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Abstract: This manual presents final, tested solutions for improved environmental control techniques (warming and CO2 enrichment, in natura and in vitro) in terms of realism of simulated atmospheric scenarios and cost. It also presents final and tested new experimental approaches for ecosystem functioning research and for studying biodiversity under environmental change.

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1. Executive summary

In this report we explain how designs were optimized, hypotheses were tested and results were disseminated. Regarding the design of more realistic warming experiments, artefacts of infrared heaters on the water balance were quantified and solutions were proposed in a published paper, and a new control for infrared heaters was tested and refined. Moreover, we also tested if temperatures in enclosures deviated from what they would be in a naturally warmed environment, and suggested ways of limiting potential problems. Finally, artefacts encountered regarding excessive and seasonally uneven warming in *in situ* lysimeters were reduced. Improvements for another environmental control technique, namely atmospheric CO_2 addition, were likewise pursued. The new "GradFACE" design, making use of Computational Fluid Dynamics, was tested both *in silico* and in the field. The results suggest that it is possible to obtain a consistent gradient of CO_2 concentrations under different wind directions and wind speed situations. Regarding the design of new approaches for experimental ecosystems, a test was carried out in several labs throughout Europe to see whether the reproducibility of results between laboratories will increase when controlled systematic heterogeneity is incorporated. Experiments were conducted in well controlled growth chambers and in greenhouses. Introduced heterogeneity was biotic (one or three lines for the plant species) or abiotic (soil texture patches). The fourth major task involved envisaging a new generation of experiments dealing with biodiversity and/or climate change. One paper was written on challenges and opportunities that arise in global change experiments, which focused on a wide range of problems (e.g. soil heterogeneity, factors that are interdependent, the stepwise nature of many imposed treatments, etc.), and how to overcome them. Another manuscript focused on how biotic interactions and feedback effects are dealt with in manipulation experiments. Finally, the most promising of two field techniques regarding biodiversity research (cf. results of pilot projects in D 8.3), namely a new method to characterize species interactions in plant communities, was further fine-tuned and a manuscript was written on the advantages and limitations. Overall, we think that WP8 was successful in yielding several advancements spanning a range of techniques and approaches used in global change research.

2. Task T8.1: Designing realistic warming experiments

2.1 Unrealistic air temperatures in warming experiments

2.1.1 Warming experiments in the field with infrared irradiation: effects of high leaf-to-air VPD

(contribution by UA)

A. Background

Infrared heating as method to simulate climate warming has been applied since the end of the past century and has some important advantages: it (i) is a method that can be actively controlled, (ii) can be applied in the free air and therefore avoids most artefacts associated with enclosures, (iii) warms both vegetation and soil, and (iv) warms the surface directly, which improves both responsiveness and energy efficiency. A criticism of the technique is the changing vapor pressure differences associated with infrared heating (caused by higher temperature differences between leaf and air). As the technology to equip infrared heater systems with air humidity controls is not yet developed or implemented, infrared heating tends to increase water loss from plants and the soil surface. A full quantification of how transpiration rates may be affected by infrared heating has yet to made, however. We believe that this is a necessary step to propel the discussion on artefacts potentially associated with infrared heaters forward.

B. Quantification

Most formulas used here can be found in standard works on environmental physics. A flux is determined by a gradient multiplied by a conductance. For water losses from the leaf, this gradient is the difference in vapor pressure between the leaf's stomatal cavities and the ambient air. If leaves are irradiated by infrared heaters, they will become warmer with little change in air temperature (which is only warmed indirectly). Although climate projections on humidity contain substantial uncertainty, the suggestion of a future warmer climate in which RH is conservative (i.e. the air will contain more water vapour in the absolute but not in the relative sense), is reasonable. The humidity inside stomatal cavities is assumed to be 99.5%. We use Tetens formula to calculate the vapour pressure:

$$e_a = RH \times 0.611 e^{\frac{17.502T}{T+240.97}}$$

with e_a vapor pressure and T the Celsius temperature of either air or leaf.

Next, we have to quantify the differences in conductance for water vapour transport. For the boundary layer, this is complicated and cannot be resolved by adopting only one approach. The fundamental problem in determining boundary layer conductances is one of scale. Formulas that

work on the leaf scale cannot readily be scaled up to the canopy, and formulas on the canopy scale hinge on assumptions (infinitely long surfaces) that are incorrect when considering warming experiments. The plot size of infrared heating experiments is constrained to merely a few square meters so far (although larger plots are feasible in theory), and enclosures are also limited in size. We thus use two different approaches, one leaf-based and one canopy-based, assuming the actual water loss in the fairly small warmed plots to be somewhere in between. The formulas used can be found in the full study (see section D).

A first example is used as a base for comparison for other calculations. We quantify the effect of a 1 °C warming on water loss in a canopy that is already 1 °C warmer than the 23 °C air (which is a reasonable assumption for temperate grasslands under sunny conditions). We compare a leaf of 25 °C in air of 24 °C (air warming) with a 25 °C leaf in air of 23 °C (surface warming by infrared heaters). We consider relative humidity (RH) of the air in both cases to be 70%. The results of the calculations in terms of relative differences can be found in Fig. 1. The base conditions are repeated in other calculations that vary temperature, humidity and the amount of warming.

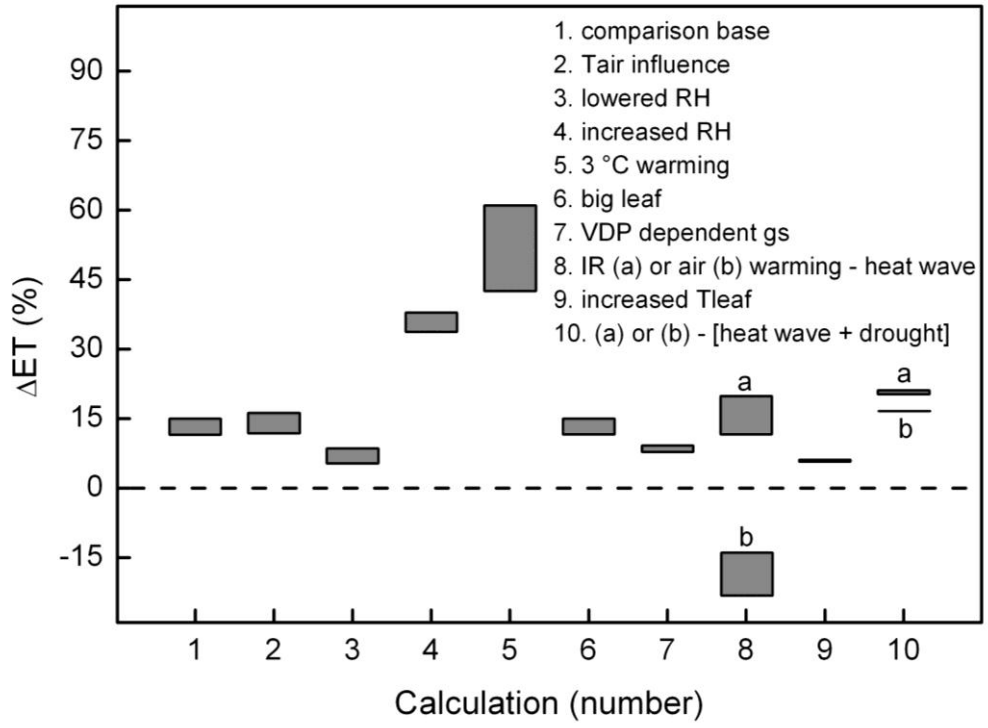

Fig. 1: *Relative differences in evapotranspiration (ΔET) between plots warmed with infrared heaters and air warming (or compared with ambient heat waves in calculations 8 and 10). Range given between results using the formulas for 'mixed' and 'endless' boundary layer approaches.*

A one degree warming led to an increase in water loss of 11 to 15% underneath infrared heaters compared to air warming, when considering only the mixed regime leaf approach and the canopy approach. Lower air temperatures resulted in a slight increase of this relative difference (calculation 2). The relative differences between infrared heating and air warming regarding evapotranspiration approximately halve when the relative humidity is decreased to 50% (calculation 3), but increase significantly at high RH values (calculation 4). For an often used 3 °C increase in canopy temperatures (a global warming scenario for the end of the century), the water loss increases more than threefold when comparing with a 1 °C warming (calculation 5). We also distinguish a case which represents a 3

meter diameter infrared heater plot. Relative differences between heating methods do not differ much between the 5 cm leaves and 3 m plot under the given (fairly average) abiotic conditions (calculations 1 and 6). The minor effect of size at sufficiently high wind speeds is also illustrated in Fig. 1.

Finally, we consider heat waves as a specific case (calculation 8). During growing season heat waves in Western Europe (and likely in many other regions), the air temperature increases are coupled to substantial decreases in relative humidity, leading to vapor pressure deficits (VPDs) that are consistently higher (111% on average) than under normal conditions. The results (calculation 8) suggest that these heat wave conditions would lead to water loss that is 20% or 12% higher than natural under infrared heaters but 23% or 14% lower than natural using air warming with constant RH, using the mixed regime and canopy equations, respectively. Note that the fact that precipitation is much lower (78%) during heat waves could decrease stomatal conductance depending on soil water reserves and root architecture and depth. It may therefore be more realistic to compare cases with closed stomates. However, closed stomates would also affect the leaf temperature. Using the base for comparison (calculation 1, Table 1), if we increase the leaf temperature in both cases by 5 °C (to 30 °C) and consider closed stomates (the cause of the leaf temperature increase), the difference between infrared heaters and warmed air approximately halves (calculation 9). If applied on our calculations for heat wave conditions, a 5 °C rise in leaf temperature caused by drought would result in a water vapor flux 20% (or 21%) higher than 'naturally' under infrared heaters using the mixed regime (or canopy) approach, and 17% (17%) lower than 'naturally' in warmed air with constant RH (calculation 10). Such deviations would evidently not only occur during heat waves, but any time the leaf temperature deviates significantly from the air temperature.

C. Discussion and recommendations

Our calculations show that, in line with assumptions, the transpiration rate under infrared heaters was higher than if the air was warmed and humidified. Driven predominantly by the increased vapour pressure difference between heated leaves and unheated air, infrared heating led to increases in water loss in the range of 11.5 to 15% for a one degree warming, assuming typical temperate summer conditions.

The 'drying' artefact in infrared heaters is less of a concern when simulating heat waves. Indeed, the naturally drier conditions during heat waves lead to water losses being underestimated when warming air at ambient relative humidity, while the greater transpiration rates under infrared heaters seem to approach reality more closely as long as water stress is not acute (i.e. stomates are not closed) (calculations 8 and 10). Heat waves or hot spells are generally associated with low precipitation and high radiation which is the result of atmospheric feedback, with the drier conditions leading to more sensible heat from plants and the surface, less cloud formation and higher air temperatures. Many studies do not account for this relationship, leading to an underestimation of potential evapotranspiration in many drought and heat wave experiments. Our results show that infrared heaters automatically generate an (artificial) atmospheric feedback by increasing potential evapotranspiration.
A side-effect of infrared heaters may be a reduction in photosynthesis caused by lower stomatal conductance in response to the increased vapour pressure gradient. Calculation 8 shows that stomatal conductance (assuming VPD driven stomatal response) is higher than during 'natural' heat waves using air warming with constant relative humidity, and lower than natural using infrared heating. In other words, deviations of photosynthetic rates from those expected naturally occur primarily underneath infrared heaters when simulating gradual climate warming, but could be expected both if warmed air (stimulation) or infrared heaters (suppression) are used to simulate heat

waves unless air humidity is altered to reflect heat wave conditions. An important remark is that, while infrared heater experiments can be conducted in the free air, experiments with warmed and humidified air in sunlit conditions have thusfar been restricted to greenhouses. These greenhouses suffer from light reductions which are typically at or above 10-15%, inevitably altering photosynthesis.

To correct drying artefacts of infrared heaters, supplemental irrigation could be administered. This could lead to deeper wetting of the soil, however, which is an artefact in itself. The practicality of such an approach, which requires no special installation or instruments, is nevertheless appealing. Another possibility is humidifying the air by a system such as “FAHM” (Free Air Humidity Manipulation). It distributes air across field plots using a central high volume blower and a ring around the plots with a number of venting pipes. The fumigation raised relative humidity with 7 % on average during two summer seasons in Estonia, with the amount of humidification dependent on wind speed (more when conditions were calmer). Such a system could potentially be employed in conjunction with infrared heaters to compensate or reduce the drying effect underneath the heaters, and prevent a potential drop in the photosynthetic rates caused by stomatal responses to an increased vapor pressure gradient. At the same time, the benefits of open air experimentation would be preserved.

In conclusion, we quantified artefacts related to heating with infrared lamps, finding that excess water loss can become problematic especially in longer term experiments. In pulse (heat wave) experiments, the increased evapotranspiration can actually be a benefit as it simulates naturally occurring feedbacks. Two possible workarounds were proposed: adding water to the soil (feasible in any experiment) or increasing the air humidity (technically more complex solution). Lastly, it should be stressed that open air systems such as infrared heating avoids many issues and artefacts related to enclosing plants and that they therefore will become ever more important tools in global change ecology.

D. ExpeER publications

De Boeck H.J., Kimball B.A., Miglietta F., Nijs I. 2012. Quantification of excess water loss in plant canopies warmed with infrared heating. Global Change Biology 18: 2860-2868.

