Publication list for INCREASE (9 November 2009)
(and earlier projects CLIMOOR and VULCAN)

Ainsworth, E. A., C. Beier, et al. (2008). "Next generation of elevated CO2 experiments with crops: a critical investment for feeding the future world." Plant Cell and Environment **31**(9): 1317-1324.

A rising global population and demand for protein-rich diets are increasing pressure to maximize agricultural productivity. Rising atmospheric [CO2] is altering global temperature and precipitation patterns, which challenges agricultural productivity. While rising [CO2] provides a unique opportunity to increase the productivity of C-3 crops, average yield stimulation observed to date is well below potential gains. Thus, there is room for improving productivity. However, only a fraction of available germplasm of crops has been tested for CO2 responsiveness. Yield is a complex phenotypic trait determined by the interactions of a genotype with the environment. Selection of promising genotypes and characterization of response mechanisms will only be effective if crop improvement and systems biology approaches are closely linked to production environments, that is, on the farm within major growing regions. Free air CO2 enrichment (FACE) experiments can provide the platform upon which to conduct genetic screening and elucidate the inheritance and mechanisms that underlie genotypic differences in productivity under elevated [CO2]. We propose a new generation of large-scale, low-cost per unit area FACE experiments to identify the most CO2-responsive genotypes and provide starting lines for future breeding programmes. This is necessary if we are to realize the potential for yield gains in the future.

Asensio, D., J. Penuelas, et al. (2008). "Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland." European Journal of Soil Science **59**(5): 878-891.

Information about soil VOC inventories and exchange rates in different soils is very scarce. Seasonality of soil VOC exchange rates is also largely unknown, despite the increasing interest in some soil volatile compounds, such as monoterpenes, because of their important role in soil ecology. We aimed to explore and quantify soil VOC exchange rates in a Mediterranean shrubland and their seasonality. Measurements of soil VOC exchange were taken using GC-MS and PTR-MS techniques, together with soil temperature, soil moisture and soil CO2 efflux measurements, during two annual campaigns with contrasting precipitation. Methanol, acetic acid, ethyl acetate, acetaldehyde, acetone, C-3 and C-4 carbonyls (such as methyl ethyl ketone), alpha-pinene and limonene, showed the highest emission rates. Maximum soil monoterpene emission rates were very low (0.003 nmol m(-2) s(-1)) compared with foliar monoterpene emission rates. The emission rates of the other VOCs were also low (maximum 0.8 nmol m(-2) s(-1)) except for methanol (1.2 nmol m(-2) s(-1)). Maximum soil uptake rates for some VOCs, such as methanol and acetonitrile (ranging from -0.1 to -0.5 nmol m(-2) s(-1)) were, however, comparable with foliar uptake rates. Further studies are needed to corroborate these results and the possible importance of the soil VOC sink in regional chemistry-climate models. Long-term severe drought increased soil monoterpene emission rates in this Mediterranean shrubland. The increases seem to be linked to changes in the soil's physical properties induced by low soil moisture. Unlike monoterpenes, other soil VOC emission rates decreased when soil moisture was low. The results suggest a seasonal control of soil temperature on the emission rates of monoterpenes and other VOCs. The emission rates increase with soil temperature. Positive correlations between the VOC exchange rates and the soil CO2 fluxes suggest that phenology of roots and microorganisms also controls seasonal changes in soil VOCs in this Mediterranean shrubland.

Bahrndorff, S., V. Loeschcke, et al. (2009). "The rapid cold hardening response of Collembola is influenced by thermal variability of the habitat." Functional Ecology **23**(2): 340-347.

1. It has been argued that species living under unpredictable thermal conditions need to have more flexible physiological capabilities to meet with thermal stress than species living in thermally stable environments. Here we investigate if the ability to rapidly cold-harden in Collembola is influenced by thermal conditions of the habitat. 2. Collembola exploit diverse habitats and are therefore exposed to different thermal environments: soil dwelling (euedaphic) species occupy relatively stable environments, whereas surface dwelling (epedaphic) species can be exposed to more fluctuating thermal environments, but a single species can also be found in diverse thermal habitats within its geographic distribution. 3. We compared the inherent cold shock tolerance and ability to rapidly cold-harden in three epedaphic, two near surface dwelling (hemiedaphic) and four euedaphic species of Collembola using a similar experimental approach for all species. Additionally we compared three populations of the epedaphic species, Orchesella cincta, sampled along a climatic gradient (Norway, Denmark, Italy). 4. Inherent cold shock tolerance was estimated as LT50 by assaying cold shock survival following a 2 h exposure to a range of temperatures from 1 degrees C to -12

degrees C. Rapid cold-hardening (RCH) was induced by cooling individuals from 20 degrees C to a temperature 7 degrees C above the LT50 during 80 min, followed by 1 h at the specific cold shock temperature, which was close to the LT50 of the particular species. 5. There was large variation in cold shock survival among species. The capacity to rapidly cold-harden was found in all three ecotypes. 6. Genetic difference in the ability to rapidly cold-harden was seen in O. cincta from different climatic regions, consistent with the predictability of the thermal environment of their habitat. Population differences matched the daily fluctuations in temperature (CV) recorded at the site of collection as well as the day-to-day predictability (autocorrelation). The role of phylogenetic inertia was tested using sequence data from the cytochrome-c oxidase I (COI) gene and no signal of phylogeny was detected that could explain these population differences. 7. Our results show that genetic differences in RCH ability exist, consistent with latitudinal gradients in thermal fluctuations and predictability; thus comparative studies can provide important insight when exploring the role of acclimation in the geographical distribution of species.

Bakonyi, G., P. Nagy, et al. (2007). "Soil nematode community structure as affected by temperature and moisture in a temperate semiarid shrubland." Applied Soil Ecology **37**(1-2): 31-40.

Nematodes are key agents in important soil processes, such as decomposition, mineralization and nutrient cycling. Therefore, alterations of the nematode community structure induced by global change may have a considerable influence on ecosystem functioning. However, it is not clear whether minor changes in soil temperature and/or moisture have any significant effect on nematode community structure. A field experiment was performed in a mosaic of open sand grassland and Juniper-Poplar woodland (VULCAN Project). Soil temperature and moisture were modified to the extent expected for the near future due to global changes. Community diversity and multivariate structure of the nematode community proved to be more sensitive to minute changes in soil temperature and moisture than different indices, such as specific richness (SR), maturity index (MI), plant parasite index (PPI), enrichment index (EI), channel index (CI), fungal feeder to bacterial feeder ratio (F/B) and nematode channel ratio (NCR). Nematode genera with high densities (> 0.1 individual g(-1) soil) were better indicators of the temperature and moisture changes than those of low density (< 0.1 individual g(-1) soil) in this sandy soil. Both drying and warming had significant influence on low density Wilk's lambda: 0.02) and high density (Wilk's lambda: 0.002) genera according to canonical variate analysis. Cephalobus and Plectus were associated with the dried plots, while Cervidellus, Ditylenchus, Eudorylaimus, Seinura and Thonus were favoured by warming. Drying induced the development of a more structured nematode community in the bare soil compared to the control. Drying and warming effects on the soil nematode community were most pronounced in bare soil, less so in soil under poplar, while no significant effect was found in the fescue grass soil.

Beier, C. (2004). "Climate change and ecosystem function - full-scale manipulations of CO2 and temperature." New Phytologist **162**(2): 243-245.

Beier, C. (2004). "Interactions of elevated CO2 and temperature on terrestrial ecosystem structure and functioning - the role of whole-ecosystem manipulation experiments - Commentary." New Phytologist **162**(2): 243-245.

Beier, C., B. Emmett, et al. (2004). "Novel approaches to study climate change effects on terrestrial ecosystems in the field: Drought and passive nighttime warming." Ecosystems **7**(6): 583-597.

This article describes new approaches for manipulation of temperature and water input in the field. Nighttime warming was created by reflection of infrared radiation. Automatically operated reflective curtains covered the vegetation at night to reduce heat loss to the atmosphere. This approach mimicked the way climate change, caused by increased cloudiness and increased greenhouse gas emissions, alters the heat balance of ecosystems. Drought conditions were created by automatically covering the vegetation with transparent curtains during rain events over a 2-5-month period. The experimental approach has been evaluated at four European sites across a climate gradient. All sites were dominated (more than 50%) by shrubs of the ericaceous family. Within each site, replicated 4-m X 5-m plots were established for control, warming, and drought treatments and the effect on climate variables recorded. Results over a two-year period indicate that the warming treatment was successful in achieving an increase of the minimum temperatures by 0.4-1.2degreesC in the air and soil. The drought treatment resulted in a soil moisture reduction of 33%-82% at the peak of the drought. The data presented demonstrate that the approach minimizes unintended artifacts with respect to water balance, moisture conditions, and light, while causing a small but significant reduction in wind speed by the curtains. Temperature measurements demonstrated that

the edge effects associated with the treatments were small. Our method provides a valuable tool for investigating the effects of climate change in remote locations with minimal artifacts.

Beier, C., B. A. Emmett, et al. (2008). "Carbon and nitrogen cycles in European ecosystems respond differently to global warming." Science of the Total Environment **407**(1): 692-697.

The global climate is predicted to become significantly warmer over the next century. This will affect ecosystem processes and the functioning of semi natural and natural ecosystems in many parts of the world. However, as various ecosystem processes may be affected to a different extent, balances between different ecosystem processes as well as between different ecosystems may shift and lead to major unpredicted changes. In this study four European shrubland ecosystems along a north-south temperature gradient were experimentally warmed by a novel nighttime warming technique. Biogeochemical cycling of both carbon and nitrogen was affected at the colder sites with increased carbon uptake for plant growth as well as increased carbon loss through soil respiration. Carbon uptake by plant growth was more sensitive to warming than expected from the temperature response across the sites while carbon loss through soil respiration reacted to warming in agreement with the overall Q10 and response functions to temperature across the sites. Opposite to carbon, the nitrogen mineralization was relatively insensitive to the temperature increase and was mainly affected by changes in soil moisture. The results suggest that C and N cycles respond asymmetrically to warming, which may lead to progressive nitrogen limitation and thereby acclimation in plant production. This further suggests that in many temperate zones nitrogen deposition has to be accounted for, not only with respect to the impact on water quality through increased nitrogen leaching where N deposition is high, but also in predictions of carbon sequestration in terrestrial ecosystems under future climatic conditions. Finally the results indicate that on the short term the above-ground processes are more sensitive to temperature changes than the below ground processes.

