

Microbial-faunal interactions shaping soil processes

Workshop GfÖ Specialists Group Soil Ecology

Humboldt-Universität zu Berlin, 5th to 7th of October 2011



Soil ecologists have to face an extremely complex ecosystem but – on the other hand – have sufficient knowledge and methodological advances at disposal for testing and formulating unifying principles across disciplines. Special attention should be drawn to: *i*) methods that connect ecosystem compartments, *ii*) model organisms and advanced model systems, *iii*) hypotheses linked to general ecological theory. One of the biggest gaps in knowledge is localized in the processes which operate in the microbial-faunal interface. The aim of our workshop therefore was to bring together a broad expertise in soil microbiology, zoology and ecology to address the following topics:

- soil ecology is short of modelling approaches and has developed too independently from general ecology,
- advanced methodological approaches are increasingly been used in soil ecology, inter-linkage of techniques will lead to a bigger picture,
- chemical ecology is a well established topic above-ground, comparable interactions are important feature in soil systems,
- connecting microbial and faunal food web compartment has to integrate both functional groups and quantification of carbon and nutrient flux.

Based on these considerations the workshop covered the following sessions:

1. Model organisms in soil ecology
2. Linking soil properties and biota to functional networks
3. Soil chemical ecology
4. Energy channels and food webs

1. Model organisms in soil ecology

1.1. Model organisms as a tool to decrypt predator-prey interactions and evolution (Chair: Alex Jousset)

This session discussed the usefulness and limitations of model organisms as a tool to understand soil processes, in relation to both trophic (nutrient flow, predator-prey) and non-trophic (chemical communication, allelopathy) interactions. As a conclusion, the participants recommend to combine holistic and reductionist approaches to better understand ecosystem functioning and evolution.

Usefulness of model organisms

Some recognized advantages of model organisms are their easy handling and their potential to reveal principle mechanisms. Model organisms are a complementary approach, allowing investigating single interactions in detail. These distinct interactions can be measured under simplified laboratory conditions, the predictions combined, and used as null hypothesis for experiments in (semi-) natural soil systems. In combination with modeling this facilitates a good prediction of the tested hypotheses. The easy experimental set-up allows increasing the number of replicates, further enhancing statistical power. Model organisms like the nematode *Caenorhabditis elegans* or the ciliate *Tetrahymena thermophila* provide a wealth of molecular tools allowing for a detailed investigation of chemotactic behaviour, reproductive fitness or stress reactions. Overall, ecology is an integrative science and model organisms may bridge different scales to understand whole ecosystem processes.

Limitations of model organisms

One critic point in application of models is that organisms grown under laboratory conditions may be ecologically irrelevant (e.g. *C. elegans*). This hampers the way back to the “real world” as well as the up-scaling of processes. Moreover, often not single organism but groups or guilds have a particular ecosystem function as well as gained results may vary from model to model. Hence, the choice of the model is to be determined by the question, and combining different species may help to generalize the results. Thereby it is important to describe well the model system used, to be aware of both advantages and limitations. Since various mechanisms are common to many organisms (for example type III secretion system), similar models may be used with different organisms. Further problems are negative biotic interactions that exert a strong selection effect or the fact that experiments with model organisms often involve a lower population genetic diversity.

1.2. Model organisms in soil ecology - challenges and promises in the omics-age (Chair: Antonis Chatzinotas)

1. The first part of the discussion centered around the question if scientist still require model organisms to mechanistically understand organismic interactions or the response to changing environmental parameters. In particular the microbiologists among the participants were rather skeptical regarding the benefit of working with bacterial model organisms and posed the question, if model organisms indeed remain unaltered during long term storage and cultivation. For instance, keeping bacteria in cultures often results in changes of different genetic features, such as the flexible genome pool, or metabolite profiles which may not be required for survival under controlled laboratory conditions. As also presented by Kornelia Smalla in her morning lecture, however, future research should focus in particular on this genome pool (i.e. the mobilome), which encodes for a wide range of properties involved in adaptation or interactions with other organisms. The phenomenon of losing certain skills or functional traits is also known for nematodes, which for instance seem to be less aggressive towards their prey after prolonged cultivation periods.