2.2 Unrealistic canopy temperatures in warming experiments

2.2.1 Field warming experiments with infrared irradiation: control methods to apply infrared irradiation

(contribution by UA)

A. Background

In the previous section we stressed the inherent value of conducting experiments using infrared heating in free air conditions. A very important aspect is how to control the amounts of warming administered with this technique. The major difficulty here is that infrared heating warms the surface directly, not the air, while meteorological and climatological data and scenario's consider air temperatures instead of canopy temperatures. The latter reflect, in addition to the meteorological conditions, also the characteristics (e.g. bigger or smaller leaves, smooth or hairy leaves) and responses (closing stomates, changing leaf orientation) of the plants. Many current control methods for infrared heating maintain the amount the canopy warms versus control plots at a constant level (e.g. +3 °C), which also implies that plant responses to conditions that differ in the warmed plots are filtered out. For example, drought caused by extra evapotranspiration in warmed plots could trigger stomatal closure, which would in turn lead to higher leaf temperatures in natural conditions. This is not possible when canopy temperatures are increased by a fixed amount, however. An alternative method is to increase the radiation input by a fixed amount, so that canopy temperatures can vary. Unfortunately, this method is uncontrolled, meaning that no target temperature can be set. An improved method should reconcile the controllability of the constant temperature increase approach and the possibility for plant feedbacks to occur of the constant radiation flux approach (Fig. 2).

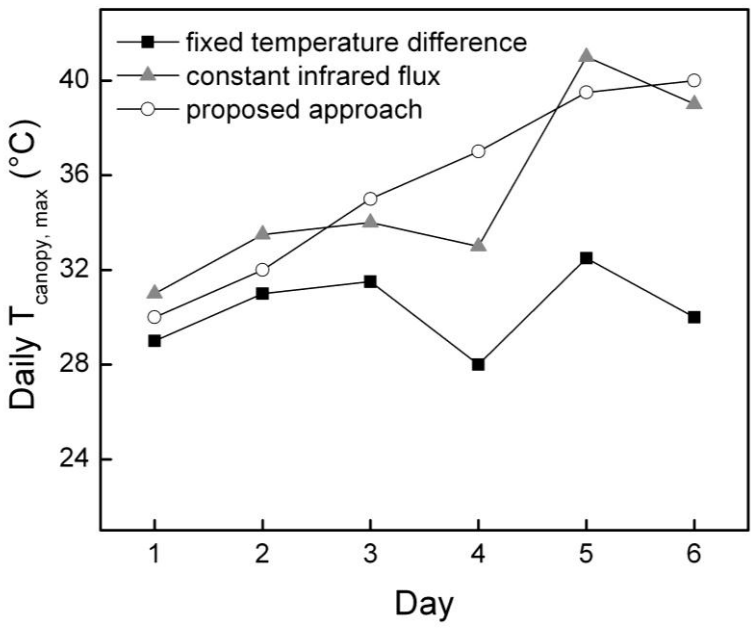

Fig. 2: *Conceptual comparison of how a heat wave with developing drought imposed by infrared (IR) heating affects canopy temperatures using: (1) a typical control that maintains a fixed difference of canopy or air temperatures between the treatment and the reference plots (black squares); (2) a predefined constant radiative flux emitted by IR heaters (grey triangles); (3) the proposed alternative approach that maintains radiative output of infrared heaters independent of plant responses by calculating a (theoretical) canopy temperature based on variable environmental conditions, a target air temperature and a reference canopy conductance (white circles).*

B. Procedure and formulas

We propose a new control method based on energy balance calculations to achieve this, which works in three steps. First, the canopy conductance of the reference plot has to be determined. Secondly, a theoretical canopy temperature associated with a given (target) air temperature needs to be calculated. Finally, the energy output of the infrared heaters required to achieve said theoretical canopy temperature has to be computed. The formulas have been given in more detail in deliverable 8.3.

Step 1: determining canopy conductance (g_v)

$$g_v = \frac{-p_a\left[(T_{canopy}-T_a)c_p(g_r+g_{Ha})-R_{abs}+\varepsilon_s\sigma T_a^4+G\right]}{\lambda D+p_a(T_{canopy}-T_a)\lambda s}$$

Step 2: calculating the theoretical canopy temperature:

$$T_{canopyt} = (T_a+o)+\frac{R_{abs}-\varepsilon_s\sigma(T_a+o)^4-G-\frac{\lambda g_\upsilon D}{p_a}}{c_p(g_r+g_{Ha})+\lambda s g_\upsilon}$$ (and $0.02 < g_v < 2.23$)

Step 3: computation of R_{abs} required to reach said theoretical canopy temperature:

$$R_{abs} = \left[(g_{Ha}+g_r)\cdot c_p+\lambda s g_v\right]\cdot(T_{canopy}-T_a)+\varepsilon_s\sigma T_a^4+G+\frac{\lambda g_v D}{p_a}$$

(increase infrared heater output until measured R_{abs} = calculated R_{abs})

This procedure requires continuous measurements of the following parameters: R_{abs} (difference between incoming radiation and reflected radiation), T_a (air temperature), T_{canopy} (canopy temperature), G (soil heat flux), RH (relative humidity), p_a (atmospheric pressure) and $u(z)$ (wind speed at height z). These have to be measured in the control plot, with the exception of R_{abs} in step 3. Furthermore, the measurement height z and vegetation height h need to manual input, with $z > h$. An offset (o) for the air temperature has to be determined manually, depending on the temperature scenario adopted and daily weather predictions. For example, if a 30°C maximum temperature is required, and 24°C is predicted for a specific day, then the offset for that day needs to be 6 °C. This also means that a natural daily course for temperature is established (the offset during the night, which can differ from that during the day, can be input separately). Constants used are surface emissivity (ε_s), the Stefan–Boltzman constant (σ), the specific heat of air (c_p) and the gravitiational acceleration (g). All other inputs can then be calculated (see deliverable 8.3).

C. Implementation

This procedure has been tested in the field, with satisfactory results (Fig. 3). We observed problems when both air humidity was very high (> 90%) and leaf temperatures were lower than air

temperatures - a situation that can occur close to sunrise. In such conditions, the control system assumed canopy conductance to be negative (i.e. implying a water flux towards the canopy), corrupting subsequent calculations. The over-sensitivity of calculated g_v values was also apparent. Both issues can be resolved by defining constraints found in literature and implementing other measures to lower this sensitivity. Final field testing will be done this spring on standard, uniform vegetation.

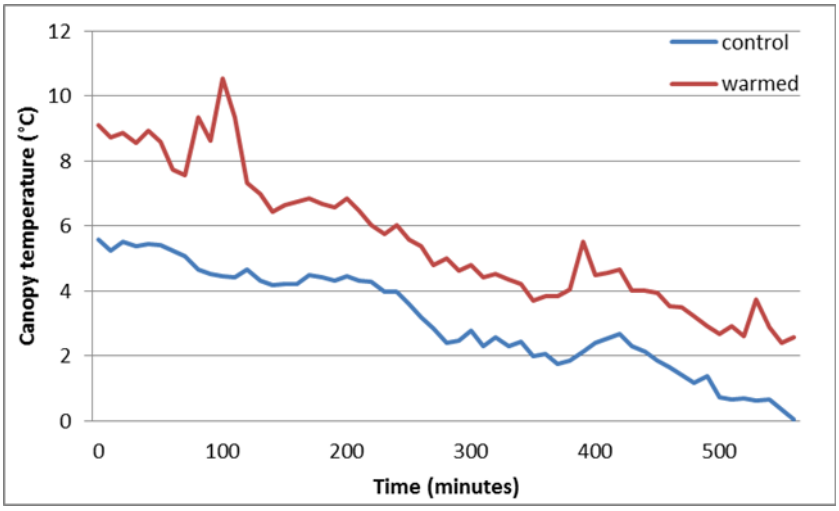

Fig. 3: *Results of a preliminary test using a scenario of 3 °C warming of air temperature. The observed difference in canopy temperature between the control and warmed plots is fairly constant, except for a short period around minute 100 where the peculiar conditions (see text) provoked the control system to calculate a g_v that was unrealistically high (around 35 mol m^{-2} s^{-1}), triggering a huge demand for infrared heater radiation (fast transpiring canopy would offset a large proportion of that energy), causing a temperature spike.*

With a planned publication on the definite algorithms, and with measurement equipment that is standard and not overly expensive, we expect that the implementation in other research groups could be swift. This control method reinforces the advantages of using infrared heaters in the open air by resolving the previous dilemma of whether to keep either the canopy temperature increase or the additional supply of (infrared) radiation constant. The two main improvements constitute: (i) custom-adjustable target air temperatures (from projections or historical records), and (ii) unconstrained vegetation responses, which can alleviate or aggravate heat stress.

D. Reference

De Boeck H.J., Nijs I. 2011. An alternative approach for infrared heater control in warming and extreme event experiments. Journal of Ecology 99: 724-728.

2.2.2 Warming experiments in enclosures: effects on tissue temperatures

(contribution by UA)

A. Background

While most researchers only consider air temperature, it is the tissue temperature that is of fundamental importance for the metabolic processes within the plant. Warming studies that report exclusively on air temperatures may therefore in some cases under- or overestimate the relevant level of warming. We specifically looked into effects of greenhouses and open top chambers on leaf temperatures. In greenhouses, the radiative environment is different from outside. The temperature of clear skies is often well below 0°C, while the 'sky' inside the greenhouse consists of the cover materials which in most circumstances will be warmer than the outside sky. Sky temperatures determine the downward longwave radiation and therefore directly affect the energy balance and thus canopy temperatures. Other properties of the greenhouse such as the total light transmission and the reflectance of long-wave radiation could also affect the leaf temperatures. In open top chambers (OTCs), a substantial proportion of the sky is not artificial, while the absence of a roof permits free convective heat exchange with the outside (the prevention of which is the most important warming mechanism in closed chambers). The side panels of the OTC inevitably affect wind speed, which in turn influences energy exchange as calmer conditions reduce heat dissipation.

If greenhouses and OTCs do indeed distort leaf temperatures significantly, this would imply that the amount and possibly the variability of true (canopy) heating achieved differs from prior estimates of attained warming. This in turn suggests that extrapolations from greenhouse and OTC studies may have to be reconsidered. Any discrepancies between leaf temperatures inside the greenhouses and outside could be buffered or mitigated by an altered heating control.

B. Quantification of artefacts

To investigate possible effects of greenhouses and OTCs on leaf temperatures we use an energy balance model that was validated empirically. We opted for a straightforward model that calculates leaf temperatures based on the environmental conditions and robust physical relationships. Although actual leaf temperatures may deviate slightly as approximations and biological variability are inevitable, the model gives a clear indication of existing trends. For greenhouses, we do simulations for different materials, based on their properties regarding reflection and transmission for short and longwave radiation (Fig. 4). For OTCs, we used 'no cover' as input in the model, but took into account the substantial changes in wind speed recorded in other OTC studies. More details on model build-up and performance can be found in the study published on this topic (see section D).

For greenhouses, the differences between the cover materials are modest, especially under cloudy conditions, with leaf temperatures underneath the LDPE foil generally being closest to those outside. On average, only small deviations of leaf temperatures inside and outside greenhouses were found. On clear days, leaves inside greenhouses were slightly warmer than outside (< 0.5 °C), especially in autumn and winter. A summer drought did not affect this inside-outside difference. On cloudy days, the leaf temperature inside greenhouses was marginally lower than outside (< 0.2 °C), with the biggest differences found in the summer. We also ran a simulation in which the wind speed was reduced to 1 m s^{-1}, compared to 2.9-4.1 m s^{-1} outside (depending on the season), and this resulted in more pronounced deviations in leaf temperatures (exceeding 1 °C in certain cases), which is

unsurprising given the sensitivity of surface temperatures on wind speed. Finally, we also ran simulations in which the percentage of radiation blocked by the greenhouse structural components (5% in the other model runs) was changed to 0% or 25%, respectively. In case of no radiation blocking, the leaf temperatures were raised by approximately 0.15 °C in comparison to 5% blocking, leading to slightly increased (decreased) differences with outside leaf temperatures on sunny (cloudy) days. When the greenhouse structure blocked 25% of incoming radiation, the leaf temperature was decreased by approximately 0.5 °C compared with a 5% blocking on both bright and overcast days. This led to leaf temperatures slightly below those outside if sunny (-0.3 °C), and somewhat more pronounced if cloudy (-0.6 °C). The results were comparable for all cover materials.