Beier, C., B. A. Emmett, et al. (2009). "Carbon and nitrogen balances for six shrublands across Europe." Global Biogeochemical Cycles **23**.

Shrublands constitute significant and important parts of European landscapes providing a large number of important ecosystem services. Biogeochemical cycles in these ecosystems have gained little attention relative to forests and grassland systems, but data on such cycles are required for developing and testing ecosystem models. As climate change progresses, the potential feedback from terrestrial ecosystems to the atmosphere through changes in carbon stocks, carbon sequestration, and general knowledge on biogeochemical cycles becomes increasingly important. Here we present carbon and nitrogen balances of six shrublands along a climatic gradient across the European continent. The aim of the study was to provide a basis for assessing the range and variability in carbon storage in European shrublands. Across the sites the net carbon storage in the systems ranged from 1,163 g C m(-2) to 18,546 g C m(-2), and the systems ranged from being net sinks (126 g C m(-2) a(-1)) to being net sources (-536 g C m(-2) a(-1)) of carbon with the largest storage and sink of carbon at wet and cold climatic conditions. The soil carbon store dominates the carbon budget at all sites and in particular at the site with a cold and wet climate where soil C constitutes 95% of the total carbon in the ecosystem. Respiration of carbon from the soil organic matter pool dominated the carbon loss at all sites while carbon loss from aboveground litter decomposition appeared less important. Total belowground carbon allocation was more than 5 times aboveground litterfall carbon which is significantly greater than the factor of 2 reported in a global analysis of forest data. Nitrogen storage was also dominated by the soil pools generally showing small losses except when atmospheric N input was high. The study shows that in the future a climate-driven land cover change between grasslands and shrublands in Europe will likely lead to increased ecosystem C where shrublands are promoted and less where grasses are promoted. However, it also emphasizes that if feedbacks on the global carbon cycle are to be predicted it is critically important to quantify and understand belowground carbon allocation and processes as well as soil carbon pools, particularly on wet organic soils, rather than plant functional change as the soil stores dominate the overall budget and fluxes of carbon.

Beier, C., B. A. Emmett, et al. (2009). "Carbon and nitrogen balances for six shrublands across Europe." Global Biogeochemical Cycles **23**.

Shrublands constitute significant and important parts of European landscapes providing a large number of important ecosystem services. Biogeochemical cycles in these ecosystems have gained little attention relative to forests and grassland systems, but data on such cycles are required for developing and testing ecosystem models. As climate change progresses, the potential feedback from terrestrial ecosystems to the atmosphere through changes in carbon stocks, carbon sequestration, and general

knowledge on biogeochemical cycles becomes increasingly important. Here we present carbon and nitrogen balances of six shrublands along a climatic gradient across the European continent. The aim of the study was to provide a basis for assessing the range and variability in carbon storage in European shrublands. Across the sites the net carbon storage in the systems ranged from 1,163 g C mâˆ'2 to 18,546 g C mâˆ'2, and the systems ranged from being net sinks (126 g C mâˆ'2 aâˆ'1) to being net sources (âˆ'536 g C mâˆ'2 aâˆ'1) of carbon with the largest storage and sink of carbon at wet and cold climatic conditions. The soil carbon store dominates the carbon budget at all sites and in particular at the site with a cold and wet climate where soil C constitutes 95% of the total carbon in the ecosystem. Respiration of carbon from the soil organic matter pool dominated the carbon loss at all sites while carbon loss from aboveground litter decomposition appeared less important. Total belowground carbon allocation was more than 5 times aboveground litterfall carbon which is significantly greater than the factor of 2 reported in a global analysis of forest data. Nitrogen storage was also dominated by the soil pools generally showing small losses except when atmospheric N input was high. The study shows that in the future a climate-driven land cover change between grasslands and shrublands in Europe will likely lead to increased ecosystem C where shrublands are promoted and less where grasses are promoted. However, it also emphasizes that if feedbacks on the global carbon cycle are to be predicted it is critically important to quantify and understand belowground carbon allocation and processes as well as soil carbon pools, particularly on wet organic soils, rather than plant functional change as the soil stores dominate the overall budget and fluxes of carbon.

Beier, C., I. K. Schmidt, et al. (2004). "Effects of climate and ecosystem disturbances on biogeochemical cycling in a semi-natural terrestrial ecosystem." Water, Air and Soil Pollution: Focus **4**: 191-206.

The effects of increased temperature and potential ecosystem disturbances on biogeochemical cycling were investigated by manipulation of temperature in a mixed Calluna/grass heathland in Denmark. A reflective curtain covered the vegetation during the night to reduce the heat loss of IR radiation from the ecosystem to the atmosphere. This `night time warming' was done for 3 years and warmed the air and soil by 1.1 ℃. Warming was comb ined with ecosystem disturbances, including infestation by Calluna heather beetles (Lochmaea suturalis Thompson) causing complete defoliation of Calluna leaves during the summer 2000, and subsequent harvesting of all aboveground biomass during the autumn. Small increases in mineralisation rates were induced by warming and resulted in increased leaching of nitrogen from the organic soil layer. The increased nitrogen leaching from the organic soil layer was re-immobilised in the mineral soil layer as warming stimulated plant growth and thereby increased nitrogen immobilisation. Contradictory to the generally moderate effects of warming, the heather beetle infestation had very strong effects on mineralisation rates and the plant community. The grasses completely out-competed the Calluna plants which had not re-established two years after the infestation, probably due to combined effects of increased nutrient availability and the defoliation of Calluna. On the short term, ecosystem disturbances may have very strong effects on internal ecosystem processes and plant community structure compared to the more long-term effects of climate change.

Cornelissen, J. H. C., P. M. van Bodegom, et al. (2007). "Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes." Ecology Letters **10**(7): 619-627.

Whether climate change will turn cold biomes from large long-term carbon sinks into sources is hotly debated because of the great potential for ecosystem-mediated feedbacks to global climate. Critical are the direction, magnitude and generality of climate responses of plant litter decomposition. Here, we present the first quantitative analysis of the major climate-change-related drivers of litter decomposition rates in cold northern biomes worldwide. Leaf litters collected from the predominant species in 33 global change manipulation experiments in circum-arctic-alpine ecosystems were incubated simultaneously in two contrasting arctic life zones. We demonstrate that longer-term, large-scale changes to leaf litter decomposition will be driven primarily by both direct warming effects and concomitant shifts in plant growth form composition, with a much smaller role for changes in litter quality within species. Specifically, the ongoing warming-induced expansion of shrubs with recalcitrant leaf litter across cold biomes would constitute a negative feedback to global warming. Depending on the strength of other (previously reported) positive feedbacks of shrub expansion on soil carbon turnover, this may partly counteract direct warming enhancement of litter decomposition.

Craine, J. M., A. J. Elmore, et al. (2009). "Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability." New Phytologist **183**(4): 980-992.

Ratios of nitrogen (N) isotopes in leaves could elucidate underlying patterns of N cycling across ecological gradients. To better understand global-scale patterns of N cycling, we compiled data on foliar N isotope ratios (delta N-15), foliar N concentrations, mycorrhizal type and climate for over 11 000 plants worldwide. Arbuscular mycorrhizal, ectomycorrhizal, and ericoid mycorrhizal plants were depleted in foliar delta N-15 by 2 parts per thousand, 3.2 parts per thousand, 5.9 parts per thousand, respectively, relative to nonmycorrhizal plants. Foliar delta N-15 increased with decreasing mean annual precipitation and with increasing mean annual temperature (MAT) across sites with MAT >= -0.5 degrees C, but was invariant with MAT across sites with MAT < -0.5 degrees C. In independent landscape-level to regional-level studies, foliar delta N-15 increased with increasing N availability; at the global scale, foliar delta N-15 increased with increasing foliar N concentrations and decreasing foliar phosphorus (P) concentrations. Together, these results suggest that warm, dry ecosystems have the highest N availability, while plants with high N concentrations, on average, occupy sites with higher N availability than plants with low N concentrations. Global-scale comparisons of other components of the N cycle are still required for better mechanistic understanding of the determinants of variation in foliar delta N-15 and ultimately global patterns in N cycling.

Damgaard, C., T. Riis-Nielsen, et al. (2009). "Estimating plant competition coefficients and predicting community dynamics from non-destructive pin-point data: a case study with Calluna vulgaris and Deschampsia flexuosa." Plant Ecology **201**(2): 687-697.

A method is proposed for estimating plant competition coefficients and predicting the dynamics of herb and grassland plant communities from non-destructive pin-point measurements. The method is applied to inter-specific competition in a natural heathland community with relatively few interacting species. The study shows that the dynamics of the heathland plant community may be thought of as essentially a two-species system of Calluna vulgaris and Deschampsia flexuosa. There were significant competitive interactions between C. vulgaris and D. flexuosa. D. flexuosa affected both the cover and compactness of C. vulgaris individuals as a function of the compactness the previous year, whereas C. vulgaris significantly affected only the compactness of D. flexuosa. There was a significant negative effect of drought on the compactness of both C. vulgaris and D. flexuosa individuals, whereas night warming had no significant effects on either species. The predicted long-term outcome of the competitive interaction between C. vulgaris and D. flexuosa was that of unstable equilibrium, where the more dominant of the two will outcompete the other. However, when both species are found at relatively high plant covers the two species are predicted to co-exist for a long time period relatively to the time scale of the ageing process of C. vulgaris. Direct analyses of the inter-specific competitive interactions in natural plant communities with non-destructive measurements can provide important new insight into the processes that determine the composition of plant communities.

de Dato, G., G. Pellizzaro, et al. (2006). "Effects of warmer and drier climate conditions in plant composition and biomass production in a Mediterranean shrubland community." Forest@ **3**(4): 511-526.