The protistologists, however, disagreed with this point of view. In particular mechanistically understanding the specific interactions between prey and predators is still an understudied topic and is best investigated with model organisms. Several research questions such as the interaction of protists with toxin producing bacterial strains or the nature of bacterial traits which confer grazing resistance represent highly relevant topics. Up to now it seems that protistan cultures remain unchanged, although it is unclear if other features are affected under laboratory conditions.

While a vast amount of work on selected trophic groups or taxa have relied substantially on model organisms like nematodes due to their relevance as plant pathogens or earthworms as a proxy for the macrofauna, the ecology of other abundant groups such the enchytraeids or viruses, phages and yeasts has been usually overlooked in soil. One explanation could be the lack of reliable identification tools which would allow also non-taxonomists to include such organisms in their studies. The recent launching of several larger bar-coding initiatives is expected to resolve some of these problems. Using molecular techniques for taxonomic purposes may also resolve nomenclatural debates on well-established model organisms: A DNA bar-coding analysis of the widely used *Lumbricus terrestris* revealed cryptic diversity within nominal *L. terrestris* and established two independent lineages.

Despite different opinions regarding the use of model organisms, one aspect was clearly emphasized by all participants, i.e. the need to transfer reductionistic systems to the complex soil matrix for testing the in situ relevance of knowledge gained under controlled lab-conditions.

2. The advance of molecular biological tools and high-throughput sequencing techniques has allowed obtaining a huge amount of genomic and transcriptomic information of many soil organisms (in particular bacteria or nematodes). However, some organisms such as protists have been usually selected only in a medical or marine biological context. Several participants pointed out the need to choose distinct groups of soil eukaryotic microorganisms for genomic and transcriptomic analysis. It is expected that similar to the genome studies of other widely used eukaryotic model organisms, a major part of the genome will be unknown. In contrast to prokaryotic organisms so far no genetic activity markers have been identified for eukaryotic microbes, which might be also used for environmental screening approaches. One particular appealing aspect would be the use of model organisms, for which the knock-out of selected genes is possible. While well-established for prokaryotes, this is not an easy task for eukaryotic organisms (see,

however, the use of *Arabidopsis* mutants), since a single knock out usually affects a network of (unknown) functions.

3. The final part of the discussion dealt with the different scales faced by soil organisms, e.g. the bacterial perspective in soil is much smaller than that experienced by an earthworm. There was general agreement during the discussion that experimental strategies as well as model organisms should cover different scales. Recent developments in the field of (non-invasive) diagnostic tools such as X-ray microCT or NanoSims enable scientist not only to reveal the 3D-structure of the soil matrix but to ultimately combine chemical, physical and biological approaches on very small scales. Conventional soil sampling, sieving and homogenization cannot provide information on the small scale distribution, response, and genetic, chemical and trophic interaction of soil organisms. Such scale dependent studies should focus not only on the presence of organisms under given conditions but in particular distinguish between active and non-active or resting organisms and complement existing methods such as stable isotope probing (SIP). From a bacterial point of view, soil may indeed be a desert with only a small part of the available surface being covered. The above mentioned approaches are promising in resolving the questions how different patches of colonies interact with each other, how substrates or chemical signals from other organisms diffuse to these patches or how soil structure affects the maintenance of microbial diversity and functions in soil. Eventually it might be useful to consider “model soil cores” as a research object in future studies addressing organisms from different trophic levels and guilds, physical and chemical constraints as well as temporal and spatial scales.

2. Linking soil properties and biota to functional networks

2.1. *The role of biotic interactions in nitrogen cycling in soil (Chair: Kristina Kleineidam)*

For all living organisms nitrogen represents an essential nutrient. Therefore, turnover processes and availability of nitrogen are of central importance for the organisms. The soil food web and hence nutrient pathways are incredibly complex and the diversity of organisms interacting in soil is huge, crossing the three domains of life. A few interactions between microorganisms and faunal organisms are known affecting N cycling products and processes, but overall, there is low conceptual and methodological overlap between the microbial and faunal research areas. It appears challenging to find a joint basis for microbiologists and zoologist to investigate the interacting networks of the very diverse organisms.