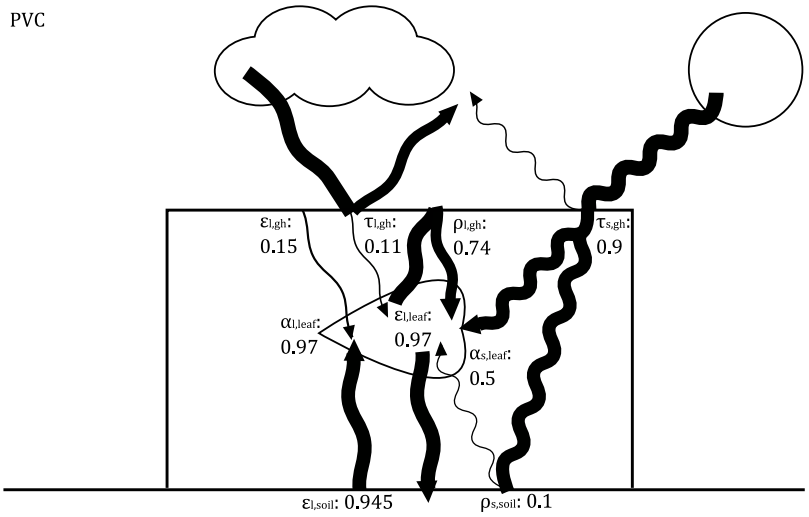

Fig. 4: *The radiation diagram for greenhouses coated with PVC in this example. The longwave (l) and shortwave (s) radiation is shown with arrows. The values for emissivity (ε), transmissivity (τ), reflectivity (ρ) and absorptivity (α) for the longwave and shortwave radiation used in the model are depicted where they affect the radiation.*

We ran a number of simulations for open top chambers under varying conditions. If air temperatures are assumed not to be increased inside OTCs (i.e. to compare net effects with climate-controlled greenhouses), our analysis shows that leaf temperatures on clear days can be expected to be 2.5 °C higher inside than outside OTCs for the conditions in Table 1. If we consider OTCs to increase air temperatures by 1.5 °C (a realistic value according to literature), the same conditions as before would lead to increases in leaf temperatures of 3.6 °C compared to outside. During clear nights, the calmer conditions inside OTCs would stimulate leaf cooling, resulting in leaf temperatures approximately 0.5 °C below those found outside. Note that we considered nights to be dark (short wave radiation set at zero), which is not necessarily the case during arctic summers, and that we did not take into account trapping of longwave radiation inside the OTCs. Nighttime air warming was not taken into account as studies documented that this is mostly absent in OTCs, which could indicate that heat trapping in these chambers is generally limited. Cloudy conditions result in only minor daytime leaf temperature increases within OTCs, while leaves inside OTCs would no longer become cooler compared to outside during the night. Under a scenario of drought stress (where stomatal conductance is significantly decreased), the leaf temperature difference is increased by a further 1-1.5 °C during the day (Table 1). Leaf temperatures are generally increased if the relative humidity is higher as this suppresses latent energy loss. Finally, the smaller the leaves, the less their temperature differs inside and outside OTCs because of their stronger coupling to the atmosphere.

C. Conclusions and suggestions

Based on the results of various simulations, we suggest that direct effects of greenhouse materials on tissue temperatures are quite small. A more important measure to prevent unrealistic canopy

temperatures inside greenhouses is to design the ventilation system in such a way that it can follow the outside wind conditions, at least up to 1.5 m s^{-1} (effects of wind speed on canopy temperatures decrease exponentially with increasing speed). Furthermore, the greenhouse structure and covering material should be designed to block and filter as little radiation as possible.

Location	T_{air} (°C)	R_s (W m^{-2})	RH	u (m s^{-1})	d (m)	$g_{vs,ad}$ (mol m^{-2} s^{-1})	$g_{vs,ab}$ (mol m^{-2} s^{-1})	Cloud cover	T_{leaf} (°C)	ΔT (OTC – outside) (°C)
Outside	10	750	0.6	1.22	0.02	0.3	0.3	0	12.43	
OTC	10	750	0.6	0.19	0.02	0.3	0.3	0	14.92	2.49
Outside	5	0	0.6	1.22	0.02	0.05	0.05	0	4.44	
OTC	5	0	0.6	0.19	0.02	0.05	0.05	0	3.84	−0.60
Outside	10	200	0.6	1.22	0.02	0.3	0.3	1	10.19	
OTC	10	200	0.6	0.19	0.02	0.3	0.3	1	10.80	0.61
Outside	5	0	0.6	1.22	0.02	0.05	0.05	1	5.30	
OTC	5	0	0.6	0.19	0.02	0.05	0.05	1	5.65	0.35
Outside	10	750	0.6	1.22	0.02	0.05	0.05	0	13.85	
OTC	10	750	0.6	0.19	0.02	0.05	0.05	0	17.75	3.90
Outside	10	750	0.6	1.22	0.02	0.3	0.3	0	12.43	
OTC	11.5	750	0.6	0.19	0.02	0.3	0.3	0	16.03	3.59
Outside	10	750	0.6	1.22	0.02	0.05	0.05	0	13.85	
OTC	11.5	750	0.6	0.19	0.02	0.05	0.05	0	18.96	5.11
Outside	10	750	0.9	1.22	0.02	0.3	0.3	0	13.31	
OTC	10	750	0.9	0.19	0.02	0.3	0.3	0	16.19	2.87
Outside	5	0	0.9	1.22	0.02	0.05	0.05	0	4.67	
OTC	5	0	0.9	0.19	0.02	0.05	0.05	0	4.31	−0.36
Outside	10	750	0.6	1.22	0.005	0.3	0.3	0	11.35	
OTC	10	750	0.6	0.19	0.005	0.3	0.3	0	12.94	1.59
Outside	5	0	0.6	1.22	0.005	0.05	0.05	0	4.69	
OTC	5	0	0.6	0.19	0.005	0.05	0.05	0	4.31	−0.38

Reduction of wind speed inside OTCs is based on data from Dalen (2004).

Table 1: Comparison of leaf temperatures (T_{leaf}) inside and outside open top chambers (OTCs) under varying conditions of air temperature (T_{air}) radiation (R_s), relative humidity (RH), leaf dimension (d), adaxial and abaxial stomatal conductance (g_{vs}), and cloud cover.

The results also demonstrate that the warming effect generated by OTCs is likely to have been underestimated. For the widely used ITEX chambers, reported increases in air temperature range from less than half a degree in forests to approximately 1.5 degrees in tundra. Our results suggest that the effective warming for plants (via the canopy temperature) could well be twice that in many ecosystems, caused by the much reduced wind speed inside OTCs. This would counter the criticism that OTC generated temperature rises are only minor. It also means that OTC studies that have extrapolated their findings on warming responses should be treated with caution. The fact that we show that OTCs create a larger increase in temperatures than previously assumed, may be considered an advantage rather than an unwanted artefact. As such, measures to minimize it seem unnecessary. However, we do advocate that researchers using OTCs to warm vegetation should provide clear data on both the amount of wind reduction in their chambers and the exact warming experienced by the plants and soils. This would allow more accurate use of the results in predicting the responses of tundra and other small-statured ecosystems (for which OTCs are being used) in a warming world.

D. ExpeER publications

De Boeck H.J., De Groote T., Nijs I. 2012. Leaf temperatures in glasshouses and open-top chambers. New Phytologist 194: 1155-1164.

2.3 Unrealistic biosphere-atmosphere exchange in monoliths

2.3.1 Artefacts from excavating, translocating and re-installing large soil cores on soil biosphere-atmosphere exchange

(contribution by KIT)

A. Background

In the framework of the German Helmholtz Society and BMBF funded infrastructure project TERENO (*TER*estrial *E*nvironmental *O*bservatories: http://www.tereno.net, KIT is running the (Pre-) Alpine Observatory covering several research sites in the Ammer catchment, South-Bavaria, Germany. TERENO was designed to study long term effects of climate change on terrestrial ecosystems. For this purpose KIT has installed 36 lysimeters with undisturbed intact grassland soil cores (diameter 1.12 m, depth 1.5 m depth) and is operating them at three sites (Graswang 860m, Rottenbuch 750m, Fendt 600m). Lysimeters were partly moved along an altitudinal gradient, with some soil cores still staying at sites as controls. In consequence, lysimeters with intact soil cores from higher elevation were translocated to sites at lower elevation with higher temperatures (δ1.5°C Graswang → Rottenbuch and δ 3.2°C Graswang →Fendt) and slightly lower mean annual rainfall (Fig. 5). In addition to the space for time in-situ climate change approach the total of 36 lysimeters are split into treatments of intensive and extensive grassland management.

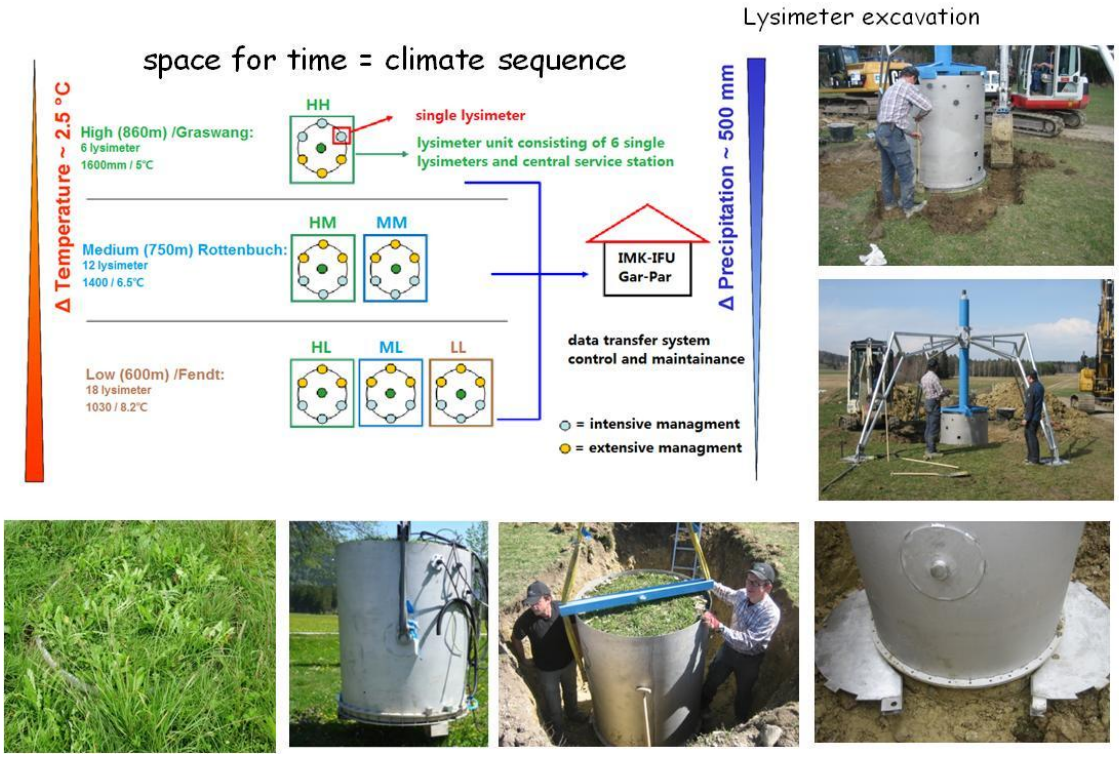

Fig. 5: *Climate change lysimeter setup of KIT in the frame of the TERENO (Pre-) Alpine Observatory*

The main research interest of this in-situ climate change experiment is to detect impacts of climate change on grassland soil hydrology, C and N turnover and associated exchange of greenhouse gases, nutrient leaching and on plant as well as microbial diversity. Since September 2011, KIT is operating 36 lysimeters (6 in Graswang, 12 in Rottenbuch and 18 in Fendt, see Fig. 5) arranged in hexagons with a central service unit hosting data loggers and other steering and sampling devices . The lysimeters have an area of 1 m² and a weight of around 3 t, which is varying due to changes of soil water content.

One of the main problems while using lysimeters for climate change studies is that the soil cores are taken out from the field, thereby cutting the soil at the base and at the sides. In consequence, boundary conditions, in particular with regard to soil water and temperature exchange, are disturbed, most severely at the lower boundary condition. In recent years the use of suction cups steered by the water tension delivered from reference tensiometers outside the lysimeter in nearby undisturbed soil significantly improved simulating the lower boundary condition leading to more realistic soil water transport and contents in the lysimeters. In contrast to soil moisture, potential differences between the lysimeter temperature profile and natural soil conditions have not been in the centre of discussion so far, though some studies have tackled the problem (e.g Todd et al., 2000; Evett et al., 1995). I.e. potential effects of temperature changes due to the transfer of intact soil cores in lysimeters have largely been neglected and not sufficiently addressed in designing lysimeters. However, in the frame of a climate change study, this is of particular interest, since soil C and N turnover processes are largely governed by temperature. For that reason and as a contribution to ExpeER KIT conducted a detailed pre-experiment which aimed at the comparison of soil temperatures in lysimeters as compared to those in soils under natural conditions. The experiment lasted from April 2010 to June 2011, thereby monitoring soil temperatures under undisturbed conditions (N=3) and in 9 lysimeters split (N=3) in sets of three different lysimeter designs: i) standard stainless steel lysimeter, (ii) stainless steel lysimeter with a new, innovative heat transfer device, consisting of metal plates dipping in a water bath which is in direct contact with the underlying soil, (iii) stainless steel lysimeter with heat transfer devices, water bath and insulation (Fig. 6). All lysimeters and in addition three natural soil profiles were equipped with temperature sensors at 10, 30, 50, 100, and 140 cm soil depth.