Abstract: The last IPCC report predicts warmer and drier conditions for the future European climate and the
Mediterranean basin could be highly sensible to future climatic change. In order to investigate how the forecast
more stressing factors could affect Mediterranean shrubland ecosystems, an appropriate manipulation of the microclimate was carried out in an area covered by Mediterranean maquis aimed at extending the drought period and increasing the night-time temperature. Soil cover, plant growth, litterfall, leaf water status, and leaf nutritional status were monitored over three growing seasons. The manipulation altered the microclimate according to common scenarios, increasing mean annual night-time air temperature by about 1 ℃ and mean annual temperature by about 0.5 ℃, and decreasing precipitation between 6-46% of the total rainfall during the growing seasons. A general increase of vegetation cover was observed in the whole community
during the three years of experimentation. This positive temporal pattern was mainly observed in control and warming treatment, whereas in the drought treatment it was less evident. At species-specific level, a clear negative effect of drought treatment was observed for C. monspeliensis percentage cover. Shoot
elongation was not significantly affected by the warming treatment. A significant negative effect of drought treatment was noticed in the 2001-2002 and 2002-2003 growing seasons. An increase of N and P concentrations
in the drought treatment in Cistus was observed and it can be explained by the reduced shoot growth induced by the water shortage that we had observed in the same treatment. The absence of a concentration

effect on the other two species could be the signal of the different behaviour with regard to a drier climate, and therefore could be a symptom of future change in species composition. We underline the need of longterm
observation, because of the different responses of plants in the short and long- term conditions.

Emmett, B. A., C. Beier, et al. (2004). "The response of soil processes to climate change: Results from manipulation studies of shrublands across an environmental gradient." Ecosystems **7**(6): 625-637.

Predicted changes in climate may affect key soil processes such as respiration and net nitrogen (N) mineralization and thus key ecosystem functions such as carbon (C) storage and nutrient availability. To identify the sensitivity of shrubland soils to predicted climate changes, we have carried out experimental manipulations involving ecosystem warming and prolonged summer drought in ericaceous shrublands across a European climate gradient. We used retractable covers to create artificial nighttime warming and prolonged summer drought to 20-m(2) experimental plots. Combining the data from across the environmental gradient with the results from the manipulation experiments provides evidence for strong climate controls on soil respiration, net N mineralization and nitrification, and litter decomposition. Trends of 0%-19% increases of soil respiration in response to warming and decreases of 3%-29% in response to drought were observed. Across the environmental gradient and below soil temperatures of 20degreesC at a depth of 5-10 cm, a mean Q(10) of 4.1 in respiration rates was observed although this varied from 2.4 to 7.0 between sites. Highest Q(10), values were observed in Spain and the UK and were therefore not correlated with soil temperature. A trend of increased accumulated surface litter mass loss was observed with experimental warming (2%- 22%) but there was no consistent response to experimental drought. in contrast to soil respiration and decomposition, variability in net N mineralization was best explained by soil moisture rather than temperature. When water was neither limiting or in excess, a Q(10) of 1.5 was observed for net N mineralization rates. These data suggest that key soil processes will be differentially affected by predicted changes in rainfall pattern and temperature and the net effect on ecosystem functioning will be difficult to predict without a greater understanding of the controls underlying the sensitivity of soils to climate variables.

Estiarte, M., J. Penuelas, et al. (2008). "Root-surface phosphatase activity in shrublands across a European gradient: Effects of warming." Journal of Environmental Biology **29**(1): 25-29.

Root-surface phosphatase activities were measured in natural and semi-natural shrublands across an European climatic gradient of temperature and rainfall including Wales (WL), Denmark (DK), Netherlands (NL), Hungary (HU), Italy (IT) and Spain (SP). In each site a warming experiment was conducted since 1999 or 2001 by means of passive night-time warming using reflective curtains that covered the vegetation at night. The treatments increased yearly average soil temperatures around 0.8 degrees C in most of sites. Root-surface phosphatase activity values ranged between 56 mg PNP g(-1) h(-1) in IT and 3,5 mg PNP g(-1) h(-1) in HU. Warming had no effect on root-surface phosphatase activity across the sites and only in Hungary a slight increase was detected. Plants at Mediterranean sites (IT, SP) showed a higher root-surface phosphatase activity than plants at temperate sites (WL, NL, DK). We suggest it might be an adaptation of plant species evolved under Mediterranean climate that allows them a) to compensate in wet period for the decrease in phosphatase activity, and thus P uptake, during drought periods, and/or b) to benefit from soluble organic P flushes following the frequent drying-rewetting episodes experienced by soils in Mediterranean ecosystems.

Filella, I., J. Penuelas, et al. (2004). "Reflectance assessment of seasonal and annual changes in biomass and CO2 uptake of a Mediterranean shrubland submitted to experimental warming and drought." Remote Sensing of Environment **90**(3): 308-318.

We aimed to evaluate how the remote sensing vegetation indices NDVI and PRI responded to seasonal and annual changes in an early successional stage Mediterranean coastal shrubland canopy that was submitted to experimental warming and drought simulating predicted climate change for the next decades. These conditions were obtained by using a new non-intrusive methodological approach that increases the temperature and prolongs the drought period by using roofs that automatically cover the vegetation after the sunset or when it rains. On average, warming increased air temperature by 0.7 degreesC and soil temperature by 1.6 degreesC, and the drought treatment reduced soil moisture by 22%. We measured spectral reflectance at the canopy level and at the individual plant level seasonally during 4 years. Shrubland NDVI tracked the community development and activity. In control and warming treatments, NDVI increased with the years while it did not change in the drought treatment. There was a good relationship between NDVI and both community and individual plant biomass. NDVI also decreased in summer seasons when some species dry or decolour. The NDVI of E. multiflora plant individuals was lower

in autumn and winter than in the other seasons, likely because of flowering. Shrubland PRI decreased only in winter, similarly to the PRI of the most dominant species, G. alypum. At this community scale, NDVI was better related than PRI to photosynthetic activity, probably because photosynthetic fluxes followed canopy seasonal greening in this complex canopy, which includes brevideciduous, annual and evergreen species and variable morphologies and canopy coverage. PRI followed the seasonal variations in photosynthetic rates in E. multiflora and detected the decreased photosynthetic rates of drought treatment. However, PRI did not track the photosynthetic rates of G. alypum plants which have lower LAIs than E. multiflora. In this community, which is in its early successional stages, NDVI was able to track biomass, and indirectly, CO2 uptake changes, likely because LAI values did not saturate NDVI. Thus, NDVI appears as a valid tool for remote tracking of this community development. PRI was less adequate for photosynthetic assessment of this community especially for its lower LAI canopies. PRI usefulness was also species-dependent and could also be affected by flowering. These results will help to improve the interpretation of remote sensing information on the structure and physiological status of these Mediterranean shrublands, and to gain better insight on ecological and environmental controls on their ecosystem carbon dioxide exchange. They also show the possibility of assessing the impacts of climate change on shrubland communities.

Gerten, D., Y. Luo, et al. (2008). "Modelled effects of precipitation on ecosystem carbon and water dynamics in different climatic zones." Global Change Biology **14**(10): 2365-2379.

The ongoing changes in the global climate expose the world's ecosystems not only to increasing CO2 concentrations and temperatures but also to altered precipitation (P) regimes. Using four well-established process-based ecosystem models (LPJ, DayCent, ORCHIDEE, TECO), we explored effects of potential P changes on water limitation and net primary production (NPP) in seven terrestrial ecosystems with distinctive vegetation types in different hydroclimatic zones. We found that NPP responses to P changes differed not only among sites but also within a year at a given site. The magnitudes of NPP change were basically determined by the degree of ecosystem water limitation, which was quantified here using the ratio between atmospheric transpirational demand and soil water supply. Humid sites and/or periods were least responsive to any change in P as compared with moderately humid or dry sites/periods. We also found that NPP responded more strongly to doubling or halving of P amount and a seasonal shift in P occurrence than that to altered P frequency and intensity at constant annual amounts. The findings were highly robust across the four models especially in terms of the direction of changes and largely consistent with earlier P manipulation experiments and modelling results. Overall, this study underscores the widespread importance of P as a driver of change in ecosystems, although the ultimate response of a particular site will depend on the detailed nature and seasonal timing of P change.

Gorissen, A., A. Tietema, et al. (2004). "Climate change affects carbon allocation to the soil in shrublands." Ecosystems **7**(6): 650-661.

Climate change may affect ecosystem functioning through increased temperatures or changes in precipitation patterns. Temperature and water availability are important drivers for ecosystem processes such as photosynthesis, carbon translocation, and organic matter decomposition. These climate changes may affect the supply of carbon and energy to the soil microbial population and subsequently alter decomposition and mineralization, important ecosystem processes in carbon and nutrient cycling. In this study, carried out within the cross-European research project CLIMOOR, the effect of climate change, resulting from imposed manipulations, on carbon dynamics in shrubland ecosystems was examined. We performed a C-14-labeling experiment to probe changes in net carbon uptake and allocation to the roots and soil compartments as affected by a higher temperature during, the year and a drought period in the growing season. Differences in climate, soil, and plant characteristics resulted in a gradient in the severity of the drought effects on net carbon uptake by plants with the impact being most severe in Spain, followed by Denmark, with the UK showing few negative effects at significance levels of p less than or equal to 0.10. Drought clearly reduced carbon flow from the roots to the soil compartments. The fraction of the C-14 fixed by the plants and allocated into the soluble carbon fraction in the soil and to soil microbial biomass in Denmark and the UK decreased by more than 60%. The effects of warming were not significant, but, as with the drought treatment, a negative effect on carbon allocation to soil microbial biomass was found. The changes in carbon allocation to soil microbial biomass at the northern sites in this study indicate that soil microbial biomass is a sensitive, early indicator of drought- or temperature-initiated changes in these shrubland ecosystems. The reduced supply of substrate to the soil and the response of the soil microbial biomass may help to explain the observed acclimation of CO2 exchange in other ecosystems.

Jensen, K. D., C. Beier, et al. (2003). "Effects of experimental drought on microbial processes in two temperate heathlands at contrasting water conditions." Applied Soil Ecology **24**(2): 165-176.