As indicated, assimilation of nitrogen is essential for microbes and faunal organisms. However, besides nitrogen assimilation into biomass, processes like nitrification, denitrification and N₂ fixation are major processes of the soil nitrogen cycle being carried out by microorganisms. Little is known about the impact of faunal organisms on nitrifiers, denitrifiers and N₂ fixers, the mechanisms of regulation at the microbial-faunal interface and hence on the overall nitrogen fluxes in soil.

We discussed how it could be possible to identify and to approach the interacting networks. Considering the enormous diversity and heterogeneity of involved groups of organisms in nitrogen cycling (bacteria, archaea, fungi, protozoa, collembola, earthworms, insect larvae...) and also feedback reactions of plants in natural ecosystems, we thought about possibilities to investigate selected functional microbes and selected faunal organisms as model organisms under controlled conditions and thus to simplify the system. Challenges and problems that could occur are, e.g., the choice of a representative organism out of a community of phylogenetically diverse organisms, the different behavior of microbes and protozoa due

to the missing associated communities in the artificial environment, different effects of protozoa species and different responses of microbial species, the (isolation and) cultivation of functional soil microbes, their re-inoculation in sterilized soil, the etc. The longer we talked about methodological approaches, the more challenges appeared. Nevertheless, it might be worth discussing this approach in more detail to find solutions for some of the difficulties and to make first steps in this direction.

Another possibility that we discussed could be a community-based approach, e.g. by using the stable isotope ^{15}N to label nitrogen-transforming organisms and subsequent organisms in the food chain as well as intermediates/products of the nitrogen cycle. However, only organisms incorporating the ^{15}N into the biomass could be traced, which is not the case for the processes of N_2 fixation, nitrification and denitrification. Nevertheless, it could be possible to gain insight into biotic interactions in N cycling in soils, notably if the ^{15}N label in the biomass could be followed by metagenomics and/or metatranscriptomics, which might remain challenging to combine.

Although techniques like high throughput sequencing on rRNA or mRNA basis, stable isotope probing, ^{15}N pool dilution technique, real-time PCR, proteomics, etc. are available by now, it is still a challenge to unravel the interactions of microbial and faunal organisms concerning nitrogen turnover. However, if microbiologists and zoologists together develop a joint basis for future experiments, it could be feasible to reveal mechanisms at the microbial-faunal interface contributing to soil functioning and delivery of ecosystem services.

2.2. Organisation of microbial life in soil – Consortia vs single cells (Chair: Michael Schloter)

It is generally accepted that certain functional traits in soil only occur if different microbial species form tight interactions and share tasks. On well studied example is nitrification, where ammonia oxidizing microbes need to meet nitrite oxidizing bacteria in order to transform ammonia into nitrate. So far no single organism has been described in nature that is able to perform both steps of nitrification, the oxidation of ammonia to nitrite as well as the transformation of nitrite to nitrate. Similar other examples, where a distribution of tasks among different microbial taxa is needed for a certain turnover process have been described recently in literature including for example anaerobic methane oxidation or mycorrhizal helper bacteria. These observations lead to the question if microbial life is generally organized as consortia of different organisms or if clonal growth a single organisms is the typical life style in soil.

To answer this question, there is a need re-define types of microbial interaction. More than a “black and white picture” including only competition or symbiosis, it has been postulated during the discussion that there might be a continuum of types of interactions including simple forms of nutrient dependency, or electron transfer up to well developed forms of interaction including communication between microbes by quorum sensing. Depending on the type of interaction the formation of consortia might be more or less randomized depending on the organisms present in a certain spatial unit or highly specialized with well defined types of interaction and acting organisms. The latter one might be comparable to biofilm structures, described from other types of ecosystems, however polysaccharides might be of minor importance. Independent from the type of interaction chemotaxis might play a key role in the formation microbial consortia.