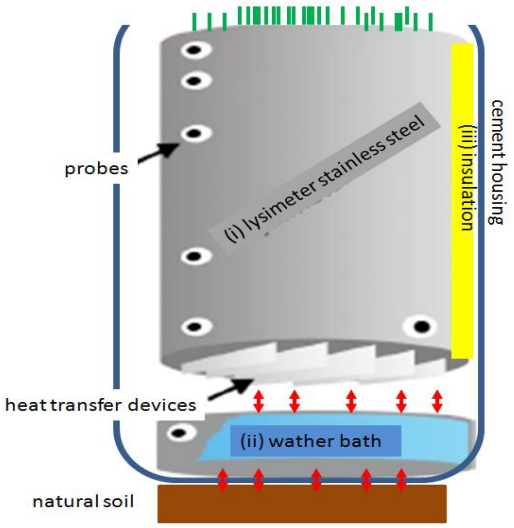

Fig. 6: *Experimental lysimeter setup with 3 different designs: (i), (ii) and (iii)*

B. Results, conclusions and recommendations

For 10 cm soil depth the temperature amplitude observed in soil cores of all 3 lysimeter designs were higher as compared to those of the natural soil. Differences were highest in summer, when soil temperatures in the lysimeters were up to 4°C warmer as compared to the directly adjacent soil. Soil temperature differences between natural soil conditions and lysimeter soil were lowest in winter (0.5-1°C). Independent from the lysimeter design, temperatures were always higher than in the natural soil with differences increasing from standard < water bath < water bath + insulation. The higher temperatures in the top soil of the lysimeters are a result of heating up the stainless steel

frame (about 5 cm higher than the vegetation surface) by solar radiation. However, heat flow might not be the only reason since reflection of radiation could also play an important role for warming. This hypothesis is supported by the fact that there was only little difference between the summer months in 2010 and 2011, though lysimeter frames were covered by white tape in spring 2011. With increasing distance from the lysimeter frame the warming effect decreases and in the centre of the lysimeter (56 cm from the lysimeter frame) temperature did not differ significantly from the natural soil. Temperature differences at 140 cm (lower boundary condition) were with max. +/- 2°C much smaller compared to 10 cm soil depth and did differ less from the maximum difference of the 3 measurements in the natural soil profile at the same depth. In contrast to the upper boundary condition the lysimeter design had a significant influence on the temperature evolution at the lower boundary condition. Best results, i.e. smallest temperature deviations of soil lysimeters temperatures at 140 cm depth from those of adjacent undisturbed soils, were obtained with the design of a water bath only. Water bath + insulation and standard lysimeters showed higher temperature differences compared to the natural soil. Taking into account the higher heat input of the insulated lysimeter (most likely due to reduced cooling at night time) also here the water bath revealed to have a positive impact at the lower boundary condition. Nevertheless temperatures of the water bath were still higher in summer and lower in winter compared to natural soil conditions. Thus, further improvement could be achieved by a more active cooling with an automated controlled continuously circulating closed water-filled serpentine coil connected to the soil outside in the same depth as published recently by Podlasly and Schwaerzel (2013).

The continuation (after March 2012) of the comparison of top soil temperatures (10cm) in lysimeters and undisturbed field conditions in the in-situ climate change experiment revealed the importance of the adequate installation of the lysimeters in the natural environment (Fig. 7).

Fig. 7: *Minimizing lysimeter housing artefacts over time*

Minimizing housing artifacts and the use of insulations shields significantly reduced temperature differences at the upper boundary conditions (Fig. 8), resulting in mean annual temperature differences of < 5%.

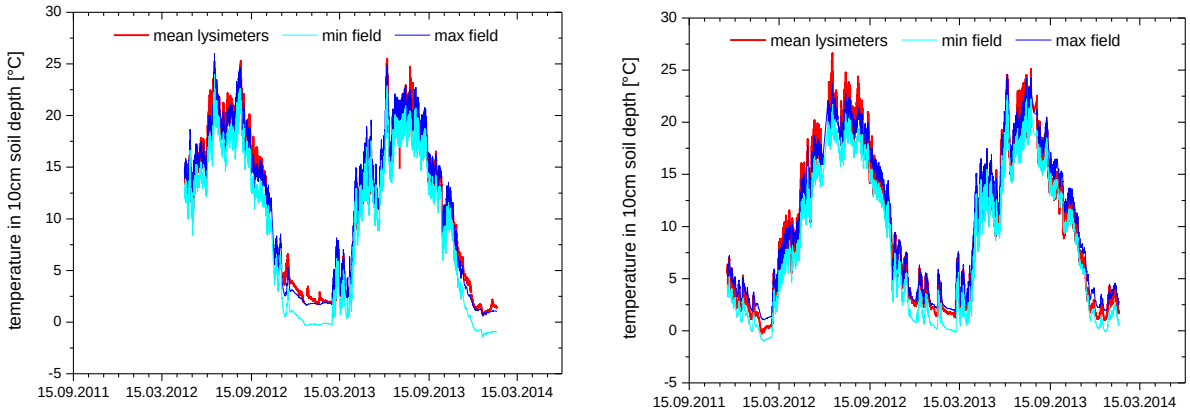

Fig. 8: *Temperature in 10 cm soil depth of lysimeters (red line N=6) and min (light blue) and max (dark blue) temperature from 3 replicated measurements in the natural soil profil. Left panel Graswang site, right panel Graswang site.*

C. References

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Todd RW, Evett SR, Howell TA, Klocke NL, 2000. Soil temperature and water evaporation of small steel and plastic lysimeters replaced daily. Soil Science 165, 890-895.

Podlasly C, Schwaerzel K, 2013. Development of a Continuous Closed Pipe System for Controlling Soil Temperature at the Lower Boundary of Weighing Field Lysimeters. Soil Science Society American Journal. doi:10.2136/sssaj2013.03.0113n.

3. Task T8.2: Using Computational Fluid Dynamics (CFD) to design CO_2 enrichment technologies

(contribution by CNR)

The GradFACE Design

The Institute of Biometeorology of CNR (Italy) began testing in Computational Fluid Dynamics framework an innovative FACE design to create a more-or-less continuous gradient of CO_2 concentrations over an experimental area of 200 m^2 (GradFACE). Starting from an original unpublished idea of Prof. Kenji Kurata (Department of Biological and Environmental Engineering, University of Tokyo, Japan), this new design was initially developed using FLUENT (www.fluent.com), a computational fluid dynamic (CFD) gas dispersion model. The CFD experiment allowed testing of a series of alternative designs, resulting in the final layout illustrated in Fig. 9. In this design, a rectangular array of laser-drilled horizontal pipes is placed over the vegetated surface, a few centimeters above the canopy height. The use of 22 automatic pressure regulators and a CO_2 injection control algorithm allow the release of different amounts of pure CO_2 from each pipe segment, depending on wind direction. In this way, more CO_2 is released at one end of the array and the amount of CO_2, which is injected along the main direction of the array, can be decreased linearly. Model experiments indicated that specific patterns of the static pressure inside the laser drilled pipes of the array permit maintenance of a relatively constant CO_2 gradient over the vegetated surface, irrespective of the wind direction (Fig. 10).

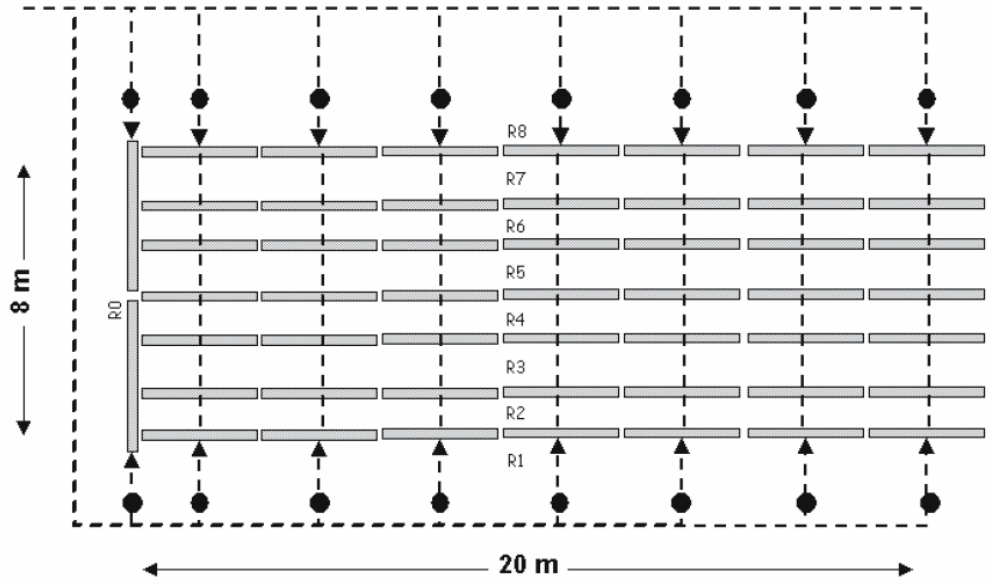

Fig. 9: *GradFACE design: the pipe array is denoted by gray bars and the position of the proportional valves controlling the release of CO_2 from the pipe array is indicated by the black dots. Dimensions of the experimental plot used for CFD simulations are shown. As discussed in the text, the direction and intensity of wind controls the pattern of valve aperture and the amount of CO_2 injected over the plot.*

Computational fluid dynamics, usually abbreviated as CFD, is a branch of fluid mechanics that uses numerical methods and algorithms to solve and analyze problems that involve fluid flows. Calculations required to simulate the interaction of liquids and gases with surfaces are made by defining adequate and realistic boundary conditions. A specific challenge of CFD simulation reproducing field situations is the proper setting of atmospheric turbulent conditions in the proximity of plant canopies. Our study made in the frame of the ExpeER project made use of different turbulent energy production/dissipation schemes, also involving parameterization of surface drag forces as discussed by Xu et al. (1997).

An initial test of the GradFACE concept was also made in a real field application at the USDA-ARS field station in Cheyenne, Colorado (USA) and the preliminary results indicate that it is possible to obtain a consistent gradient of CO_2 concentrations under different wind directions and wind speed situations as the model anticipated.

There may be a significant advantage in using this type of gradient design in elevated CO_2 studies. Tunnel experiments made in Texas (USA) created a consistent CO_2 concentration gradient (Polley et al. 2003) and demonstrated that non-linearities occur in plant responses as [CO_2] increases.

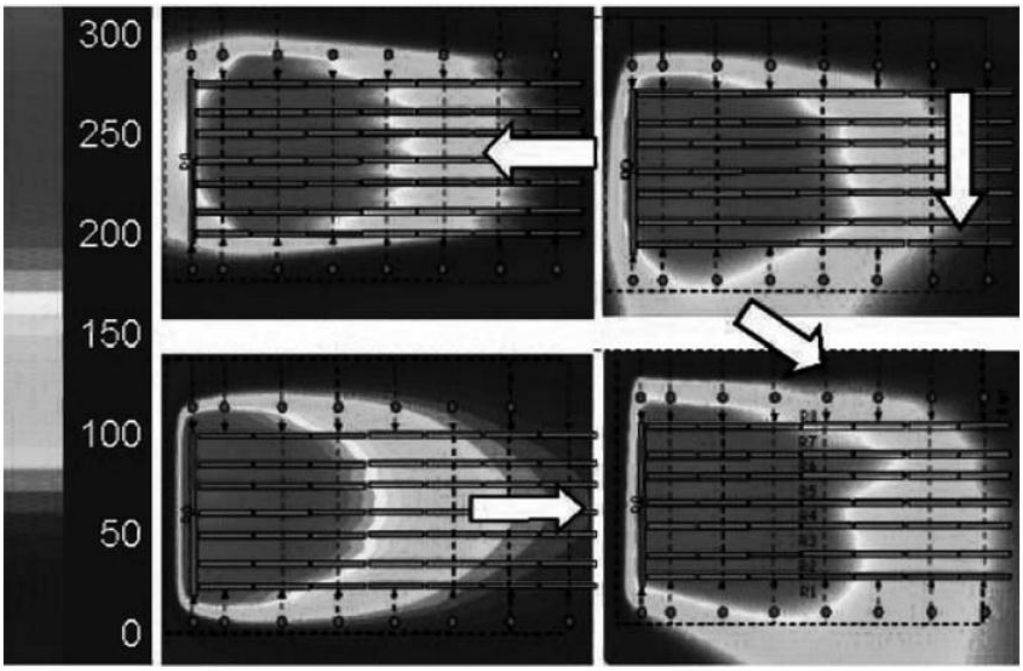

Fig. 10: *Distribution of [CO_2] in GradFACE (in ppm) above ambient [CO_2] calculated by the CFD model at 35 cm above ground in a 15 cm tall short-grass prairie with wind blowing from four different directions (arrows). In these simulations, adjustment of valve apertures allows maintenance of a well-defined elevated [CO_2]*

Such observations provide very valuable information for modelers. In addition, gradient experiments may enhance the statistical power of elevated CO_2 experiments, as they are amenable to regression analysis rather than the standard analysis-of-variance approach typically used for block experimental designs by most FACE studies.

References

Z.G. Xu, P.J. Walklate, A.R. McLeod (1997) Numerical study of a full-size free-air fumigation system. *Agric. Forest Met.* 85 (1997), pp. 159–170

Polley HW, Johnson HB, Derner JD (2003) Increasing CO2 from subambient to superambient concentrations alters species composition and increases above-ground biomass in a C3/C4 grassland. *New Phytol* 160: 319–327.

4. Task T8.3: Designing new approaches for experimental ecosystems

(contribution by CNRS)

A. Background

In ecology, experimental approaches involving simplified ecosystem models (microcosms/pots) are often use to inform global ecological problems (e.g. Benton et al. 2007). Despite oversimplification of ecological complexity, microcosms are often preferred as experimental model systems due to ease of replication, precise control of environmental variables and high feasibility of manipulating the parameters and treatments under investigation. These characteristics of microcosm experiments, together with exigent standardization of biotic and abiotic parameters are assumed to make the experiments more replicable (i.e. to facilitate exact replication of a given experimental setup and produce the exact same result in identical conditions) and more reproducible (i.e. to successfully reproduce the general findings of an experiment, implying external validity and generality of the findings).