Climate change predictions indicate that extremely dry years are likely to become more frequent in the future. In the present study the potential impacts of drought on the microbial processes and carbon and nitrogen dynamics in the soil were investigated in two heathland ecosystems in Denmark and the UK, at contrasting water inputs of ca. 750 and ca. 1700 mm per year, respectively. The drought treatment involved removal of all precipitation for 2 months during summer. In general the two ecosystems reacted differently. At the drier site in Denmark the drought reduced the microbial activity shown by a 27% reduced below ground CO2 emission, and reduced microbial and soil solution carbon (C) and nitrogen (N) levels. In contrast, microbial activity at the wetter UK site seemed to benefit from the drought as indicated by a 22% increase in below ground CO2 emission caused by the drought treatment. At both sites drought led to an increase in the microbial ON ratio suggesting a change towards a more fungal-dominated microbial community and decomposition of more complex substrates. The drought-induced reduced availability of nutrients in both heath types may counteract changes in plant competitive patterns and in species composition caused by anthropogenic N deposition in these nutrient-limited ecosystems. (C) 2003 Elsevier B.V All rights reserved.

Jump, A. S., J. Penuelas, et al. (2008). "Simulated climate change provokes rapid genetic change in the Mediterranean shrub Fumana thymifolia." Global Change Biology **14**(3): 637-643.

Rapid climate change will impose strong directional selection pressures on natural plant populations. Climate-linked genetic variation in natural populations indicates that an evolutionary response is possible. We investigated such a response by comparing individuals subjected to elevated drought and warming treatments with individuals establishing in an unmanipulated climate within the same population. We report that reduction in seedling establishment in response to climate manipulations is nonrandom and results from the selection pressure imposed by artificially warmed and droughted conditions. When compared against control samples, high single-locus genetic divergence occurred in drought and warming treatment samples, with genetic differentiation up to 37 times higher than background (mean neutral locus) genetic differentiation. These loci violate assumptions of selective neutrality, indicating the signature of natural selection by drought. Our results demonstrate that rapid evolution in response to climate change may be widespread in natural populations, based on genetic variation already present within the population.

Kovacs-Lang, E., G. Kroel-Dulay, et al. (2000). "Changes in the composition of sand grasslands along a climatic gradient in Hungary and implications for climate change." Phytocoenologia **30**(3-4): 385-407.

The primary objectives of this study were (1) to document the changes in the composition and structure of a semiarid grassland (Festucetum vaginatae) along a climatic gradient in Hungary, and (2), by applying the concept of "Space for Time Substitution", to form hypotheses on the possible effects of a predicted climate change on these grasslands. Three sites were selected along a 200 km transect in the sand forest-steppe vegetation of the Hungarian Plain. Percentage canopy cover was estimated by species in 30 pairs of randomly located 16 m(2) quadrats at each site. Species were classified based on their phytosociological character, geographic distribution, and Raunkiaer life form. We found that species richness, canopy cover, diversity, and the relative importance of dry grassland generalists, forest species, and Hemicryptophytes decreased, whereas the relative importance of sand grassland specialists, Therophytes, and species with Continental and Sub-Mediterranean distribution increased with increasing aridity. We;Found that the existing differences in climate along the gradient are comparable to the predicted climate change for the region, therefore, the changes observed along the gradient may serve as hypotheses on the possible future changes in grassland structure and composition. However, the rate of changes is unpredictable. The indirect effects of climate, such as higher forest cover and soil organic matter content :it the wet end of the transect, may be important in driving the present differences in grassland composition, and may buffer or considerably slow down the expected changes.

Larsen, K. S., A. Ibrom, et al. (2007). "Ecosystem respiration depends strongly on photosynthesis in a temperate heath." Biogeochemistry **85**(2): 201-213.

We measured net ecosystem CO2 flux (F-n) and ecosystem respiration (R-E), and estimated gross ecosystem photosynthesis (P-g) by difference, for two years in a temperate heath ecosystem using a chamber method. The exchange rates of carbon were high and of similar magnitude as for productive forest ecosystems with a net ecosystem carbon gain during the second year of 293 +/- 11 g C m(-2) year(-1) showing that the carbon sink strength of heather-dominated ecosystems may be considerable when C. vulgaris is in the building phase of its life cycle. The estimated gross ecosystem photosynthesis and

ecosystem respiration from October to March was 22% and 30% of annual flux, respectively, suggesting that both cold-season carbon gain and loss were important in the annual carbon cycle of the ecosystem. Model fit of R-E of a classic, first-order exponential equation related to temperature (second year; R-2 = 0.65) was improved when the P-g rate was incorporated into the model (second year; R-2 = 0.79), suggesting that daytime R-E increased with increasing photosynthesis. Furthermore, the temperature sensitivity of R-E decreased from apparent Q(10) values of 3.3 to 3.9 by the classic equation to a more realistic Q(10) of 2.5 by the modified model. The model introduces R-photo, which describes the part of respiration being tightly coupled to the photosynthetic rate. It makes up 5% of the assimilated carbon dioxide flux at 0 degrees C and 35% at 20 degrees C implying a high sensitivity of respiration to photosynthesis during summer. The simple model provides an easily applied, non-intrusive tool for investigating seasonal trends in the relationship between ecosystem carbon sequestration and respiration.

Lellei-Kovacs, E., E. Kovacs-Lang, et al. (2008). "Experimental warming does not enhance soil respiration in a semiarid temperate forest-steppe ecosystem." Community Ecology **9**(1): 29-37.

The influence of simulated climate change on soil respiration was studied in a field experiment on 4 m x 5 m plots in the semiarid temperate Pannonian sand forest-steppe. This ecosystem type has low productivity and soil organic matter content, and covers large areas, yet data on soil carbon fluxes are still limited. Soil respiration rate-measured monthly between April and November from 2003 to 2006-remained very low (0.09 - 1.53 mu mol CO2 m(-2) s(-1))in accordance with the moderate biological activity and low humus content of the nutrient poor, coarse sandy soil. Specific soil respiration rate (calculated for unit soil organic matter content), however, was relatively high (0.36 - 7.92 mu mol CO g(-1) C(org)h(-1)) suggesting substrate limitation for soil biological activity. During the day, soil respiration rate was significantly lower at dawn than at midday, while seasonally clear temperature limitation in winter and water limitation in summer were detected. Between years, annual precipitation appeared to be important in determining soil carbon efflux intensity. Nocturnal warming increased soil temperature in 1 cm depth at dawn by 1.6 degrees C on the average, and decreased topsoil (0-11 cm) moisture content by 0.45 vol%. Drought treatment decreased soil moisture content by an average of 0.81 vol%. Soil respiration rate tended to decrease by 7-15% and 13-15% in response to heat and drought treatment, respectively, although the changes were not statistically significant. Nocturnal warming usually prevented dew formation, and that probably also influenced soil respiration. Based on these results, we expect a reduction in the volume and rate of organic matter turnover in this ecosystem in response to the anticipated climate change in the region.

Llorens, L. and J. Penuelas (2005). "Experimental evidence of future drier and warmer conditions affecting flowering of two co-occurring Mediterranean shrubs." International Journal of Plant Sciences **166**(2): 235-245.

We aimed to assess the impact of drier and warmer conditions on flowering traits of two common species of the coastal Mediterranean shrublands, Erica multiflora and Globularia alypum, which flower in autumn and winter. To achieve this goal, we performed a field experiment over two years, using a novel nonintrusive technique to prolong the drought period or to create passive nighttime warming. Drier conditions (17%-29% reduction in annual mean soil moisture) delayed most of the studied flowering phenophases, altered the length of the flowering period, decreased the functional flower production, and extended the flower life span in both species. Warmer conditions delayed and advanced, respectively, the onset of autumn and winter flowering of G. alypum in the first year. Higher temperatures also decreased the number of G. alypum plants with functional autumn flowers by 20%, whereas they increased the number of G. alypum plants with functional winter flowers by 28%. Therefore, our results indicate that drier and warmer conditions might alter the flowering phenology and production of these Mediterranean species. This, in turn, might lead to a change in the species composition and structure of these Mediterranean shrublands in the long term.

Llorens, L., J. Penuelas, et al. (2004). "Effects of an experimental increase of temperature and drought on the photosynthetic performance of two ericaceous shrub species along a north-south European gradient." Ecosystems **7**(6): 613-624.

Plant ecophysiological changes in response to climatic change may be different in northern and southern European countries because different abiotic factors constrain plant physiological activity. We studied the effects of experimental warming and drought on the photosynthetic performance of two ericaceous shrubs (Erica multiflora and Calluna vulgaris) along a European gradient of temperature and precipitation (UK, Denmark, The Netherlands, and Spain). At each site, a passive warming treatment was applied during the night throughout the whole year, whereas the drought treatment excluded rain events over

6-10 weeks during the growing season. We measured leaf gas exchange, chlorophyll a fluorescence, and leaf carbon isotope ratio (delta(13)C) during the growing seasons of 1999 and 2000. Leaf net photosynthetic rates clearly followed a gradient from northern to southern countries in agreement with the geographical gradient in water availability. Accordingly, there was a strong correlation between net photosynthetic rates and the accumulated rainfall over the growing season. Droughted plants showed lower leaf gas exchange rates than control plants in the four sites. Interestingly, although leaf photosynthetic rates decreased along the precipitation gradient and in response to drought treatment, droughted plants were able to maintain higher leaf photosynthetic rates than control plants in relation to the accumulated rainfall over the months previous to the measurements. Droughted plants also showed higher values of potential photochemical efficiency (F-v/F-m) in relation to controls, mainly at midday. The warming treatment did not affect significantly any of the studied instantaneous ecophysiological variables.

Llorens, L., J. Penuelas, et al. (2002). "Developmental instability and gas exchange responses of a heathland shrub to experimental drought and warming." International Journal of Plant Sciences **163**(6): 959-967.

The effects of predicted climatic changes on the physiological stress of bilberry (Vaccinium myrtillus L.) were investigated in a field experiment in a heathland of northern Wales (U. K.). In the experiment, drought was increased during the growing season by excluding precipitation, and night warming was created by reducing heat loss. To assess physiological stress, leaf size and leaf fluctuating asymmetry (FA) as integrative measures of stress during leaf development and leaf gas exchange as a classical instantaneous method to detect physiological stress, were measured. Since leaf FA increased with increasing size, comparisons of asymmetries were made for relative rather than absolute asymmetry. Relative leaf FA was calculated as the absolute difference between leaf size (area or width) of left and right halves, corrected for trait size. Drought treatment increased the relative leaf area FA in 2000 and not in 1999, in agreement with a stronger treatment in 2000. Conversely, the warming treatment decreased the relative leaf area FA in 1999 and not in 2000, coinciding with the lower minimum temperatures in the growing season of 1999. Differences in water availability and temperature between years were related with changes in the relative leaf area FA, which were consistent with the treatment effects. In contrast, leaf gas exchange rates and leaf size showed no significant response to the environmental manipulations, although there was a slight decrease of photosynthetic values and leaf size in drought treatments in both years. Leaf FA therefore appeared to be a more sensitive indicator of physiological stress than leaf size or gas exchange measurements. Our results indicate that a future increase in the severity of drought during the growing season will increase physiological stress of V. myrtillus, whereas warming will decrease physiological stress during leaf development because of the alleviation of temperature constraints.