A second point that has been considered during the discussion was related to the question which conditions in soil are needed to induce the formation of microbial consortia and if these structures are dynamic in time. There has been an agreement that biotic and abiotic stressors in soil enhance the formation of microbial consortia to be more competitive and to protect individual cells from the

corresponding stress conditions. Therefore microbial consortia may play also an important role during initial soil development (“soil crust formation”). If changes in environmental conditions automatically induce shifts in microbial consortia is an open question and might be addressed in future research.

Overall there was an agreement that studying microbial consortia in soil may not help us to understand their role for soil stability and ecosystem functioning but might be also a possibility to reduce the complexity of soils related to diversity by studying functional related units and operating consortia. Therefore the methods that we have in hand at the moment (including stable isotopes, NanoSIMS and high throughput sequencing) may form a well suited toolbox.

3. Soil chemical ecology

3.1. Soil organisms’ interactions and the provision of ecosystem services – knowledgegaps and management options (Chair: Silvia Pieper)

Species interactions and ecosystem services

The concept of 'ecosystem services' is currently widely utilized in basic and applied soil ecological research to describe not only soil resources and functions, but also their usefulness for mankind. While the use of the ecosystem service concept is acceptable as a tool to transfer complex knowledge into more simple descriptors, it should be kept in mind that the valuation of ecosystem performance only in terms of human convenience might be ineffective in the identification of essential processes and their drivers. For example, as the participants to the workshop pointed out, a 'good' service is not obviously a process that runs at high parameter range. A high stability and resilience of the process outcome was identified as a more pertinent characterization of service provision.

Remarkably, there is a focus on species, populations or functional groups of organisms as being the drivers of ecosystem services –without addressing interactions between organisms as the essential determinants of ecological processes. The discussion at species or functional group level was not considered to be promising, since typically it's a complex system performing a function that might in turn be a provisioning, regulating or supporting service for man. It was stressed that functions in soils depend on the diversity of species and their interactions, and that diversity delivers functions.

As long as not all functions of species in soil are known, it is not possible to decide if single species (interactions) or groups of species are responsible for specific services in soils. The workshop participants felt that –more than in aboveground ecology – the notion of redundancy has been (mis)used in soil ecology when coming to the uncertain attribution of traits to single species. While a species will be important *per se* in some processes, it might be a part of a redundant complex in other processes. Moreover, the same function might be performed by different species at different abiotic ranges.

Only in respect to microorganisms, it seems that the dependency of function delivery from high diversity has not been consistently demonstrated for bacteria. Since few strains might be responsible for specific processes, the characterization of the benefits that an organism obtains in delivering a certain service would be an effective way to identify their role.

'Open the box' of soil functions

At present, most of the soil ecosystem services recurrently addressed are based on very crude functions that integrate over a wide range of soil processes. Typically, services like 'primary production' or 'provision of fresh water' foot on processes like dead organic matter turnover, nutrient cycling or soil formation. At such low level of discrimination it is not possible to link the highly diverse soil organisms to their functions –and the relationship points inevitably but erroneously to redundancy.

To establish the correct links between diversity and functions, it will be imperative in the future to 'open the box' of general function descriptions and to split them to 'subfunctions' with high resolution. In so doing, the high specificity of species impact on ecosystem functions will become visible. A promising tool for high resolution characterizations is the further investigation of genes responsible for metabolic functions. Here, even if some of the key genes are identified, the knowledge is still fragmentary.

An open point of debate was the hypothesis claiming that the availability of 'recurrent' resources in the soil environment might have led to the existence of more species evolved to use them. Along with this thesis, 'rare' events often coupled to negative interactions (e.g. the upcoming of pathogens) should refer to fast evolving species and lead to fewer species involved in the resource use. As evidence of the contrary, the process of nitrification can be adduced, since in this case a widespread resource in the soil environment, like nitrogen compounds, is utilized by a relatively small group of organisms.