Recently, the issue of poor reproducibility of experiments has been given significant attention in prominent journals such as Nature and Nature Methods due to mounting evidence of poor reproducibility of many studies, leading to a "reproducibility crisis" in many fields of science (Drummond 2009, Richter et al. 2009, 2010, 2011, Begley and Ellis 2012). Several studies point out that exigent standardization, typically assumed to increase the statistical power (by reducing within treatment standard deviation) and replicability, might be responsible for generating results lacking in external validity (local truths). However, despite this standardization, laboratories inherently vary in many experimental conditions, such as experimenter, environmental conditions, physical characteristics of the experimental set-up, conditioning of biota, etc. The emerging hypothesis is that issues with replicability are caused by exaggerated and oversimplified standardization, which removes any source of biological and environmental variation. By artificially narrowing the experimental conditions, the model system under study becomes more prone to being influenced by any unaccounted local particularity, which increases the likelihood to find local truths (Fig. 11). Recently, several studies argue that the introduction of controlled systematic variation (heterogenization) is the answer for obtaining increased reproducibility (Richter et al. 2009, 2010, 2011, Würbel et al. 2013).

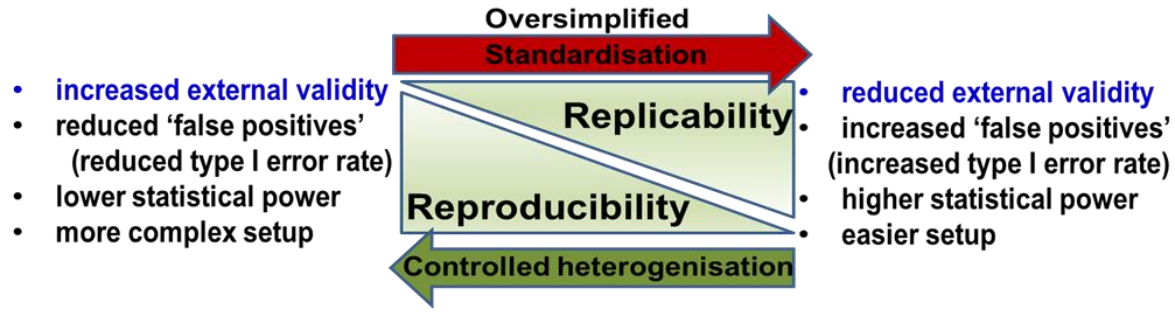

Fig. 11: *Overview of potential trade-offs between replicability and reproducibility.*

B. Multi-laboratory experiment

We initiated a multi-laboratory experiment (14 laboratories in France, Germany, Switzerland, England, Italy) to test the overarching hypothesis that the reproducibility of results between laboratories will increase when controlled systematic heterogenization (both biological and environmental) is incorporated. Specifically, we address the following research questions?

1) Does introducing controlled heterogenization increase the reproducibility of results between several laboratories?
2) If so, what level of heterogenization (biological and/or environmental) is needed to achieve a detectable increase of reproducibility?
3) What is the relative importance of heterogenization introduced at the pot/microcosm level (within a treatment) vs. heterogenization between individual replicate pots?

The set-up is fairly simple, and is aimed towards quantifying the well-known legume-grass facilitation effect by comparing mixtures of a grass (model grass Brachypodium distachyon) and a legume (the model legume Medicago truncatula). Measurements were: Plants height and biomass, Brachypodium shoot C, N and 15N content, Brachypodium gene expression (N uptake related) in roots, pots evapotranspiration and soil organic matter decomposition rate.

The following treatments were established:

I. Presence and absence of nodulation in Medicago truncatula in microcosms with a grass species (2 levels, with the non rhizobia-nodulating Medicago truncatula mutant and without, but with a closely related nodulating variety).

II. Controlled systematic variation at the pot level with 6 levels:

1) Standardized microcosms/pots with typical standardization procedure, i.e., everything is homogenized/ standardized, e.g. one seed source, homogenized soil (Ctr).
2) Environmental heterogenization introduced at the pot level, e.g. pots with more heterogeneous soil conditions (e.g. with introduced patchiness). This is achieved by working with two soil types (or with soil and sand), with thoroughly homogenized soil (or soil-sand) mixtures for the control and non-homogenized mixtures (patches) for this treatment.
3) Environmental heterogenization introduced between replicate pots. For this treatment, three levels of soil heterogenization are introduced between the replicate pots of this treatment (more or less patches).
4) Biological variation introduced at the pot level (e.g. heterogenized seed source), i.e. instead of one seed source/variety, three seed sources/varieties are introduced at the pot level.
5) Biological variation introduced between replicate pots. Three levels of biological variation, in this case the seed source, are introduced between pots by setting up microcosms with one, two or three sources/varieties of seeds.
6) Environmental and biological variation at pot level (i.e. combining the setup from point (2) and (4) to include two sources of heterogenization, one biological and one environmental.

In addition to the above mentioned gradient of six levels of heterogeneity, an additional, (7th) level of inter-pot heterogeneity can be derived by including, in the statistical analysis, individual replicates from the levels one to six as replicate pots as indicated in the figure below by the rectangle selecting pots across the six initial treatments (Fig. 12).

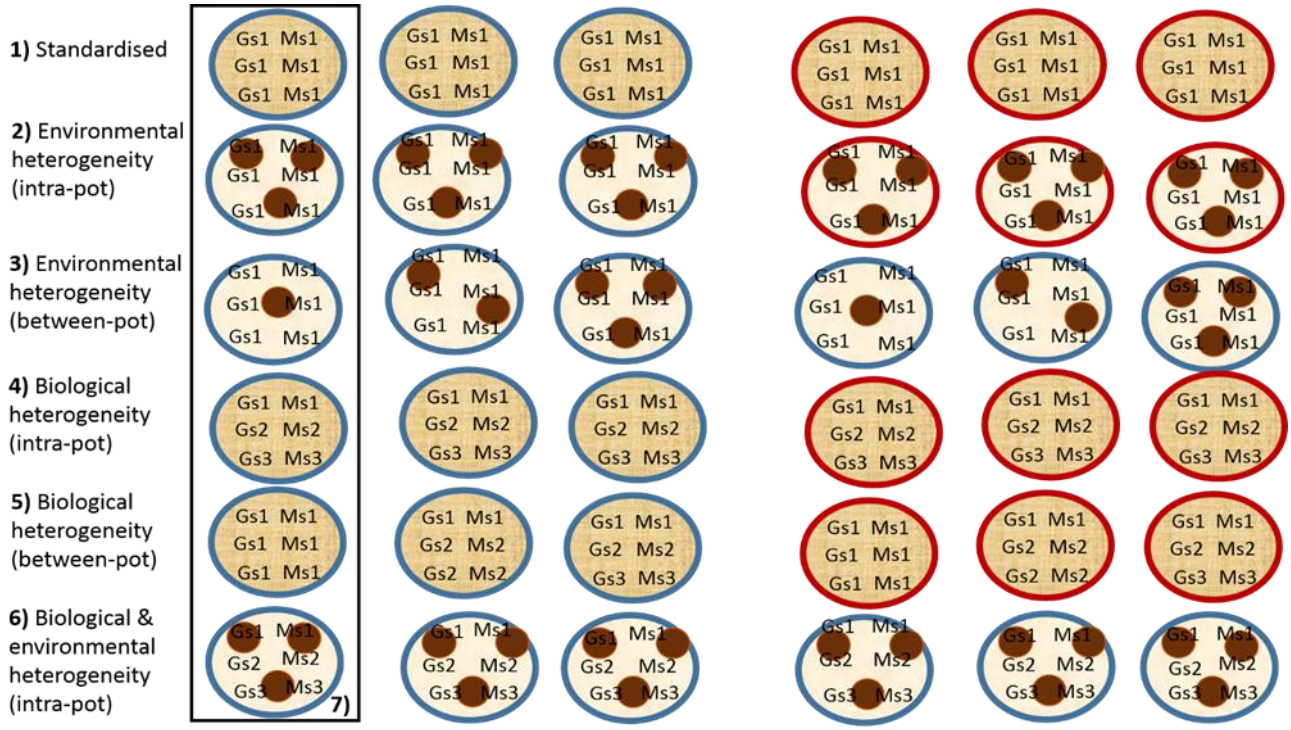

Figure 12. *Simplified schematic depicting the experimental setup at the pot/microcosm level for the seven levels of heterogeneity mentioned above. For simplicity the schematic includes just three replicate pots, but the idea is to multiply the below setup by two in order to achieve a replication of 6 pots per treatment.*

C. ExpeER publications

Milcu et al. Do we need to introduce controlled systematic variation in microcosm experiments to improve their reproducibility? In preparation

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5. Task T8.4: New generation biodiversity/climate change experiments

5.1 Global change experiments: challenges and opportunities

(contribution by UA)

A. Background

Manipulation experiments have become an increasingly important scientific tool in research focusing on understanding the ecological consequences of global changes such as climate change, nitrogen deposition and biodiversity loss. The oldest of those is the Park Grass experiment, which has been running for over 150 years, providing a wealth of data against a background of environmental change. In contrast to traditional field surveys and monitoring in natural and semi-natural ecosystems, manipulation experiments enable researchers to specifically test hypotheses. Hundreds of ecological experiments are being initiated each year, varying widely in scale and design. Their ultimate goal is to acquire fundamental understanding of ecological processes to enable the extrapolation of case-specific results. Unfortunately, flawed designs and experimental artefacts can lead to misinterpretations and may severely limit generalizations to wider contexts. Therefore, artefacts should be identified and then avoided if possible.

Scientists may be aware of potential problems and eager to improve the methodology, but unable to resolve this issue because of financial or logistical constraints. For example, it is well known that passive open-top chambers are an imperfect warming technique, but these chambers require no power and hardly any maintenance and are therefore highly practical in warming ecosystems in remote and inaccessible areas. In such cases the challenge is then to be aware of the drawbacks of the applied method, formulate hypotheses that reflect these and extrapolate findings cautiously. On occasion, however, researchers may not realize that the designs and infrastructures they use are reason for concern or that solutions exist to solve or circumvent artefacts. Knowledge on experimental artefacts and workarounds is scattered throughout the literature, often reaching only part of the scientific community and not crossing interdisciplinary boundaries.

To transcend the very detailed difficulties found in specific domains, we address issues that are relevant to a wider range of global change ecologists. We consider major choices and challenges that experimentalists are confronted with, discuss whether they can significantly impact the studies' conclusions, and if that is the case, we suggest ways forward. Some artefacts may be circumvented, other issues are inherent and cannot be avoided altogether. Even then, choices can be made to ensure that experimental artefacts do not become detrimental to the conclusions of a study. Our aim is thus to refine the general approach, as fundamentally, the manipulation experiment is an invaluable tool to identify processes underlying change and gain process understanding sufficient to drive models of ecosystem response to global change.

B. Challenges and suggestions

Important for all issues discussed below are the fundamental trade-offs that exist between external and internal validity (Fig. 13). The "external validity" relates to the experimental realism and the applicability of a result to other conditions or systems. On the other hand, more stringent control

over variables stimulates “internal validity”, i.e. the confidence that the treatment is the cause of the observed effects. Indeed, allowing more processes and factors to vary freely in an experiment makes it reflect reality better, but also renders attributing changes to specific processes more difficult.

In this regard it is crucial to recognize that the questions that can be answered by simplified experiments or those involving more complexity will and have to be different. More control by blocking realistic but unwanted sources of variation allows researchers to uncover fine-scale or specific mechanisms, while a better representation of natural complexity allows for more straightforward extrapolation of results and trends but also less understanding of the underlying mechanisms. The position of the study on the internal to external validity gradient (Fig. 13) inherently constrains it, and failing to recognize the limitations, leads to a number of pitfalls, of which the most important is the inappropriate extrapolation of findings. On the other hand, awareness of the strengths and weaknesses of an experiment also generates opportunities. It allows for more streamlined hypotheses and well-grounded conclusions, and also enables devising explicit combinations with experiments positioned at other places along the gradient, creating added value by transcending limitations posed by single experiments.

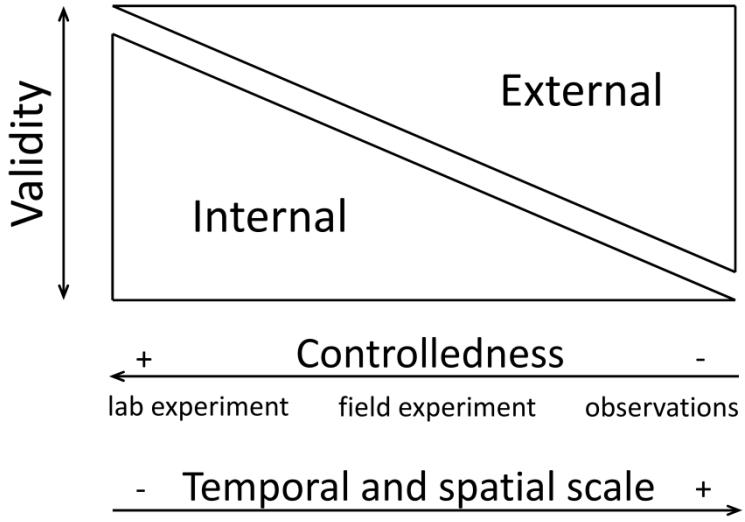

Fig. 13: *Schematic representation of the trade-off between internal validity (being able to attribute a change to a specific factor) and external validity (the confidence with which results can be generalized).*

We discuss issues related to three main topics: treatment choices, spatial scale and time scale. The most important aspects are summarized below and in Fig. 14.