Llorens, L., J. Penuelas, et al. (2003). "Ecophysiological responses of two Mediterranean shrubs, Erica multiflora and Globularia alypum, to experimentally drier and warmer conditions." Physiologia Plantarum **119**(2): 231-243.

A new approach was used to experimentally dry and warm a Mediterranean shrubland. By means of automatically sliding curtains, the drought period was extended by excluding rain over the two growing seasons (spring and autumn), and passive warming was created by avoiding infra-red dissipation at night over the whole year. The aim of the study was to test how a future extended drought period and an increase in temperatures could affect the photosynthetic and water use strategies of two co-occurring Mediterranean shrubs, Erica multiflora and Globularia alypum, which are common species of the dry coastal shrublands. The shoot water potential, leaf gas exchange rates and chlorophyll a fluorescence of plants was monitored seasonally during two years (1999-2001). In addition we measured the photosynthetic response curves to light and CO2 in autumn 2001 and the foliar N concentration and leaf C and N stable isotopes in summer 1999 and 2000. Droughted plants of both shrub species showed lower shoot water potentials, transpiration rates and stomatal conductances than control plants, although there was a high seasonal variability. Drought treatment reduced significantly the overall leaf net photosynthetic rates of E. multiflora, but not of G. alypum. Droughted plants of E. multiflora also showed lower leaf net photosynthetic rates in response to light and CO2 and lower carboxylation efficiency than controls, but there was no significant effect of drought on its overall photosystem II (PSII) photochemical efficiency. Although warming treatment did not affect the leaf net photosynthetic rates of the two species overall the study, it increased significantly the carboxylation efficiency and leaf net photosynthetic rates of G. alypum plants in response to CO2 levels in autumn 2001. In addition, warming treatment increased the potential photochemical efficiency of PSII (F-v/F-m) of both species (but especially of G. alypum) at predawn or midday and mainly in autumn and winter. Thus, the results suggest that drier conditions might decrease the annual productivity of these Mediterranean

shrubs, particularly of E. multiflora, and that future warming could alleviate the present low temperature constraints of the photosynthetic performance of the two studied species, but especially of G. alypum, during the colder seasons. Ultimately, drier and warmer conditions in the near future may change the competitive relationship among these species in such Mediterranean ecosystems.

Llorens, L., J. Penuelas, et al. (2004). "Contrasting growth changes in two dominant species of a Mediterranean shrubland submitted to experimental drought and warming." Annals of Botany **94**(6): 843-853.

Background and Aims Climate projections predict drier and warmer conditions in the Mediterranean basin in the next decades. The possibility of such climatic changes modifying the growth of two Mediterranean species, Erica multiflora and Globularia alypum, which are common components of Mediterranean shrublands, was assessed. Methods A field experiment was performed from March 1999 to March 2002 to prolong the drought period and to increase the night-time temperature in a Mediterranean shrubland, where E. multiflora and G. alypum are the dominant species. Annual growth in stem diameter and length of both species was measured and annual stem biomass production was estimated for 1999, 2000 and 2001. Plant seasonal growth was also assessed. Key Results On average, drought treatment reduced soil moisture 22%, and warming increased temperature by 0(.)7-1(.)6degreesC. Erica multiflora plants in the drought treatment showed a 46% lower annual stem elongation than controls. The decrease in water availability also reduced by 31% the annual stem diameter increment and by 43% the annual stem elongation of G. alypum plants. New shoot growth of G. alypum was also strongly reduced. Allometrically estimated biomass production was decreased by drought in both species. Warming treatment produced contrasting effects on the growth patterns of these species. Warmer conditions increased, on average, the stem basal diameter growth of E. multiflora plants by 35%, raising also their estimated stem biomass production. On the contrary, plants of G. alypum in the warming treatment showed a 14% lower annual stem growth in basal diameter and shorter new shoots in spring compared with controls. Conclusions The results indicate changes in the annual productivity of these Mediterranean shrubs under near future drier and warmer conditions. They also point to alterations in their competitive abilities, which could lead to changes in the species composition of these ecosystems in the long term. (C) 2004 Annals of Botany Company.

Lloret, F., J. Penuelas, et al. (2005). "Effects of vegetation canopy and climate on seedling establishment in Mediterranean shrubland." Journal of Vegetation Science **16**(1): 67-76.

Question: Does the influence of plant canopy on seedling establishment interact with climate conditions, and particularly. do intensified drought conditions, enhance a positive effect of the vegetation canopy on seedlings in Mediterranean-type ecosystems. Location: Mediterranean shrubland near Barcelona, Spain at 2 10 m a.s.l. Methods: Over the Course of four years we recorded seedling emergence and survival in open areas and below vegetation under control. drier and warmer experimental climatic conditions. Results: Seedling emergence is more sensitive to climate conditions than later stages of growth. When considering the whole set of species. the total number of established seedlings at the end of the experiment was lower in the drought and warming stands than in control ones, and vegetation canopy increased the number of these seedlings in the drought stands. Drought reduced seedling emergence but not warming, while the interaction between climate treatments and vegetation canopy was not significant. Seedling survival was lower in the warming treatment than in the control. Under drought conditions. vegetation canopy increased seedling emergence of the dominant Globularia alypum. In control stands, vegetation canopy reduced their survival. Vegetation canopy increased the survival of the dominant Erica multiflora in warming stands, and it reduced the survival of G. alypum in drought stands. No significant effects of drought and warming were observed in the seed rain of these two species. Conclusions: The balance of the facilitation-competition interactions between vegetation canopy and seedling establishment in Mediterranean-type ecosystems determined by water availability, and drought conditions enhance the positive effect of vegetation canopy. This interaction is species-specific and shows important between-year variability.

Lloret, F., J. Penuelas, et al. (2009). "Plant community changes induced by experimental climate change: Seedling and adult species composition." Perspectives in Plant Ecology Evolution and Systematics **11**(1): 53-63.

Experimental manipulation of climate provides a powerful tool for studying plant community dynamics with respect to current climate change. We experimentally investigated the vegetation dynamics of a Mediterranean shrubland under directional climate change by manipulating rain and temperature at stand level throughout 7 years. We focused on seedling establishment in relation to the between-year variability of drought conditions. We also compared seedling dynamics to changes in the established adult vegetation to assess the coupling between both dynamics. We used multivariate techniques (principal response curves

(PRC) and redundancy analysis (RDA)) to explore changes in the whole community, and Generalized Linear Model (GLZM) to analyse the influence of drought on the abundance and survival of the most abundant species. Drought treatment induced significant changes in the species composition of the seedlings, via a differential decrease in the seedling density of most species. No species was particularly favoured in terms of seedling abundance under water-deficit conditions. Warming only explained a low percentage of the variability in seedling species composition. The emergence of seedlings in control plots - which may be considered an estimation of the between-year variability in the conditions for seedling establishment - was a better predictor of seedling emergence in experimental plots than climate manipulation treatments. The PRC analysis of the adults showed dynamics that were different from those recorded for seedlings, and it also showed that drought treatment significantly explained species composition. This result is reinforced by the change in the relative abundance of seedling and adults of the more common species in the drought and warming treatments, supporting the hypothesis that climatic directional change heightens discrepancies between recruitment and the adult performance. The RDA analysis applied to species composition at the end of the experiment failed, however, to attain any statistical significance. The warming treatment did not produce any significant shifts in adult vegetation. In conclusion, directional climate change - particularly drier conditions in Mediterranean shrublands, - would result in a change in the recruitment of the plant community. This change in seedling recruitment tends to be different from the dynamics of adults, suggesting that potential adult mortality would not be compensated by actual seedling recruitment, thus enhancing shifts in community composition.

Llusia, J., J. Penuelas, et al. (2006). "Seasonal contrasting changes of foliar concentrations of terpenes and other volatile organic compound in four domiant species of a Mediterranean shrubland submitted to a field experimental drought and warming." Physiologia Plantarum **127**(4): 632-649.

To test the effect of forecasted drought and warming conditions for the next decades by GCM and ecophysiological models on foliar concentrations of volatile organic compounds (VOCs) and especially of volatile terpenes, we studied four typical Mediterranean woody plants Winus halepensis L., Pistacia lentiscus L., Rosmarinus officinalis L. and Globularia alypum U under a field experimental drought and warming generated using automatically sliding curtains. Terpenes were detected in the four studied species (R. officinalis L., P halepensis L., Pistacia lentiscus L. and G. alypum L.). In general, maximum concentrations of terpenes were found in the coldest periods and minimum concentrations in the summer. Their concentrations ranged between 0.003 mg g(-1) DM (eugenol) in G. alypum under drought conditions and 37 mg g-1 DM in R. officinalis under control conditions. Main volatile terpenes found in all studied species except in G. alypum were alpha-pinene, camphene, beta-pinene, beta-phellandrene and caryophyllene. In general, VOC leaf concentrations increased when soil moisture increased and decreased when air temperature increased. However, contrasting not consistent responses to the drought and warming treatments were found among species, seasons and years. For example, in P. halepensis, the concentrations decreased in response to drought in winter and instead increased in summer. Contrarily, drought decreased concentrations in summer and increased them in winter in Pistacia lentiscus. In any case, the data on seasonal VOC concentration in Mediterranean woody species provided here will add new knowledge of seasonal variation in essential oil contents of these species. These data might help in the study of flammability of Mediterranean ecosystems and in improving prediction algorithms, inventories and modelling of monoterpene emissions in response to climate change, which mostly do not consider the changes in concentration under drought stress. However, the lack of general and consistent response patterns to increasing drought and warming among species, seasons and years found here makes this task difficult.

Llusia, J., J. Penuelas, et al. (2008). "Contrasting species-specific, compound-specific, seasonal, and interannual responses of foliar isoprenoid emissions to experimental drought in a mediterranean shrubland." International Journal of Plant Sciences **169**(5): 637-645.