Indicating risk for ecosystem service loss

There is an evident large knowledge gap regarding the consequences that might arise from the loss of species and the disruption of interactions in relation to service provision. Even when addressing a relatively circumscribed functional group as the 'nitrifiers', we still ignore what would happen if 'they go extinct'. The participants emphasized that concepts of redundant traits allocated to several species and the consequent 'insurance hypothesis' due to the high soil biodiversity shouldn't hide the fact that –even if certain functions are more crucial in some environments than in others –we will never be able to compensate for the loss of services delivered by species and their interactions in soils.

It is therefore extremely worthy to identify indicators that point at imminent risks of disrupting important functions in soil. The usefulness and completeness of concepts regarding indicator organisms for such risk has been widely discussed. Clearly, the identification of indicators is more difficult for microorganisms than for soil macrofauna, at least as long as the functions to be indicated are so broadly defined. Again, a more detailed description of functions in soil will be the basis for the identification of specific and sensitive organisms. Since, especially for microorganisms, the detection of presence does not mean functioning, a good indicator set would combine verification of species' presence and of their functions with the help of descriptors of activities. The group felt confident about the good indicator potential of earthworm communities, even if the extrapolation possibilities from earthworms to the respectively associated microbial community were still considered as uncertain.

Extrapolation over scales –knowledge gaps to be filled

In the attempt to extrapolate from one temporal or spatial scale to another, most difficulties arise from the uncertainties in predicting the process outcome. No careful examination at one level of biological organization can foresee the impact of properties that arise in a more complex system. This holds true not only for the results of short-term lab experiments, but also for models built on that results. One possible way out of this well-known dilemma would be to follow the way from the lab to the field on the basis of hypotheses and models generated at the smaller/shorter scale and to verify how good the prediction of processes magnitude is. But, then, the way should also lead back to the lab, so to adjust the experiments to

the field conditions. Regarding the extrapolation along temporal scales, the survey of established chronosequences (e.g. after species invasion) bears most optimal and comfortable starting points for investigations. Even if there will always be a gap between 'real' field environments and predictions from experimental set-ups, this gap is deemed to become smaller.

One area of investigation that is considered extremely significant for the understanding of mechanisms is the resolution at different scales of chemical signaling in soil ecosystems. Following the principle 'no sound, no view in soil? Say it chemically', the initiation of most interactions in the soil matrix are determined to be of chemical origin. On top of that, soil ecosystems turn to deliver the perfect matrix for chemical signaling: If substances tend to evaporate, moist soils will keep the signal, as the water film in pores will be the medium for all water-soluble compounds and the water surface the transport interface. Soil air filled voids will support the spread of volatiles.

There are several experimental findings that describe chemical gradients at small scales, but the characterization of possible interactions over greater distances (10-30 cm, mesoscale) and between aggregated communities is still unexplored. Also the investigation of electric signals in soils shouldn't be neglected (fungal hyphae?), as they are known to run in sediments.

Can interaction between species be managed?

At larger scale, management option can only mean to have the general feature of maintaining or improving the diversity of the soil organisms' community (e.g. no tillage practices, green manures and legumes in agricultural land). However, it should be kept in mind that optimizing general conditions is not an option in endangered natural or extreme environments, where rare and stenocious species will be vulnerable to standard management practices.

The possible management of interactions between species has been impressively illustrated for processes running at the mesoscale for predatory/parasite prey/host interactions (e.g. nematodes parasitising *Diabrotica* larvae). This direct management will be however technically intricated at the microscale and almost impossible to perform.

3.2. Chemical and molecular mechanisms in soil food web regulation – conceptual and methodological challenges (Chair: Martin Schädler)

Unique features of belowground chemical interactions

Soil food webs are characterized by a high importance of local, short-distance interactions between less mobile species. These short-distance interactions are usually mediated by chemical communication which is especially efficient through water films in the soil matrix. The role and mechanisms of long-distance chemical communication in soil are rather unexplored. It is known that volatiles in soil can be used for indirect defence (attraction of predators) of plants. It is, however, not known if this is a common phenomenon and if this may also work under a wide range of soil conditions (e.g. in water-saturated soils). These belowground plant volatiles may also influence aboveground biota and represent another linkage between above- and belowground processes. Another possible mechanism of long-distance chemical interactions in soil may be systemic responses in plant roots, e.g. induced by herbivore damage.