- <u>Treatment choices and resulting challenges:</u>

  Global change involves interacting drivers of ecological responses, both environmental and biotic, that can generate non-additive effects through positive and negative feedbacks.). Testing a complete set of projected future conditions is not practically feasible, which means priority should go to manipulating factors that are most likely to change, preferably in full-factorial combination. Single factor experiments be used to improve mechanistic understanding under tightly framed hypotheses, but extrapolation should be done cautiously.
  New and alternative approaches have recently been suggested in global change ecology. One interesting new direction is the use of regression/gradient approaches as an alternative to replicated ANOVA-designs, in order to maximize the number of interactions and levels testable. Such experiments would result in response surfaces to be analyzed by regression

techniques and might reveal thresholds and tipping points better than classical replicated setups, which is particularly important in extreme event research. Furthermore, promising new methods have been developed to help elucidate process dynamics and sensitivities related to biotic and/or abiotic feedbacks. For example, plant-soil feedback treatments (growing a plant community on soil that was previously used for another community so that soil characteristics changed) generate valuable insights into the processes driving ecosystem succession and invasion. Finally, making use of 'natural laboratories' offers a number of unique possibilities. Geologically active locations harbor areas where CO_2 is released (CO_2 springs), or where soils and streams are warmed. These could be used to test long term effects of CO_2 addition or warming in a natural setting. Embedding highly controlled experiments within such settings could create unique possibilities, such as studying effects of long term acclimation to warmer conditions on the sensitivity to temperature pulses (for example by heat waves).

In full-factorial experiments, isolating the single factor effects of the imposed treatments is challenging, as manipulating one factor can also change others. Consequently, the response to a treatment often includes not only direct but also co-occurring changes, rendering it more difficult to attribute changes to a specific factor. In other words, interdependences increase the external validity at the expense of the internal validity of the study. Co-varying factors can be separated using explicit designs, which due to their complexity and artificiality are warranted only if researchers want to keep full control of major factors in order to precisely attribute responses. In other cases, it may be more appropriate to aim for high external validity by allowing naturally occurring interdependence to develop. Increased awareness of co-varying factors and their quantification through measurements should always be pursued as it helps to avoid attributing responses to the wrong factor (i.e. being right for the wrong reason) and to understand direct versus indirect mechanisms of treatment responses.

Island effects are another mechanism that can influence imposed treatments. These are a direct result of the inherent limitations regarding scale, making outdoor experiments islands in a wider landscape that remains unchanged. Whenever experiments alter conditions in the study area, these conditions may be perceived as more or less attractive to animals, changing plant-animal interactions. Island effects can also lead to a reduction of the effect size by pushing mesocosms closer to the conditions of their surroundings, both in the abiotic and the biotic sense. Some of these issues could be overcome by increased isolation of the communities from the outside. However, isolation may therefore an artefact whenever processes such as colonization and herbivory are important, rendering the potential for making long-term predictions from systems in isolation challenging. One solution to such issues is to artificially include biotic changes at the larger scales. In the case of drought experiments, this could encompass adding seeds in quantities that reflect drought effects in the entire landscape. Obviously, this requires a priori knowledge, which could be gained from observational studies or many small experiments. A solution such as this remains a simplification of natural processes, but at least it acknowledges processes beyond the island that is the experiment in order to increase its realism and potential for extrapolation. Comparisons with natural systems could indicate how important feedbacks and mechanisms not represented in treated 'islands' may be. It is in any case necessary to quantify which treatment levels are ultimately realized because, as we have demonstrated, island effects can alter the intended treatment.

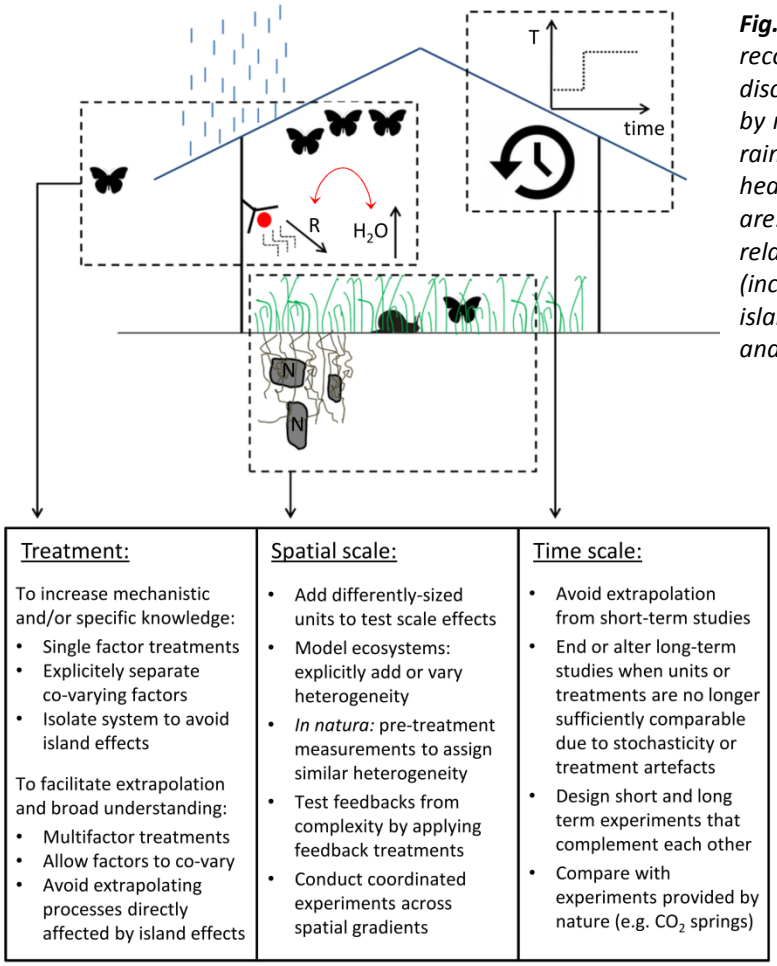

Fig. 14: *Overview of main recommendations for issues discussed in our study, visualized by means of an experiment using rainout shelters and infrared heaters. Depicted and discussed are: choices and challenges related to the treatment (including co-varying factors and island effects), the spatial scale and the time scale.*

- Spatial scale:

The spatial scale of the experiment is a fundamental choice that directly influences internal and external validity. This implies that by using an inappropriate scale relative to the studies' aims, either too much or not enough biotic complexity and spatial heterogeneity is taken into account, undermining the conclusions. Therefore, the spatial scale needs to be carefully matched to the processes and ecosystems under study. For experiments highly focused on specific processes or mechanisms, such as the role of the spatial patterning of plant species in invasion resistance, the scale can be kept limited as in such studies high internal validity (low 'noise' of uncontrolled aspects or processes) is key. When external validity gains importance because we want to understand how given small scale responses can be generalized or lead to larger scale responses, the scale of the experiment has to be large enough to feature higher levels of complexity. Challenges are often different for *in natura* and assembled (model) ecosystems.

Artificially assembled systems strive for large internal validity and will decouple many of the interactions between the scale of the mesocosm and complexity and heterogeneity. Enlarging plots in these highly-controlled experiments would only make sense if this significantly affects key response variables. Minimal sizes can be and have been defined regarding surface area and volume. Like minima for plot or mesocosm dimensions, minimum amounts of resource heterogeneity may also be required as heterogeneity in the distribution of nutrients can for example modulate interactions between climatic changes and

biodiversity. In assembled systems it may thus generally be advisable to actively include sources of heterogeneity in a controlled manner, for example patchy nutrient and water supply, if these are considered important for the processes being studied (Fig. 14). On the other hand, some solutions may surpass practical feasibility. In those cases, applying the same experiments also *in natura* could give information about the generality of the findings. Alternatively, using intact samples of established ecosystems keeps most heterogeneity near its natural levels.

In natura experiments are often limited in scale by practical considerations, as many manipulations are difficult or even impossible to carry out across large areas. Even when the manipulation is provided for free by natural laboratories (see earlier), the large scale can pose challenges regarding work load. It would therefore be useful to establish minimum plot sizes, i.e. the minimum of a range in which processes are independent of scale. However, such knowledge is scarce and often very specific. Including plots or mesocosms of different spatial scale in the same experiment would help to elucidate whether scale affects the results and where (and whether) scale saturation occurs, facilitating interpretation and increasing comparability. This would also be valuable in artificially assembled ecosystems. Such a multi-scale approach has mostly been used in aquatic systems so far. Whatever the size of the plots or mesocosms, the heterogeneity of *in natura* systems in question should be captured adequately, avoiding results being skewed by over-representing some patches and under-representing others. This requires using an adequate number of replicates (i.e. more if heterogeneity is more variable) and well-reasoned distribution across the landscape and across treatments. Pre-treatment assessment or characterization of physical, chemical and/or biological heterogeneity can be very valuable in this regard.

Biological complexity, defined here as the whole of biotic interactions within an ecosystem including food web structure, community composition and their interactions, can interact with ecosystem responses to global changes. It is inherently included in experiments *in natura*, although within limits. Sometimes these limits result mainly from active measures such as fencing, but mostly they stem from the inability to extend the manipulation to the entire landscape (cf. island effects). Including all aspects of complexity can only be approached by means of observation studies on long time scales, however. In assembled systems, many aspects of complexity are not included (e.g. colonization and/or herbivory) in order to safeguard high internal validity. Experiments could gain in realism if aspects such as more natural demography (particularly in regions characterized by individuals with long life spans), colonization rates (e.g. by providing natural propagule influx) or intraspecific variation were introduced, albeit at the cost of internal validity.

An overarching recommendation regarding scale issues is to increase the scale of the experiment instead of the plot/unit. This can be achieved by combining experiments and gradient studies by conducting the same experimental manipulations along climatic gradients, with manageable cost, work and space requirements for each site (Fig. 14). This reconciles safeguarding internal validity at the local scale with increased external validity across all sites. Trends and relationships found across study sites would substantially increase confidence that these are not merely a product of local conditions and thus improve the potential for extrapolation. At the same time, locations where diverging trends are found can reveal specific mechanisms and place limits on the generality of uncovered trends and processes. Asking the same research question in studies differing in realism, i.e. along the gradient in Fig. 13, could work in a similar way: identifying robust mechanisms when similar results are found, or, when results differ, marking areas where improved process understanding is needed.

- Time scale:

  Whether the duration of an experiment can be deemed "sufficient" depends on the hypotheses the experimental study aims to test and the amount of extrapolation envisaged. Short term experiments can be important for process-understanding and identifying thresholds and tipping points. However, examples from CO_2 research have taught us that extrapolating from short experiments may be misleading, with short term conclusions on the stimulating effect of CO_2 on growth and productivity being contested by observations following a longer period of treatment. Part of the problem here may be that many aspects of global change manifest slowly and incrementally in time, while experiments are typically imposed in a stepwise manner. Stepwise and gradual changes may trigger different responses, as shown by both modelling and experimental studies, suggesting that stepwise changes could be considered as a perturbation, to which systems cannot acclimate in the short term. Obviously, there are few alternatives to step change experiments. The challenge is then to correctly assess and more fully exploit their potential. To achieve this, a number of recommendations can be made. Firstly, experiments can be specifically designed to assess to which extent stepwise treatments differ from treatments imposed more gradually. Alternatively, observations from long-term monitoring studies (e.g. tree ring analysis, ecosystem stocks and phenological records) and studies involving natural gradients could be used as comparison (Fig. 14). For example, the FORHOT experiment in Iceland uses natural increases in soil temperature caused by volcanic activity to study soil warming effects. As these hot spots are variable in time and emerge in new places, researchers can compare how short term (~ 5 years) and longer term (> 30 years) effects differ, and thus filter out perturbation-induced transient responses resulting from the stepwise change. The mechanistic understanding thus gained can then be used to inform and correct existing models to better predict responses to gradual scenarios.
  The stepwise nature of imposed treatments is not the only reason to be cautious in extrapolating from short term studies. For example, the importance of species richness may be underestimated if inferred from short term data, as the effects of biodiversity loss can escalate through time because important plant-soil feedbacks develop only slowly. Based on our current knowledge, long term responses can often not be derived from short term effects, hence warranting experiments on longer time scales. An advice to simply conduct experiments longer is not a miracle solution, however. Apart from logistical constraints, one complicating factor is that the amount of variation will increase the longer the experiment is running. This can occur because random events or stochastic trajectories of succession may make replicates in a study less comparable (i.e. a loss of internal validity). The degree to which communities are affected by treatments should therefore be quantified, for example by means of similarity measures. The decision can then be made to end the experiment (if communities are no longer sufficiently comparable) or to shift the focus to a more individualistic perspective at the level of communities. Furthermore, unavoidable imperfections in the experiment such as island effects and other artefacts may be proliferated as the length of the treatment increases (i.e. a loss of external validity). Researchers therefore need to be aware of the drawbacks of the set-up and techniques used, which is often not readily apparent.
  Ultimately, short and long term experiments are both needed to link short and long term responses, and this requires experiments dealing with the same question along the gradients of scales. More specifically, we make the following suggestions. Firstly, if an experiment focuses on short term responses, the signal from the imposed environmental change must be strong enough to be detected against the natural intra- and inter-annual variation. If this signal-to-noise ratio is low, short experiments run the risk of concluding that 'no changes are detected', leading to erroneous extrapolation. This logically leads to the next

recommendation: design short and long term experiments that complement each other (Fig. 14). For example, extreme events often act like a disturbance allowing two complementary strategies to be adopted: (i) a short term experiment focusing on the processes related to the extreme event and its immediate effects on the ecosystem and (ii) a long term experiment to evaluate fundamental changes in the ecosystem structure and functioning, for example through changes in biodiversity and community composition or food-web complexity. This would help develop the knowledge needed to predict long term effects based on shorter term observations, and which could be verified in the same set-up. Finally, compare the findings with experiments provided by nature (see earlier), where acclimation to the local conditions has developed across decades. These recommendations again illustrate how important awareness of the study's limitations and possibilities (cf. Fig. 13) is in avoiding several pitfalls, and highlight the opportunities that arise when combining experiments across scales.

