics

mRNA shotgun metatranscriptomics

A metatranscript of the soil communities will be conducted at each timepoint for the different treatment. A shotgun sequencing approach will be used so that a profile of the full mRNA sequences of the communities is obtained. Sequencing will be performed using 454 GS FLX platform with Titanium chemistry. Although 454 sequencing obtains a lower number of reads per sample

compared to Illumina sequencing, the reads are longer (500 bp) and therefore the success of assigning function to the contigs is more successfull (Myrold et al. 2014). This method has been proven successful for several soil studies in the past (Carvalhais et al. 2012). Sequences assembly will be performed and the obtained contigs will be compared to databases that contain genes with known functions (NCBI non-redundant (nr) database (http://www.ncbi.nlm.nih.gov), the Intergrated Microbial Genomes database (http://img.jgi.doe.gov), cAZy ((Lombard et al. 2014) and the Metagenomics Analysis Server (MG-RAST, http://metagenomics.anl) (Carvalhais et al. 2012).

Analysis of chemical composition of plant litter: Py-MBMS

Pyrolysis – molecular beam mass spectrometry will be performed at the National Renewable Energy Laboratory (Colorado) to study the chemical composition of the plant litter substrate during the experiment. Methods as described by (Wallenstein et al. 2013; Hoover et al. 2002) will be implemented. The method produces pyrolysis products through heating which are then examined by means of a mass spectrometer. The relative abundance of a range of compounds can be detected through this method, like lignin, cellulose, alkahyl aromatics, carbohydrates, peptides, N compounds and nucleic acids (Wallenstein et al. 2013).

Complementary to the chemical and meta-transcriptome study the CO_2 production over time will be monitored by means of a respiration monitor (BiometricSystems, Darmstadt).

Creating an artificial soil

Soils are highly complex environments due to their heterogeneous nature and high diversity of organic compounds, minerals and organisms. In this project we aim to study the functional response, in terms of mRNA-gene transcript to plant litter chemistry. Creating an artificial soil, with known diversity allows for in dept examination of this process while minimizing the variables to be considered. We will use the protocol developed by (Guenet et al. 2011) which is based on mixing the elementary components, rewetting and creating aggregates. The advantage of building a 'soil' from scratch is that no unrealistic share of organic matter is introduced, which happens if a soil is sterilized due to the death of microorganisms. The protocol was designed and tested for a study were six strains of bacteria were inoculated in the soil. Further testing is necessary to adjust the protocol for a mixed community of bacterial and fungal species.

Preliminary data

To answer these questions we will use soils for which we detected functional dissimilarity in a previous study (Neilen et al, unpublished). In this study we focused on potato and maize leaf decomposition in different soils. Two types of soils were used, one with a history of potato cultivated in Buinen ((52°55'0"N, 6,49'0"E) and the other with a history of maize cultivated in Helvoirt (51°38'0"N, 5°14'0"E). Mesh bags with dried potato or maize litters were supplied to the soils as substrate for decomposition. All possible combinations of soil and substrate were applied and the activity of the communities was measured by means of a respiratory experiment and pyrosequencing of 16S (bacteria) and 18S (fungi) rRNA genes. The maximum respiration rate at both sites was higher when potato litter was added, as expected from the more labile nature of the potato leaf litter. However, the difference in respiration rate between maize and potato in the same soil were much smaller in Helvoirt compared to Buinen. Moreover, the respiration rate when maize straw was applied was much higher in Helvoirt compared to Buinen. These respiration results indicate higher

microbial activity when soils are subjected to plant litter that was encountered by the community before. For the bacterial but not the fungal communities we were able to detect differences in communities between sites, and also between applied substrates (fig. 1). In the currently proposed project we aim to further study this pattern by looking at it from a functional perspective and for a longer period of time. Also, we will include an additional soil and substrate type for a broader coverage. Moreover, the change of chemical composition of the substrate will be taken into account to provide a comprehensive view of the dynamics of plant litter decomposition.