From aboveground chemical interactions it is known that they are often triggered by specific blends (mixtures) of different chemical compounds. Soil interactions, however, are usually characterized as mediated by single specific compounds. It is an open question if this reflects an important difference between the above- and belowground system. Moreover, despite there are sometimes only few common signalling pathways for the establishment of species interactions in soil, there seems to be a high specificity of signals which mediate interactions. These specific signals, however, can be exploited by further biota.

An unique trait of soil food webs is the crucial functional role of fungi. Fungi form symbiotic interactions with plants, contribute to the decay of organic matter, are grazed by fauna and can be pathogenic. Despite this multiple functions with central importance in food webs the integration of fungi in chemical interaction webs is rather unexplored. For instance, we lack any knowledge on possible systemic responses in fungal hyphae, and chemical communication between different fungi and between fungi and other biota.

Lack of co-evolutionary perspective in soil chemical ecology

In aboveground species interactions, chemical ecology is usually interpreted as a consequence and an important mediator of a co-evolutionary history. A long standing view in soil ecology is that there is not much coevolution since selective forces may not act on dead organic matter and the mobility of soil biota is low. The specificity of interactions is usually seen as an indicator of co-evolution between biota. For soil biota, however, we often lack detailed information on diet or other indicators for the specificity of association between species. Thus, the question if the degree and frequency of specificity in belowground species interactions generally differs from aboveground food webs can not be answered currently.

Future studies have further to explore the role of food web-evolution in plant-microbe interactions. For instance, the fact that some bacteria promote plant growth whereas others do not may indicate trade-offs in this relationship, which again may act as selective pressures.

The use of agricultural plants as model systems in many studies may further hamper any evolutionary thinking in soil ecology. The loss of selective pressures during many generations of artificial breeding is very likely to mask any signs of co-evolution. Paradoxically, the current trend to genetically modified crops counteracts the loss of defence mechanisms during the history of breeding.

Availability of tools – methodological challenges

The general availability of tools and methods which can be used for soil chemical ecology is not seen as a central issue. A wide range of knowledge and methods for the work with aboveground biota can be adopted for soil ecology. However, a number of methodological problems have to be solved: For instance, the identification of key functional genes in some groups (e.g. AMF) is still a problem. Further, mutants (e.g. knock-out mutants) are an established tool for the investigation of mechanisms in aboveground interactions but are rather unavailable for soil biota. As key problems we identified (1) the low number of chemical ecologists working with belowground biota, (2) the weak communication between the many different disciplines of research on the field of soil sciences and (3) the neglect of the role of roots even in plant sciences.

4. Energy channels and food web

4.1. Integrating stoichiometry into food web models (Chair: Robert Koller)

There is a need for a more integrative view of C: N: P ratios in terrestrial ecology

General perspective

The general concept that most terrestrial ecosystems are C:N and not C:P limited, needs to be revisited. Recent findings indicate that terrestrial ecosystems are co-limited by N:P. N:P availability determined organic matter accumulation and biomass production. P-limitation might be of prime importance especially for fast growing organisms because P-rich ribosomes make up to 50% of dry weight of bacteria. Additionally, resource availability may fundamentally affect DNA and protein evolution and amount of RNA/ protein ratio in organisms. Knowledge on the proportions of C, N and P in specific groups of soil organisms is scarce but may represent a useful tool for assessing nutrient limitation of ecosystem processes in terrestrial ecosystems. We assumed that higher trophic levels should not be limited in P but levels in-between.