We aimed to test the effect of soil drought conditions projected by general circulation models and eco-physiological models for the next few decades on emission rates of isoprenoids by Mediterranean shrublands. We conducted a field experiment in which we generated soil drought (ca. 12% - 20% decreased soil moisture) using automatically sliding curtains, and we measured foliar isoprenoid emissions in the three dominant species of the studied shrubland (Erica multiflora L., Globularia alypum L., and Pinus halepensis L.) in two different annual periods. Monoterpene emissions were detected in the three studied species, but isoprene emissions were significantly detected only in E. multiflora. Main volatile terpenes emitted by the three species were alpha-pinene, beta-myrcene, limonene, and Delta(3)-carene. In general, maximum

isoprenoid emission rates were found in the hottest periods, and minimum emission rates were found in winter. Isoprene emissions in E. multiflora ranged between practically 0 mu g g(-1) (dry matter) h(-1) in winter 2005 and 57 mu g g(-1) (dry matter) h(-1) in summer 2003. Isoprene emissions were 75% lower during the dry second annual period, 2004 - 2005, than during the first year, 2003 2004. In E. multiflora, there was an overall decrease of 19% in isoprene emissions in response to soil drought. Conversely, monoterpene emissions increased by 26.4% in drought treatment. In G. alypum, there was an overall increase of 75% in terpene emissions in response to soil drought. In P. halepensis, drought treatment increased terpene emission rates by 156%. Drought treatment affected the emissions mainly in the hottest seasons, spring and summer. There were strong compound-specific, species-specific, interannual, and seasonal changes in the emission rates and in their response to the treatments. These data might help to improve prediction algorithms, inventories, and modeling of isoprenoid emissions and of their response to climate change (decreased isoprene emissions and increased monoterpene emissions under moderate or short-term drought and decreased emissions under severe or long-term drought), but the great variability highlights the difficulty of the task.

Llusia, J., J. Penuelas, et al. (2009). "Net ecosystem exchange and whole plant isoprenoid emissions by a mediterranean shrubland exposed to experimental climate change." Russian Journal of Plant Physiology **56**(1): 29-37.

We tested the effect of forecasted soil drought and warming climate conditions for the next decades on emission rates of isoprenoids by mediterranean shrublands. We measured isoprenoid emissions by whole dominant mediterranean woody plants (Erica multiflora L. and Globularia alypum L.) inhabiting the studied shrublands. Monoterpene emissions were detected in both species, but isoprene was emitted only by E. multiflora. Maximum emission rates were found during the hottest periods (except for G. alypum, in which they occurred in autumn), and minimum emission rates in winter in E. multiflora. Terpene emission rates ranged from 0.08 mu g/(g dry wt h) in winter in E. multiflora to 8.8 mu g/(g dry wt h) in G. alypum in autumn. In E. multiflora, the terpene emission rates decreased in response to soil drought only in summer, but increased in response to warming in spring and autumn. Isoprene emissions ranged from 0.1 mu g/(g dry wt h) in spring to 4.4 mu g/(g dry wt h) in summer. The effect of the treatments was only detected in autumn when soil drought and warming had a negative effect on isoprene emission rates. These data might improve our knowledge of isoprenoid emissions at the canopy level and in response to climate change, soil drought, or warming.

Luo, Y. Q., D. Gerten, et al. (2008). "Modeled interactive effects of precipitation, temperature, and CO2 on ecosystem carbon and water dynamics in different climatic zones." Global Change Biology **14**(9): 1986-1999.

Interactive effects of multiple global change factors on ecosystem processes are complex. It is relatively expensive to explore those interactions in manipulative experiments. We conducted a modeling analysis to identify potentially important interactions and to stimulate hypothesis formulation for experimental research. Four models were used to quantify interactive effects of climate warming (T), altered precipitation amounts [doubled (DP) and halved (HP)] and seasonality (SP, moving precipitation in July and August to January and February to create summer drought), and elevated [CO2] (C) on net primary production (NPP), heterotrophic respiration (R-h), net ecosystem production (NEP), transpiration, and runoff. We examined those responses in seven ecosystems, including forests, grasslands, and heathlands in different climate zones. The modeling analysis showed that none of the three-way interactions among T, C, and altered precipitation was substantial for either carbon or water processes, nor consistent among the seven ecosystems. However, two-way interactive effects on NPP, R-h, and NEP were generally positive (i.e. amplification of one factor's effect by the other factor) between T and C or between T and DP. A negative interaction (i.e. depression of one factor's effect by the other factor) occurred for simulated NPP between T and HP. The interactive effects on runoff were positive between T and HP. Four pairs of two-way interactive effects on plant transpiration were positive and two pairs negative. In addition, wet sites generally had smaller relative changes in NPP, R-h, runoff, and transpiration but larger absolute changes in NEP than dry sites in response to the treatments. The modeling results suggest new hypotheses to be tested in multifactor global change experiments. Likewise, more experimental evidence is needed for the further improvement of ecosystem models in order to adequately simulate complex interactive processes.

Maraldo, K., I. K. Schmidt, et al. (2008). "Can field populations of the enchytraeid, Cognettia sphagnetorum, adapt to increased drought stress?" Soil Biology & Biochemistry **40**(7): 1765-1771.

The ability to evolve increased drought tolerance in response to climate change was investigated in the enchytraeid, Cognettia sphagnetorum. Populations exposed to reduced precipitation or

increased night time temperature for more than six years were collected in mixed Calluna/grass heathland at the Mols Laboratory, Denmark. The level of prolonged drought and increased temperature corresponded to a predicted climate change scenario and has been applied since 1999. In autumn 2005, enchytraeids were sampled in 3 cm intervals down to 9 cm depth and total number, biomass, diversity and soil organic matter were determined. The drought treatment resulted in a significant reduction of the density and biomass of enchytraeids, as well as changes in the species composition. In total, five different genera were found at the site in all three treatments (control, temperature and drought). C. sphagnetorum was the dominant species, especially in the upper 0-3 cm, and was clearly affected by the drought treatment. C. sphagnetorum from all plots were cultured in the laboratory to rear second or third generation adults. Results showed that populations of drought treated plots had not developed an increased drought resistance compared to populations of control or warming plots even after several years of a putative severe selection. Lack of adaptive potential in C. sphagnetorum suggests that more frequent periods with drought in the future will have a very strong negative influence on enchytraeid density, biomass and diversity.

Mikkelsen, T. N., C. Beier, et al. (2008). "Experimental design of multifactor climate change experiments with elevated CO2, warming and drought: the CLIMAITE project." Functional Ecology **22**(1): 185-195.

Recent findings indicate that the interactions among CO2, temperature and water can be substantial, and that the combined effects on the biological systems of several factors may not be predicted from experiments with one or a few factors. Therefore realistic multifactorial experiments involving a larger set of main factors are needed. We describe a new Danish climate change-related field scale experiment, CLIMAITE, in a heath/grassland ecosystem. CLIMAITE is a full factorial combination of elevated CO2, elevated temperature and prolonged summer drought. The manipulations are intended to mimic anticipated major environmental changes at the site by year 2075 as closely as possible. The impacts on ecosystem processes and functioning (at ecophysiological levels, through responses by individuals and communities to ecosystem-level responses) are investigated simultaneously. The increase of [CO2] closely corresponds with the scenarios for year 2075, while the warming treatment is at the lower end of the predictions and seems to be the most difficult treatment to increase without unwanted side effects on the other variables. The drought treatment follows predictions of increased frequency of drought periods in summer. The combination of the treatments does not create new unwanted side effects on the treatments relative to the treatments alone.

Penuelas, J. and I. Filella (2001). "Phenology - Responses to a warming world." Science **294**(5543): 793-+.

Penuelas, J., C. Gordon, et al. (2004). "Nonintrusive field experiments show different plant responses to warming and drought among sites, seasons, and species in a north-south European gradient." Ecosystems **7**(6): 598-612.

We used a novel, nonintrusive experimental system to examine plant responses to warming and drought across a climatic and geographical latitudinal gradient of shrubland ecosystems in four sites from northern to southern Europe (UK, Denmark, The Netherlands, and Spain). In the first two years of experimentation reported here, we measured plant cover and biomass by the pinpoint method, plant C-14 uptake, stem and shoot growth, flowering, leaf chemical concentration, litterfall, and herbivory damage in the dominant plant species of each site. The two years of approximately PC experimental warming induced a 15% increase in total aboveground plant biomass growth in the UK site. Both direct and indirect effects of warming, such as longer growth season and increased nutrient availability, are likely to be particularly important in this and the other northern sites which tend to be temperature-limited. In the water-stressed southern site, there was no increase in total aboveground plant biomass growth as expected since warming increases water loss, and temperatures in those ecosystems are already close to the optimum for photosynthesis. The southern site presented instead the most negative response to the drought treatment consisting of a soil moisture reduction at the peak of the growing season ranging from 33% in the Spanish site to 82% in The Netherlands site. In the Spanish site there was a 14% decrease in total aboveground plant biomass growth relative to control. Flowering was decreased by drought (up to 24% in the UK and 40% in Spain). Warming and drought decreased litterfall in The Netherlands site (33% and 37%, respectively) but did not affect it in the Spanish site. The tissue P concentrations generally decreased and the N/P ratio increased with warming and drought except in the UK site, indicating a progressive importance of P limitation as a consequence of warming and drought. The magnitude of the response to warming and drought was thus very sensitive to differences among sites (cold-wet northern sites were more sensitive to warming and the warm-dry southern site was more sensitive to drought), seasons (plant processes were more sensitive to

warming during the winter than during the summer), and species. As a result of these multiple plant responses, ecosystem and community level consequences may be expected.

Penuelas, J., P. Prieto, et al. (2007). "Response of plant species richness and primary productivity in shrublands along a north-south gradient in Europe to seven years of experimental warming and drought: reductions in primary productivity in the heat and drought year of 2003." Global Change Biology **13**(12): 2563-2581.