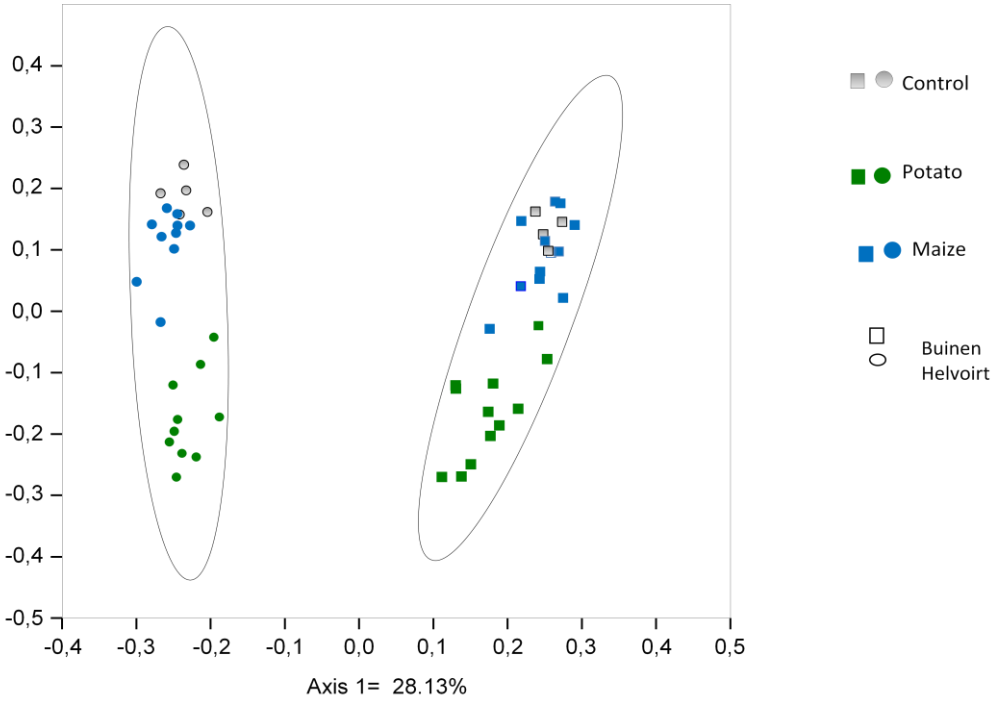

Figure 1. NMDS plot of bacterial communities based on 454 pyrosequencing of 16s rRNA genes. Circles enclose the OTUs extracted from Helvoirt (circles, left) and Buinen (squares, right). The colors indicate the type of plant litter substrate was applied to the soils during the experiment, green (potato), blue (maize) and grey (control).

Impact

Understanding the functional dynamics of decomposition in terrestrial ecosystems is of fundamental importance for understanding global carbon cycle dynamics. Currently, the assumption of functional equivalence among soils is embedded in the majority of terrestrial carbon models (Keiser et al. 2011). The project proposed here aims to study the microbial decomposition in agricultural systems from a functional point of view. By combining several state of the art methods, like studying artificial soil communities, shotgun mRNA meta-transcriptomics and py-MBMS, our study will allow for linking soil function to species diversity. This link will directly enhance our ability to predict ecosystem decomposition rates. This way our study will add to the development of future, more reliable terrestrial carbon models.

References

Allison, S.D. & Martiny, J.B.H., 2008. Colloquium paper: resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*, 105 Suppl (May), pp.11512–9.

Baldrian, P. et al., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME journal*, 6(2), pp.248–58.

Balser, T.C. & Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry*, 73(2), pp.395–415.

Bastida, F. et al., 2009. Soil metaproteomics: a review of an emerging environmental science. Significance, methodology and perspectives. *European Journal of Soil Science*, 60(6), pp.845–859.