C. Conclusions

To enable understanding of ecosystem responses to global change and making predictions that are valuable also to local stakeholders confronted with global changes that trigger very site-specific responses, we need experiments to test specific hypotheses. As we have argued throughout this review, the answers that single experiments can provide are confined by the trade-off between internal and external validity (Fig. 13). The experimental challenges often differ for studies on opposite sides of the gradient. Highly controlled studies aiming to uncover specific processes could for example gain from explicitly separating co-varying factors (to attribute responses more precisely) and sticking to short time frames (to keep maximum control over treatments), while opposite recommendations would benefit studies targeting realism and broad understanding. Some recommendations, such as including additional experimental units of different size to reveal scale-dependencies, are relevant to any study, however. Multiple problems could be remedied by combining studies. Firstly, this could encompass the combination of studies across spatial gradients, using coordinated experiments. This would facilitate identifying broad trends (when effects are similar across sites) and factors overruling these trends (when effects differ on one or more sites). Secondly, studies of different duration can be combined, such as short term (imposed) warming experiments and studies on locations where natural warming (for example in geothermal streams) has been present for decades. Thirdly, bringing together experiments carried out across the gradient of internal to external validity would reconcile both highly specific and broader understanding. These recommendations would help identify overarching patterns and strengthen process-based models, improving both our understanding of global change impacts and the confidence of our predictions on ecosystem responses.

D. ExpeER publications

De Boeck HJ, Vicca S, Roy J, Nijs I, Milcu A, Kreyling J, Jentsch A, Chabbi A, Campioli M, Callaghan T, Beierkuhnlein C, Beier C. 2015. Global change experiments: challenges and opportunities. BioScience, in press.

5.2 Biotic interactions and feedback effects in manipulative experiments in ecology

(contribution by UFZ)

A. Background

Besides observational approaches a great part of ecological theory and concepts bases on manipulative experiments. Such experiments are of course often artificial systems which necessarily lack a certain degree of natural complexity but are nevertheless used to explain processes and mechanisms in natural systems. Whilst the use of simple systems in manipulative experiments is useful for determining the mechanisms behind certain ecosystem functions they also ignore mediating effects of interacting processes like biotic interactions. On the other hand, many experiments are conducted in complex natural systems and treatment effects may depend on specific environmental settings resulting in "context-dependent" results in manipulative experiments.

Species interactions like competition and trophic interactions are known to be important drivers of community dynamics and structure, thereby affecting energy flow and element cycles in ecosystems and consequently ecosystem functioning. There is strong evidence that the outcomes in ecological experiments dealing with the effects of global change (e.g. diversity change, climate change) strongly depend on the presence and the manipulation of trophic interactions. Numerous studies showed that the influence of global change factors is importantly mediated by their effects on biotic interactions like pollination, herbivory, symbioses etc. However, biotic interactions have been turned out to be one of the sources of uncertainty in predicting the consequences of global change since it has been shown that these indirect interaction-mediated affects may overwhelm direct effects (Thylianakis et al. 2008). Experiments documented strongly interacting effects between global change and trophic interactions for a wide range of systems ranging from vertebrate grazing system (Post & Pedersen 2008, Espinosa et al. 2013, Kaarlejärvi et al. 2013, Porensky et al. 2013, Post 2013) and invertebrate herbivores (Gianoli et al. 2009, Moise et al. 2012) to soil food webs (Crowther et al. 2012). Moreover, Lau & Lennon (2012) showed that plants under global change may benefit from associations with the interacting soil microbial communities due to their ability to adapt and respond to global change.

B. Methodological challenges

Can we explain complex communities with simplified food webs?

Of course, the importance of biotic interactions for given ecosystem functions are easiest to investigate in very simple artificial communities. However, this does not reflect the complexity of natural systems, especially the mediating effects of simultaneously operating further interaction types. This approach is not only true for experiments lab or field experiments, it also applies to modelling approaches. Therefore, since interaction motifs are extracted from complex networks they provide just basic information on the type of interaction and underlying mechanisms which have to be verified in a more complex community context.

Are the model organisms representative?

Manipulative experiments on trophic interactions are often done with standard organisms which are easily available and easy to rear under lab conditions. For instance, the role of earthworms as

decomposers and ecosystem engineers is often investigated for Lumbricus terrestris which differs quite a lot from most earthworm species in regards to ecology and behaviour (Fründ et al. 2010). For AM fungi Glomus intraradices is often used as model organism whilst Hartley & Gange& (2009) found that the effect of G. intraradices on plant-herbivore interactions consistently differs from other mycorrhizal fungi. Interestingly, whilst G. intraradices induced similar effects on the anti-herbivore resistance across several plant species (Kempel et al. 2009), different AMF isolates may induce different resistance responses in a single plant species (Roger et al. 2013). Therefore, authors and readers have to be careful when interpreting the results as they have to separate species identity effects from potential effects of functional groups.

Manipulation of trophic interactions in natural (complex) communities

Manipulation of biotic interactions in complex communities is much more challenging and nearly impossible without any side effects. Common methods involve the exclusion of certain species groups e.g. by the application of aboveground and belowground pesticides (possible toxic effects on non-target organisms), caging, bagging of plants (possible changes in microclimate etc.). Other approaches might be useful, for instance the enhancement of the relative importance of given interaction types in natural communities, e.g. by litter addition etc. to increase the abundance of detritivorous species.

The long-time overlooked importance of microbial players in ecosystems further calls for methods which can be applied for the manipulation of microbiota. Usual methods to exclude soil microbiota include sterilization by steam, gamma rays, autoclaviation, drying and pesticides (e.g. fungicides). All these methods are considered to have substantial side effects and are known to affect soil properties like nutrient availability which have been shown to potentially feedback on plant communities and may also differ between different soils (He & Cui 2012).

Pesticides have been used for the exclusion of a wide array of organisms ranging from plants (Stein et al. 2009), insects, molluscs and microorganisms. Common criticism on the use of pesticides is related to the (lack of) specificity and the possibility of side effects. E.g., fungicides against fungal diseases have been shown also to affect AM-fungi (Jin et al. 2013). The drawbacks of sterilization approaches as an alternative to manipulate soil microbiota have been outlined by He & Cui (2012). Addition experiments may circumvent some of the disadvantages of exclusion techniques but may also interfere with (other) species interactions. E.g., additions of mycorrhizal inoculums in pre-established fungal communities may lead to increased competition between fungi with (partly negative) consequences for plant growth (Januskova et al. 2013).

Even in field experiments, experimental manipulations of environmental factors may have the “hidden treatments” affecting the density of interacting species unintentionally. For instance, physical barriers (e.g. open-top chambers, fences) may prevent colonization by animals (e.g. herbivores) and some of the commonly measured treatment effects may be attributed in part to the absence of these consumers (Moise & Henry 2010). Thus, the density of consumer species on differentially treated plots can be affected by the specific experimental set-up or even by the treatments themselves.

C. Conceptual challenges

Do we lack knowledge about important linkages?

Food webs are complex and consist of a multitude of direct and indirect interactions between species. In many food webs, a great number of players is still largely unknown – especially with

regards to soil food webs and microbiota. New (molecular) tools mainly expand our knowledge on biodiversity but not (that much) on function of trophic interaction webs. This is especially true with regard to rather "enigmatic" species (e.g. in soil) or microorganisms. Even if the role of soil biodiversity received much attention in recent years it is still not clear if the extremely high number of species in soil is related to a high degree of functional redundancy or to a multitude of unexplored species interactions in that system. Further, the function of highly diverse cryptic groups in ecosystems (small invertebrates, microflora – 'hidden players'. see Thompson et al. 2001) is often not well explored and many of these species cannot be cultured and are therefore hardly amenable for experimental manipulation

How can we consider complex interaction networks?

The fact that there is no consensus on the relative importance of specific biotic interactions in ecosystems may simply reflect the high degree of context dependency of the strength and outcome of functional links between species. A given biotic interaction can be affected by direct and indirect effects of other associated species, the genotype or genetic make-up of involved species and abiotic conditions. For instance, Haase et al (2008) demonstrated that the effects of herbivores on plant communities is strongly driven by the activity of decomposers and vice versa. Moreover, the availability of nutrients strongly mediated the relative importance of functional links. Whilst in low fertile soils the influence of decomposers on plants and associated herbivores was strongest, the negative top-down effects of herbivores were much more pronounced in highly fertile soil. Also, the predominance of positive and negative interactions can be shaped by resource availability (Jousset et al. 2011 Ecol Lett) or the availability of distinct niches (Eisenhauer et al. 2012 Funct Ecol).

Whilst plant competition is considered in many studies as one factor strongly mediating the effects of trophic interactions (Schädler et al.2008), direct and indirect competition between phytophages is known to be important in natural communities (Kaplan &Denno 2007) but is largely neglected in experimental studies. Indirect competition is often mediated by plant responses which therefore determine the spatial and temporal aspects of such interactions. For instance, local defence responses may importantly differ in their effects on communities from systemic plant responses and changes in volatile production. Especially the latter mechanisms are known to affect the food web structure of communities importantly. Recent studies demonstrate that induced plant responses also drive above-belowground interaction (van der Putten et al. 2001, Wardle et al. 2004, Bezemer& van Dam 2005, Erb et al. 2008) and indirect defences via volatile production also shapes soil food webs (Hiltpold etc.). Local belowground interactions are known to induce changes in nectar and volatile production aboveground which mediate species interactions at larger spatial scale (Wäckers&Bezemer..).

Does the scale of an experiment match the scale of relevant interactions (spatially and temporarily)?

There is a wide range of approaches in experimental interaction ecology which differ fundamentally regarding the addressed species, temporal as well as spatial scales, complexity and consequently representativeness for natural systems. Simple lab experiments usually focus on specific, often pairwise species interactions under standardized conditions. Of course, they are not designed to reflect any natural conditions or the complexity of interaction networks in ecological communities but rather focus on the presence and mechanisms of specific interactions (Fig. 15). In contrast, field experiments are usually designed to assess the importance of interactions in complex communities and under natural conditions. This necessarily leads to confounding patterns regarding the different approaches, the body size of the investigated players and temporal as well as spatial scale. Considering this, the scale (both spatial and temporal) may modify the degree to which an experiment covers the potential interactions within a system. For instance, whilst the total local

species pool of microbiota in soil may be available on a scale of a few centimetres, it might take tens or hundreds of meters to cover all plant species of a community. As a consequence, an experiment on the scale of centimetres or a few meters can not consider all species interactions of this ecological community and might even miss some of the more important ones. This also applies to temporal scales since a number of interactions are clearly realized only at specific time points (e.g. flowering and pollination) which might also be missing in short-term experiments (Fig. 16). Lab experiments (e.g. using microcosms) usually deal with small sized species, e.g. insects or microorganisms. Moreover, the duration of such experiments is only rarely longer than a few weeks what does not cover the life span of most insects (but may allow thousands of generations in the case of microorganisms). However, the importance of the rather chronic effects of smaller sized organisms for ecosystems has been found to accumulate over longer time scales. In contrast, the effects of larger organisms (e.g. vertebrate grazers) are often spatially and temporally less predictable but may cause changes which are pretty obvious even on shorter time scales. Thus, experiments which are designed to investigate the effects of small organisms may often not fit to the relevant time scale of their effects in real ecosystems.