We used a nonintrusive field experiment carried out at six sites - Wales (UK), Denmark (DK), the Netherlands (NL), Hungary (HU), Sardinia (Italy - IT), and Catalonia (Spain - SP) - along a climatic and latitudinal gradient to examine the response of plant species richness and primary productivity to warming and drought in shrubland ecosystems. The warming treatment raised the plot daily temperature by ca. 1 degrees C, while the drought treatment led to a reduction in soil moisture at the peak of the growing season that ranged from 26% at the SP site to 82% in the NL site. During the 7 years the experiment lasted (1999-2005), we used the pin-point method to measure the species composition of plant communities and plant biomass, litterfall, and shoot growth of the dominant plant species at each site. A significantly lower increase in the number of species pin-pointed per transect was found in the drought plots at the SP site, where the plant community was still in a process of recovering from a forest fire in 1994. No changes in species richness were found at the other sites, which were at a more mature and stable state of succession and, thus less liable to recruitment of new species. The relationship between annual biomass accumulation and temperature of the growing season was positive at the coldest site and negative at the warmest site. The warming treatment tended to increase the aboveground net primary productivity (ANPP) at the northern sites. The relationship between annual biomass accumulation and soil moisture during the growing season was not significant at the wettest sites, but was positive at the driest sites. The drought treatment tended to reduce the ANPP in the NL, HU, IT, and SP sites. The responses to warming were very strongly related to the Gaussen aridity index (stronger responses the lower the aridity), whereas the responses to drought were not. Changes in the annual aboveground biomass accumulation, litterfall, and, thus, the ANPP, mirrored the interannual variation in climate conditions: the most outstanding change was a decrease in biomass accumulation and an increase in litterfall at most sites during the abnormally hot year of 2003. Species richness also tended to decrease in 2003 at all sites except the cold and wet UK site. Species-specific responses to warming were found in shoot growth: at the SP site, Globularia alypum was not affected, while the other dominant species, Erica multiflora, grew 30% more; at the UK site, Calluna vulgaris tended to grow more in the warming plots, while Empetrum nigrum tended to grow less. Drought treatment decreased plant growth in several studied species, although there were some species such as Pinus halepensis at the SP site or C. vulgaris at the UK site that were not affected. The magnitude of responses to warming and drought thus depended greatly on the differences between sites, years, and species and these multiple plant responses may be expected to have consequences at ecosystem and community level. Decreases in biodiversity and the increase in E. multiflora growth at the SP site as a response to warming challenge the assumption that sensitivity to warming may be less well developed at more southerly latitudes; likewise, the fact that one of the studied shrublands presented negative ANPP as a response to the 2003 heat wave also challenges the hypothesis that future climate warming will lead to an enhancement of plant growth and carbon sequestration in temperate ecosystems. Extreme events may thus change the general trend of increased productivity in response to warming n the colder sites.

Penuelas, J., T. Rutishauser, et al. (2009). "Phenology Feedbacks on Climate Change." Science **324**(5929): 887-888.

Penuelas, J. and J. Sardans (2009). "ECOLOGY Elementary factors." Nature **460**(7257): 803-804.

Penuelas, J., J. Sardans, et al. (2008). "Nutrient Stoichiometric Relations and Biogeochemical Niche in Coexisting Plant Species: Effect of Simulated Climate Change." Polish Journal of Ecology **56**(4): 613-622.

Here we define a "biogeochemical niche" characterized by the species position in the multivariate space generated by its content not only of macronutrients like N, P or K, but also of micronutrients such as Mo, Mg and Ca, and trace toxic elements such as Pb and As. We then hypothesize that the flexibility of the species "biogeochemical niche" will influence the quality of plant tissue, which may have implications for herbivores, and will affect the species capacity to respond to disturbances and climate change and to adapt to the new climate conditions. We show with a simple multivariate procedure, a principal component analysis (PCA), first, that there is a strong differentiation in the total and relative (stoichiometry) content of the different elements in coexisting plant species, and, second, that there is

species-specific plasticity in the response of this elemental composition to experimental climate change. The concentrations of foliar macro and micronutrients, as well as trace elements were measured in several tree species (Quercus ilex L., Phillyrea latifolia L. and Arbutus unedo L. in a Mediterranean broad leaf forest (Prades Mts) and in shrub species (Erica multiflora L., Globularia alypum L. and Dorycnium pentaphyllum Scop.) in a Mediterranean shrubland (Garraf Mts) in control plants and in plants grown in experimental drought and warming plots. The climate conditions were monitored during the period 1999-2005. During this period, in the Prades experiment the drought plots had on average a soil moisture content 9% lower than the control plots, whereas in the Garraf experiment the drought treatment led to a mean reduction in soil moisture of 21% and the warming treatment to 0.9 degrees C rise. The species with greater changes in biogeochemical niche under increased warming or drought, Arbutus unedo, Erica multiflora and Globularia alypum, were those that were also more affected in growth, photosynthetic capacity and other eco-physiological traits. The species differentiations indicate a strong biogeochemical niche, and that the changes in biogeochemical niche are probably an underlying factor in community structure shifts.

Prieto, P., J. Penuelas, et al. (2009). "Experimental drought and warming decrease diversity and slow down post-fire succession in a Mediterranean shrubland." Ecography **32**(4): 623-636.

Plant community recovery (species richness, diversity and composition) of a post-fire Mediterranean shrubland was monitored over a seven year period (1998-2005) under experimental drought and warming that simulated the environmental conditions forecast for this area in the coming decades. Species richness and Shannon's index were positively correlated with accumulated precipitation in the growing season and both variables were negatively affected by reduced water availability in drought plots. The relative abundance of the different species in both treatments was linearly correlated with their relative abundance in control plots. Moreover, we found species-specific responses to treatments. Drought and warming treatment reduced the competitive ability of the obligate seeder tree Pinus halepensis against native resprouter shrubs and consequently, the transformation from shrub to pine tree dominated vegetation was slowed down. Conversely, the water use strategy of Globularia alypum may allow this species to maintain a dominant position in drought plots. Therefore, future drier and warmer conditions in Mediterranean areas may severely affect plant community recovery after a disturbance, due to the existence of both abundance-dependent and species-specific responses that may change inter-specific competitive relationships.

Prieto, P., J. Penuelas, et al. (2009). "Effects of long-term experimental night-time warming and drought on photosynthesis, Fv/Fm and stomatal conductance in the dominant species of a Mediterranean shrubland." Acta Physiologiae Plantarum **31**(4): 729-739.

We conducted a night-time warming and drought field experiment for 7 years (1999-2005) in a Mediterranean shrubland. We focused on the two dominant shrub species, Erica multiflora L. and Globularia alypum L. and the tree Pinus halepensis L. and the final years to study the effects of the experimental night-time warming and drought on Fv/Fm, photosynthesis, and stomatal conductance. Warming treatment increased mean air temperature and mean soil temperature through the years by an average of 0.7 and 0.9A degrees C respectively, and drought treatment reduced soil moisture through the years by an average of 19%. Warming tended to increase photosynthetic rates in E. multiflora, G. alypum and P. halepensis mostly in the cold seasons, when plants were more limited by temperature, as shown by the lowest values of Fv/Fm being detected in winter in the three studied species. A negative effect of warming was only detected for E. multiflora in summer 2003. Drought treatment generated different responses of net photosynthetic rates depending on the species, season and year. Stomatal conductance showed the same pattern as photosynthesis for the three studied species, displaying seasonal and inter-annual variability, although with an overall negative effect of drought for P. halepensis. Photosynthetic rates decreased significantly in the dry winter 2005 and spring 2005 in comparison to the same seasons of 2003 and 2004. There were positive correlations between the photosynthetic rates in different seasons for E. multiflora, G. alypum and P. halepensis and the soil moisture of the week prior to measurements. The great variation in the photosynthetic rates was thus explained in a significant part by soil moisture levels. The lowest Fv/Fm values usually corresponded with lowest stomatal conductances suggesting that drought stress could be associated to stress by low temperatures in winter.

Prieto, P., J. Penuelas, et al. (2009). "Changes in the onset of spring growth in shrubland species in response to experimental warming along a north-south gradient in Europe." Global Ecology and Biogeography **18**(4): 473-484.

Aim To test whether the onset of spring growth in European shrublands is advanced in response to the warmer conditions projected for the next two decades by climate models, and, if there is a change, whether it differs across Europe. Location The studied sites spanned a broad north-south European gradient with average annual temperatures (8.2-15.6 degrees C) and precipitation (511-1427 mm). Methods 'Bud break' was monitored in eight shrub and grass species in six European sites under control and experimentally warmer conditions generated by automatic roofs covering vegetation during the night. Results Species responsive to increased temperatures were Vaccinium myrtillus and Empetrum nigrum in Wales, Deschampsia flexuosa in Denmark, Calluna vulgaris in Netherlands, Populus alba in Hungary and Erica multiflora in Spain. Although the acceleration of spring growth was the commonest response to warming treatments, the responses at each site were species specific and year dependent. Under experimental warming 25% of cases exhibited a significantly earlier onset of the growing season and 10% had a significantly delayed onset of vegetative growth. No geographical gradient was detected in the experimental warming effects. However, there was a trend towards a greater dominance of phenological advances with more intense the warming treatments. Above 0.8 degrees C warming, only advancements were recorded. Main conclusions Our results show that warmer temperatures projected for the next decades have substantial potential effects on the phenology of the spring growth of dominant species in different European shrublands, with a dominant trend towards advancements the more intense the warming is. However, our study also demonstrates the overall difficulties of applying simple predictive relationships to extrapolate the effects of global change on phenology. Various combinations of environmental factors occur concurrently at different European sites and the interactions between different drivers (e. g. water and chilling) can alter phenology significantly.

Prieto, P., J. Penuelas, et al. (2008). "Precipitation-dependent flowering of Globularia alypum and Erica multiflora in Mediterranean shrubland under experimental drought and warming, and its inter-annual variability." Annals of Botany **102**(2): 275-285.