Baumann, K. et al., 2009. Residue chemistry and microbial community structure during decomposition of eucalypt, wheat and vetch residues. *Soil Biology and Biochemistry*, 41(9), pp.1966–1975.

Carvalhais, L.C. et al., 2012. Application of metatranscriptomics to soil environments. *Journal of microbiological methods*, 91(2), pp.246–51..

Cleveland, C.C. et al., 2014. Litter quality versus soil microbial community controls over decomposition: a quantitative analysis. *Oecologia*, 174(1), pp.283–94.

Dilly, O. et al., 2004. Bacterial Diversity in Agricultural Soils during Litter Decomposition Bacterial Diversity in Agricultural Soils during Litter Decomposition. , 70(1).

Flores, S., Saxena, D. & Stotzky, G., 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology and Biochemistry*, 37(6), pp.1073–1082.

Freschet, G.T., Aerts, R. & Cornelissen, J.H.C., 2012. Multiple mechanisms for trait effects on litter decomposition: moving beyond home-field advantage with a new hypothesis. *Journal of Ecology*, 100(3), pp.619–630.

Gholz, H.L. et al., 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Global Change Biology*, 6(7), pp.751–765.

Gießelmann, U.C. et al., 2011. Lack of home-field advantage in the decomposition of leaf litter in the Atlantic Rainforest of Brazil. *Applied Soil Ecology*, 49, pp.5–10.

Guenet, B. et al., 2011. A new protocol for an artificial soil to analyse soil microbiological processes. *Applied Soil Ecology*, 48(2), pp.243–246.

Hanson, C. a et al., 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nature reviews. Microbiology*, 10(7), pp.497–506.

Hättenschwiler, S., Fromin, N. & Barantal, S., 2011. Functional diversity of terrestrial microbial decomposers and their substrates. *Comptes rendus biologies*, 334(5-6), pp.393–402.

Van der Heijden, M.G. a, Bardgett, R.D. & van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, 11(3), pp.296–310.

Hoover, C.M., Magrini, K. a & Evans, R.J., 2002. Soil carbon content and character in an old-growth forest in northwestern Pennsylvania: a case study introducing pyrolysis molecular beam mass spectrometry (py-MBMS). *Environmental pollution (Barking, Essex : 1987)*, 116 Suppl , pp.S269–75.

St. John, M.G., Orwin, K.H. & Dickie, I. a., 2011. No "home" versus "away" effects of decomposition found in a grassland–forest reciprocal litter transplant study. *Soil Biology and Biochemistry*, 43(7), pp.1482–1489.

Kagata, H. & Ohgushi, T., 2012. Home-field advantage in decomposition of leaf litter and insect frass. *Population Ecology*, 55(1), pp.69–76.

Keiser, a. D. et al., 2011. The effect of resource history on the functioning of soil microbial communities is maintained across time. *Biogeosciences*, 8(6), pp.1477–1486.

Keiser, A.D. et al., 2014. Disentangling the mechanisms underlying functional differences among decomposer communities F. de Vries, ed. *Journal of Ecology*, 102(3), pp.603–609.

Lombard, V. et al., 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic acids research*, 42(Database issue), pp.D490–5.

Myrold, D.D., Zeglin, L.H. & Jansson, J.K., 2014. The Potential of Metagenomic Approaches for Understanding Soil Microbial Processes. *Soil Science Society of America Journal*, 78(1), p.3.

Ng, E.-L. et al., 2014. Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? *Soil Biology and Biochemistry*, 70, pp.54–61.

Scharroba, A. et al., 2012. Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth. *Soil Biology and Biochemistry*, 50, pp.1–11.

Strickland, M.S. et al., 2009. Testing the functional significance of microbial community composition. *Ecology*, 90(2), pp.441–51.

Wallenstein, M.D. et al., 2013. Litter chemistry changes more rapidly when decomposed at home but converges during decomposition–transformation. *Soil Biology and Biochemistry*, 57, pp.311–319.