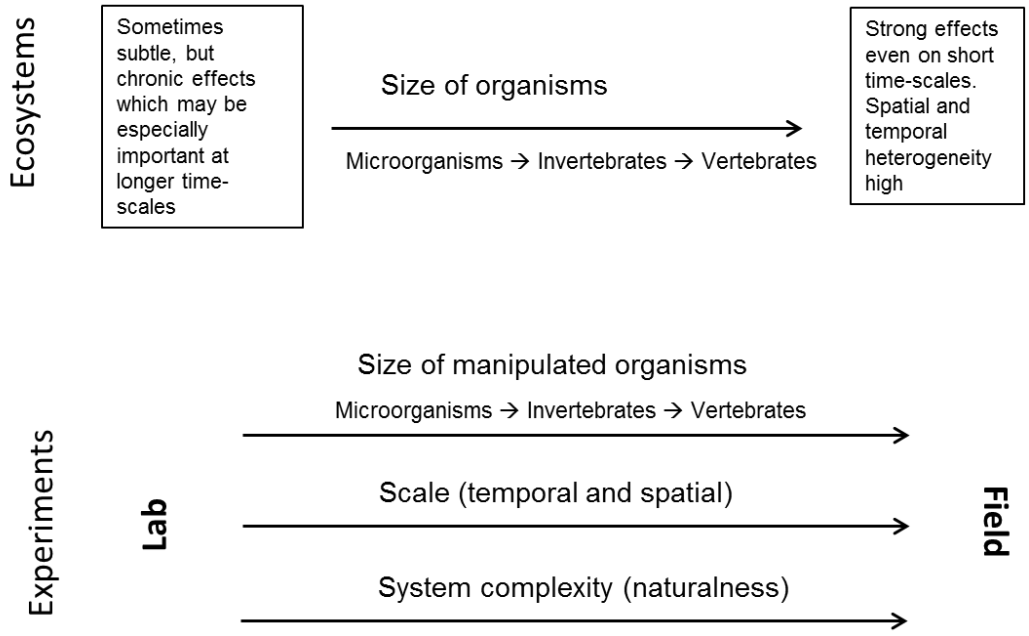

Fig.15: *Interacting biota may show dynamics on differing temporal and spatial scales. They may further show different patterns of heterogeneity and spatial patterns in nature. Therefore, the scale of an experiment involving given interacting biota may not (or may) reflect the scale where this specific interaction is realized in natural ecosystems.*

D. Conclusions for future studies

a) Comparisons between lab studies and field are useful to identify the potential role of biotic interactions in relation to their relative contribution to ecosystem effects in natural system.
b) Comparison between short-term studies and long-term studies are useful to evaluate transient dynamics of ecosystem change.
c) Life-history traits of the involved species and the (expected) dynamics of the evaluated effects determine the spatial and temporal scale of manipulative experiments.

d) Statistical methods can be used to (i) disentangle indirect and direct effects of trophic interactions and (ii) use context-dependency as information for mediating effects of environmental conditions for the variability of observed effects. For (i) the common experimental statistics can be supplemented by multivariate approaches. For instance, structural equation modelling can be used to clarify but also to model causal connections between different observed variables and in response to factors like biotic interactors in experiments. For (ii) meta-analytical approaches are suitable tools to overcome "context-dependency" and to investigate mediating effects of environmental variables as well as to evaluate overall-effects of trophic interactions in different systems and under different enviuronmental settings.

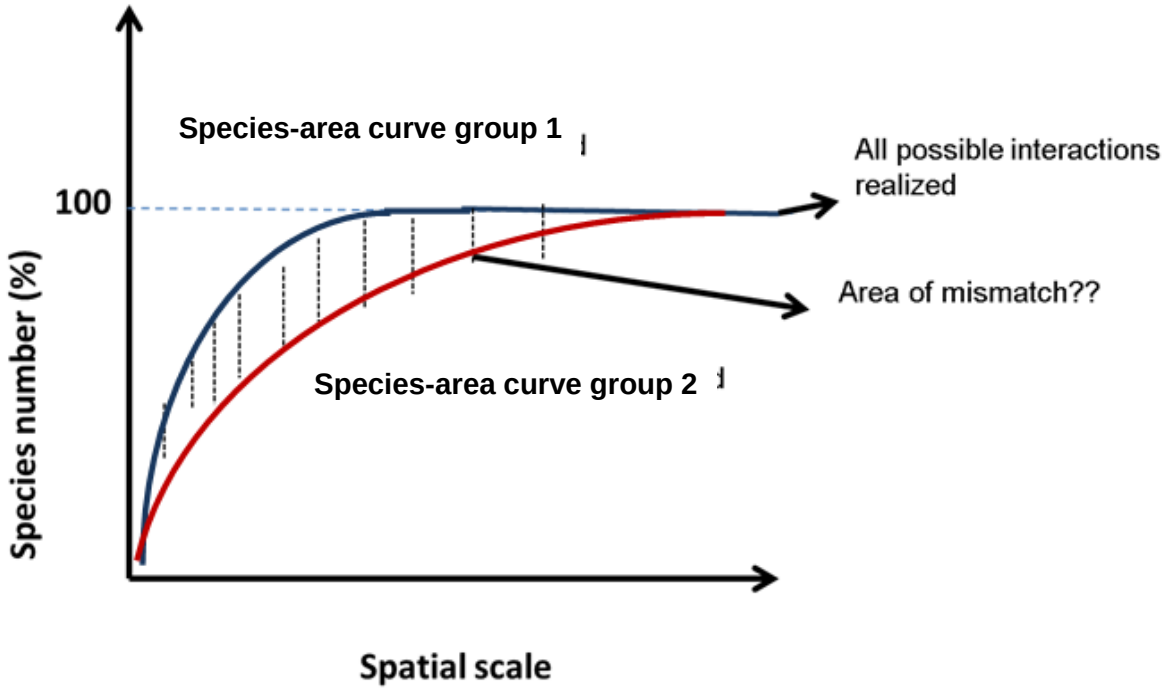

Fig. 16: *Different interacting groups (this can be above- and belowground biota, plants and herbivores, plants and pollinators, predators and prey…) may show different patterns of species-area-curves. Only if both taxa reach the plateau phase the full number of possible interactions can be realized. Depending on the scale of a field experiment the percentage of realized biotic interactions is decreased and there might be something like an "unsaturated interaction space" (area of mismatch). Thus, the scale on which an experiment is performed determines the number of links etc. This problem might also be applicable to the time-scale of experiments.*

E. ExpeER publications

Martin Schädler, Stefan Klotz, Jutta Stadler, Nico Eisenhauer. Biotic interactions and feed-back effects in manipulative experiments in ecology (manuscript).

5.3 New method to characterize species interactions in plant communities

(contribution by UA)

A. Background & challenges

Interactions between plant species shape communities and are essential for a range of characteristics and processes (invasibility, stability, extinction, etc.), yet mapping such interactions is often a challenge. When landscapes host suitable patches in a matrix of unsuitable habitat (e.g. forest remnants surrounded by agricultural land), the interactions between the species can be studied by comparing the observed co-occurrence of species in patches with an expected co-occurrence in null models (i.e. randomized species presence across the patches). However, many landscapes consist of continuous vegetation such as grassland, heathland, forest, etc. In such cases, methods to explore interactions usually entail fully mapping the vegetation. Perhaps this is the reason why thousands of studies have made vegetation surveys, but few have focused on detecting all possible interactions (e.g. in a 20-species community, there are 400 potential interactions).

B. New procedure: methodology and real-life example

To avoid the high workloads associated with quantifying all interactions, we designed a simpler and faster method based on the principle that abundant species have a greater probability to be a neighbour of a target individual than rare species. The method consists of two steps: (i) constructing the rank-abundance curve, which can be done with a classical vegetation survey, and (ii) scoring the nearest neighbours of target individuals. The rank-abundance curve plots the relative abundances of the species in the community, ranked from high (dominant species) to low (rare species). The relative abundance of species X is defined as the fraction of the individuals of species X relative to the total number of individuals of all the species in the community, which can be measured by laying out a number of (usually square) plots of suitable dimension. The second step consists of randomly selecting a series of individuals of species X and each time recording the identity of the nearest neighbour. This is repeated for all species in the community. Subsequent comparison of the observed relative frequency of neighbourships of a species with what is expected based on the relative abundance of each species in the community, provides information on the nature of the interactions between the species.

Plots per species can then be compiled, where every symbol represents a species of the community (example in Fig. 17). The abscissa of each symbol is the relative abundance of the species, and the ordinate is the relative frequency of that same species as neighbour of species X. Imagine that species A is the most abundant species, with a relative abundance of 30%. We then expect 30% of the neighbours of species X to be species A, provided that contacts between species X and species A are not overrepresented or underrepresented in the community. If B is the next most abundant species represented by 15% of the individuals, then we expect 15% of the neighbours of species X to be species B, again assuming no over- or underrepresentation. The same expectation applies to all the other species neighbouring species X, such that the expected plot for species X with neighbourship frequency as a function of relative abundance is a straight line going through the origin (0%, 0%) and the point (100%, 100%). Deviations between the observed values (abundance %)

and the expected values (neighbourship %) signify over- or underrepresentation of the contacts between species X and its neighbouring species (including itself, as same-species contacts are also recorded). Note that the sum of the contact frequencies (Y-axis values) is always 100%, which implies that species that have more contacts with species X compared with their relative abundance, must be compensated by other species that have less contacts with species X than anticipated based on their relative abundance. Neighbourships that are overrepresented suggest facilitation, those that are underrepresented point to competition. Separate plots are made for every species in the community, and in each of these plots, the abscissa values (i.e. the relative abundances) are the same. Indeed, regardless of whether species X is a dominant species or a rare species, it is always expected to 'meet' species according to their observed relative abundances.

Data were collected in a real community to illustrate the method. A ruderal field in the province of Antwerp (Belgium) was selected, where species present were: *Trifolium pratensis* L., *Lotus corniculatis* L., *Chenopodium album* L., *Malva sylvestris* L., *Solanum nigrum* L., *Taraxacum officinale* Wigg., *Leucanthemum vulgare* Lam., *Cirsium arvense* L. (Scop)., *Achillea millefolium* L., *Lolium perenne* L., *Holcus lanatus* L., and *Conyza canadensis* (L.) Cronq (see rank-abundance curve in Fig. 17). We used a homogenous subsection of the field of 18 m long and 3.2 m wide. To assess the relative abundance of each species, 46 plots of 0.25 × 0.25 m were (regularly) distributed across the subsection. In each plot the number of individuals of each species was counted. Neighbourships were determined only for the six most abundant species (rarer species had insufficient individuals to assess the contact distribution). Per species, 91 neighbours were identified, which were likewise distributed homogeneously across the sampling area.

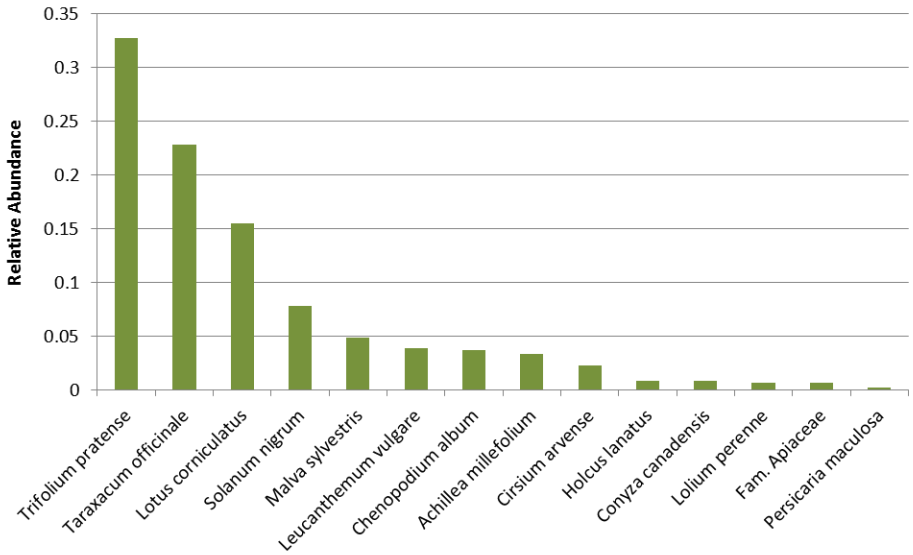

Fig. 17: *Rank abundance curve of the species observed in a test experiment carried out in a ruderal field in Northern Belgium. Details: see text.*

Data were processed for all species and the significance of the deviations between the observed and the expected associations were tested with a Chi-square test. Of the six species tested (others were too rare), only the graph for *Malva sylvestris* showed significant deviations from the 1:1 line, i.e. a combination of positive and negative associations (Fig. 18). For example, *Trifolium pratensis* occurred significantly more as neighbour of *Malva sylvestris* then expected by chance, suggesting positive interaction. Conversely, *Taraxacum officinale* occurred significantly less as neighbour of *Malva sylvestris* then expected by chance, suggesting negative interaction. The data collection for the

method was completed in three person-days, which makes the method feasible for rapid screening of possible interactions in multi-species communities.

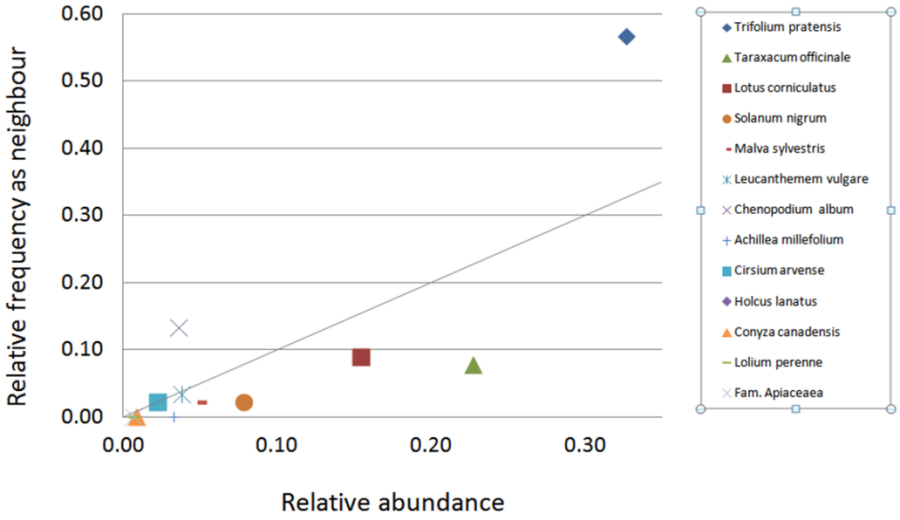

Fig. 18: *Example of the relationship between relative neighbourship frequencies and relative abundances, for target species Malva sylvestris. Deviations from the 1:1 line suggest either competition or facilitation to be dominant (details: see text).*

C. ExpeER publications

Van den Berge J, Liao J, De Boeck HJ, Nijs I. A quick method for detecting spatial associations and interactions between plant species (manuscript).