Consequences of P-limitation

Remobilization of P is from critical importance because P reserves are becoming depleted. Given that protozoa are most important bacterial grazer do release N but not P opened the question how P can be mobilized. Remobilization of P by microbes might be an important issue for future research because due to fertilization, agricultural soils are relative P rich and may represent an important P source in future. Bacteria are able to incorporate virus' derived P underlining an urged need for a better understanding of how virus' are involved in ecosystem processes.

We concluded that we need to improve our knowledge on (1) C:N:P ratios of organisms in different terrestrial ecosystems and (2) mechanisms involved in the regulation of P (re-) mobilization across multiple terrestrial ecosystems.

Plant association with mycorrhizal fungi fosters plant P uptake (Smith & Read, 1997). We questioned whether there is knowledge on the mechanisms how plants acquire P from mycorrhizal fungi and whether it is known if C:N:P ratio of mycorrhizal hyphae is depending on the soil environment or homeostatic? Additionally we asked the questions how P transport in mycorrhizal fungi is controlled and whether this is highly dependent on the P-form. The follow up questions was how P can be stored in organisms of higher trophic levels.

As plants are major source of C input for soil food webs they may have a strong impact on soil stoichiometry. It is still an open question whether root derived C is an important recourse for soil food webs and whether this C is important for driving decomposition processes. Interestingly plant possess of a large range of C:N:P ratio on the species level but does the C:N:P ratio shift during the life cycle?

The connection of C source: sink relation to biodiversity was discussed from an earthworm's perspective. Especially earthworm – microbe interactions might be important for C fluxes and we need to consider soil physics and the role aggregates production in relation to C mineralization. Aggregate production may fundamentally feed back on N and P mineralization. Moreover we highlighted that earthworms may strongly depend on root derived C as energy recourse.

C: N: P ratio and 'Global change'

Under elevated CO₂, plant growth is increased which leads to an enhanced demand for N by plants. Consequently, soil N pools may deplete and multiple ecosystems may run into N limitation. Additionally terrestrial ecosystems may be co-limited by P (see above) because air derived N-input may play a significant role for N fertilization. This opens the question which nutrients limits plant growth and whether P may become the dominant limiting nutrient in the conditions of a “global change world”. We pointed out that under elevated CO₂ bacteria are known to produce less phosphatase which may subsequently intensify P limitation under changed conditions.

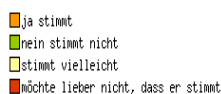
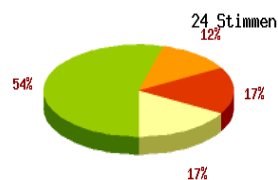
Stoichiometry and signals & toxins production are strongly linked

The C backbone is major component of toxic products such as antibiotics and consequently toxin production might be a “useful” sink of supplement C. The release of toxins can mobilize N and P from dead prey, which directly links stoichiometry and toxin production. There is experimental evidence that P solving products of bacteria can kill protozoa, which indicate an overlap in functions and displays another example of a direct link of stoichiometry and toxin production. The follow up question was whether ectomycorrhizal enzymes/products like organic acids involved in mineral weathering can directly feed back on microbial community composition. Finally, we discussed the consequences that P activity in amoeba is increased in the presence of nematodes and concluded that chemical cues may strongly control the P activity in amoeba.

4.2. Stable isotope techniques – light in the “black box” soil? (Chair: Liliane Rueß)

Soil organisms and the structure and function of their food webs play a key role in soil carbon dynamics. However, we lack sufficient inside in belowground trophic interactions to address this issue. Recent developments have seen a tremendous increase in the applicability of a suite of (stable) isotope tracer methods to assess dynamics of soil carbon pools. Moreover, for food web analysis stable isotope composition in animal tissues has been employed, with nitrogen applied to rank relative trophic levels and carbon to assign food sources. Hence stable isotopes provide a high resolution method to define feeding strategies of decomposer invertebrates *in situ*. Based on these findings this discussion round has focussed on 3 major questions:

1. “You are what you eat” – a good guess or a good working base?



Cross feeding as problem

- secondary links difficult to follow
- can be levelled out with high numbers of trophic links, i.e. in natural food webs

Whole animals/ tissues

- approach works well with whole animals
- enzymatic discrimination in metabolism
- caution with biomarkers for specific tissues or pathways

2. SIP, Rhaman, NanoSims – will they reveal new key-findings or do we end up with the same food webs as in 1980ies, yet just more detailed?

Techniques available

On the microbial level stable isotope and molecular techniques are combined in nucleic acid-based stable isotope probing (NA-SIP, carbon or nitrogen) to determine trophic connectivity between soil biota, length of food chains, and microorganisms actively involved in specific metabolic processes. Comparably, phospholipid fatty acid based stable isotope probing (PLFA-SIP, carbon) provides quantitative and chemotaxonomic information on resource allocation in soil microbial communities.

In Rhaman spectroscopy a laser interacts with molecular vibrations, phonons or other excitations in the probe, resulting in the energy of the laser photons being shifted up or down. It offers several advantages for microscopic analysis. Since it is a scattering technique, specimens do not need to be fixed or sectioned. Raman spectra can be collected from a very small volume (< 1 μm in diameter).

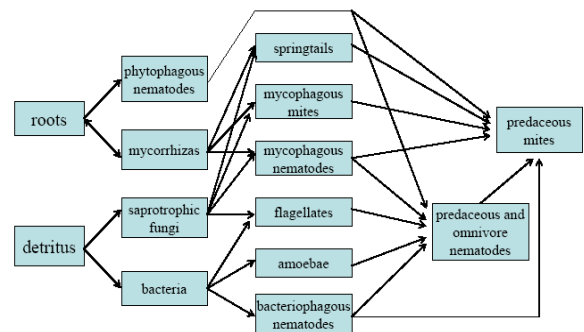
Secondary ion mass spectrometry (SIMS) is a surface analysis technique. It provides information about the lateral distribution of any element and its isotopes as well as quantitative information about the isotopic composition of a sample. NanoSIMS is used for microbes and microfauna to trace an isotopic label in single individuals and its different organs.

Do we need such a resolution?

Stable isotopes offer several advantages such as: 1) *in situ* approach, i.e. field application, 2) detection of trophic interactions that cannot be seen in the lab, 3) estimation of the active part of microbes in the soil, i.e. to distinguish biomass of “sit and wait” from the metabolically active carbon assimilators.

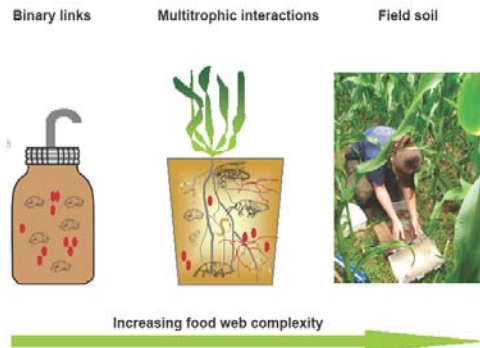
Favourable is also the application of a time resolution approach. Long-term isotope incorporation gives insight into the re-use of N or C by organisms, and which are part of the process at different stages. Further it allows the connection of energy channels and long-term incorporation of specific plant resources. Short-term isotope assimilation can assign binary food web links.

However - if we ask a simple question such as “What does XY feed” do we need to apply sophisticated methods, to e.g. determine the position of each ^{13}C atom in a glucose molecule? For simple questions simple methods work best!



(modified after Hunt et al. 1987)

3. What approach works in the field (complex systems), i.e. with label dilution or mixing and with many replicates?



In the field (long term, i.e. vegetation periods):

- N fixation by rhizobia
- old/new carbon usage by microbes and fauna
- mycorrhiza < > plant carbon exchange
- turnover rates in food web models

In food webs (short term allocation):

- determine a trophic group/top predator
- estimate carbon flux

The final conclusion of this discussion round was that stable isotopes need to be combined with molecular techniques to identify the organisms involved in uptake of C and N with a high taxonomic resolution.

We hope you have enjoyed our workshop! Best wishes from Berlin

Liliane Rueß and the Session Chairs