Background and Aims Relationships between autumn flowering, precipitation and temperature of plant species of Mediterranean coastal shrublands have been described, but not analysed experimentally. These relationships were analysed for two species of co-occurring, dominant, autumn-flowering shrubs, Globularia alypum and Erica multiflora, over 4 years and in experimentally generated drought and warming conditions. The aim was to improve predictions about the responses and adaptations of flowering of Mediterranean vegetation to climate change. Methods Beginning of anthesis and date of maximum flowering intensity ('peak date') were monitored over 4 years (2001-2004) on a garrigue land type in the noth-east of the Iberian Peninsula. Two experimental treatments were applied, increased temperature (+0.73 degrees C) and reduced soil moisture (-17%) relative to untreated plots. Key Results Flowering of Globularia alypum and Erica multiflora differed greatly between years depending on the precipitation of the previous months and the date of the last substantial rainfall (> 10 mm). Globularia alypum flowered once or twice (unimodal or bimodal) as the result of differences in the distribution and magnitude of precipitation in late-spring and summer (when floral buds develop). The drought treatment delayed and decreased flowering of Globularia alypum in 2001 and delayed flowering in 2002. Warming extended the period between the beginning of flowering and the end of the second peak for autumn flowering in 2001 and also increased peak intensity in 2002. Flowering of Erica multiflora was unaffected by either treatment. Conclusions Autumn flowering of Globularia alypum and Erica multiflora is more dependent on water availability than on temperature. Considerable inter-annual plasticity in the beginning of anthesis and peak date and on unimodal or bimodal flowering constitutes a 'safe strategy' for both species in relation to varying precipitation and temperature. However, severe changes in precipitation in spring and summer may severely affect flowering of Globularia alypum but not Erica multiflora, thus affecting development/structure of the ecosystem if such conditions persist.

Riera, P., J. Penuelas, et al. (2007). "Valuation of climate-change effects on mediterranean shrublands." Ecological Applications **17**(1): 91-100.

In general, the socioeconomic analysis of natural systems does not enter into the realms of natural science. This paper, however, estimates the human-welfare effects of possible physicochemical and biological impacts of climate change on Mediterranean shrublands over the coming 50 years. The contingent choice method was applied to elicit the trade-offs in perceived values for three climate-sensitive attributes of shrubland (plant cover,. re risk, and soil erosion) and for the costs of programs designed to mitigate changes. Soil erosion was found to be the attribute of shrubland that most concerned the population, followed by. re risk and then plant cover. An increase of 1% in the shrubland area affected by erosion was estimated to cost each person on average 2.9 euros per year in terms of lost welfare, a figure that is

equivalent in terms of perceptions of social welfare to an increase of 0.24% in the shrub area burned annually and a decrease of 3.19% in the area of plant cover. These trade-off values may help ecologists, policy makers, and land managers to take social preferences into account.

Sardans, J., J. Penuelas, et al. (2006). "Warming and drought alter soil phosphatase activity and soil P availability in a Mediterranean shrubland." Plant and Soil **289**(1-2): 227-238.

We conducted a field experiment simulating the warming and drought in a Mediterranean shrubland dominated by Erica multiflora and Globularia alypum with the aim to simulate the next future climate conditions predicted by the IPCC and ecophysiological models. As P is frequently a limiting nutrient in Mediterranean ecosystems, we investigated the drought and warming effects on soil phosphatases activities, soil P contents and availability, litter and leaf P concentration, and the capacity of this community to maintain soil P reserves and retain this nutrient in the ecosystem. Warming treatment increased soil and air temperature (an average of 1 degrees C) and drought treatment decreased soil water content in one of the seasons analysed (28% in autum 2004). Warming increased (68%) the activities of soil acid phosphatases in summer and alkaline phosphatase activity (22%) in spring 2004, and increased P concentrations in E. multiflora. Instead, warming decreased P concentrations in litterfall of this same species, E. multiflora, and soil HCO3-extractable P-i (Olsen-P-i) in some seasons, decreasing total P soil concentration (37%) after 6 years of treatment. The drought treatment did not change soil phosphatase activities, nor available P-i. The effects of climate change on soil P dynamics in Mediterranean areas will thus be strongly dependent on whether the main variable involved in the local change is warming or drought. If warming is the main change without significant changes in water availability, the increases of biological activity can accelerate plant growth, P capture by plants and increase soil-phosphatase activity, altogether decreasing P contents in soil. If drought is the main change, a reduction in P demands by plants is expected, increasing P stocks in soils.

Sardans, J., J. Penuelas, et al. (2007). "Seasonal patterns of root-surface phosphatase activities in a Mediterranean shrubland. Responses to experimental warming and drought." Biology and Fertility of Soils **43**(6): 779-786.

Mediterranean ecosystems are water limited and the current general circulation Models (GCM) and ecophysiological models forecast a warming and a further increase of drought in the next decades. A stronger water stress can decrease the capacity for nutrient absorption by plants. We conducted a field experiment to simulate forecasted drought and warming in a Mediterranean calcareous shrubland to assess the performance of root-surface phosphatase activities of the dominant shrub Globularia alypum. These enzyme activities were higher in autumn and spring, when the climate conditions were optimal for plant activity, than in summer or winter, when there was either lack of water or cold temperatures. A decrease in soil moisture in drought plots decreased root-surface phosphatase activity (29% in summer and 25% in autumn). The decrease in root-surface phosphatase activity in drought plots coincided with a decrease in P leaf concentrations and P accumulation in aboveground biomass and loss of photosynthetic capacity of some dominant shrub species of this ecosystem, and with a tendency to increase total soil-P. These results suggest that the expected drier conditions in this Mediterranean shrubland in the next decades will slow down the P uptake by plants, thereby, diminishing the P contents in biomass and increasing total P contents in soil in non-available forms and that this can be, in part, attributable to a result of the decrease in root-surface phosphatase activity.

Sardans, J., J. Penuelas, et al. (2008). "Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland." Applied Soil Ecology **39**(2): 223-235.

In a Mediterranean shrubland, we investigated the effects of the projected warming and drought on soil urease, protease and P-glucosidase activities and the relation of the possible changes in the activities of these enzymes with the observed changes in soil moisture, soil pH and in C and N stocks in soils, leaves and leaf litter during 1 year (April 2004-May 2005). This investigation was conducted in a long-term experiment of warming and drought manipulation that began in 1999 and is lasting until now. Warming increased soil urease activity by 10% in the study period, mainly by increasing soil urease activity 30% in winter and 10% in spring, and increased p-glucosidase activity 38% in spring. Soil urease and beta-glucosidase activities were positively correlated with soil temperatures in winter and negatively in summer. Warming increased soil enzyme activities in winter when soil moisture was highest and in spring coinciding with the greatest biological activity. Warming decreased NH4+ Soil concentration in the spring of 2004 (by 30%) and 2005 (by 72%), in consonance with the increase in N uptake by plants. Warming decreased N

concentration in Giobularia alypum leaf litter, increasing C/N leaf ratio by 30% showing an increase in N mobilization and contributing to a greater total N accumulation in plants. However, the greater NO3- availability in soil observed under warming, probably by an increase in nitrification, may lead to a net N loss by leaching under the torrential rainfalls typical of the Mediterranean climate regions. Drought reduced soil protease activity (9%) in the study period, mainly by decreasing it in spring by 13-21%, but did not affect N soil contents because N turn-over reduction was counterbalanced by a decrease in N leaf concentrations. Soil protease activity was positively correlated with soil water content showing a strong dependence of this enzyme on soil water content. Drought did not affect p-glucosidase activity but tended to increase C contents in soils, which together with the increase in C/N in leaves indicate a reduction of C turn-over and a trend to increase C stocks in soil at long term. The effects of warming and drought on soil enzyme activities were due to a direct effect on soil temperature and soil water content, respectively, and not to changes on soil organic matter quantity and nutritional quality.

Sardans, J., J. Penuelas, et al. (2008). "Warming and drought change trace element bioaccumulation patterns in a Mediterranean shrubland." Chemosphere **70**(5): 874-885.

A field experiment consisting of drought and warming manipulation was conducted in a Mediterranean shrubland dominated by the shrubs Erica multiflora and Globularia alypum. The aim was to investigate the effects of the climatic changes predicted by IPCC models for the coming decades on trace element concentration and accumulation in aboveground biomass, plant litter, and soil. Warming increased concentrations and aboveground accumulation of some trace elements related to plant root uptake, such as Al, As, Cr, Cu, and partially Pb. This effect was more general in E multiflora than in G. alypum. The stronger effects were increases in Al leaf concentrations (42%) and aboveground accumulation (500 g ha(-1)) in E multiflora, in As stem biomass accumulation (0.2 g ha(-1)) in E. multiflora, and in Cr leaf concentrations (51%) in G. alypum and stem aboveground accumulation in E multiflora (1.1 g ha(-1)). These species-specific increases were related to greater retranslocation, photosynthetic capacity and growth in E multiflora than in G. alypum. Warming decreased the concentrations of some trace elements in leaf litter, implying the existence of an increased leaf retranslocation. Drought increased As (40%) and Cd (55%) in E multiflora stems, whereas it decreased Cu (50%) in leaves, Ni (28%) in stems and Pb (32%) in leaf litter of G. alypum. The increasing concentrations of some trace elements in E multiflora and not in G. alypum were related to a greater growth reduction in E multiflora than in G. alypum. Warming increased As soil solubility (67%) and decreased total soil As (21%). Those changes were related to a greater Fe mobilization in warming plot and to a greater plant capture. Drought increased Hg (350%) concentrations in soils but had no significant effects on trace element accumulation in aboveground biomass. The different response to warming and drought in the two dominant species implies uneven changes in the quality of the plant tissues that may have implications for herbivores. This may be specially important for the performance of the studied Mediterranean ecosystems under the warmer and drier conditions predicted by the next decades by the GCM and ecophysiological models.

Sardans, J., J. Penuelas, et al. (2008). "Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland." Global Change Biology **14**(10): 2304-2316.

We investigated the effects of warming and drought on C and N concentrations, nitrogen use efficiency (NUE), and C and N accumulation in different ecosystem compartments. We conducted a 6-year (1999-2005) field experiment to simulate the climate conditions projected by IPCC models for the coming decades in a Mediterranean shrubland. We studied the two dominant species, Globularia alypum and Erica multiflora, and an N-fixing species, Dorycnium pentaphyllum, also abundant in this shrubland. Warming (1 degrees C) decreased N leaf concentrations by 25% and increased N stem concentrations by 40% in G. alypum. Although warming changed the available ammonium in soil in some seasons, it did not increase total soil N contents. Drought (19% average reduction in soil moisture) decreased leaf N concentrations in the two dominant shrub species, E. multiflora and G. alypum by 16% and 19%, respectively, and increased stem N concentrations by 56% and 40%, respectively. Neither warming nor drought changed the leaf N concentrations in the N-fixing species D. pentaphyllum, although warming increased stem N concentration by 9%. In G. alypum, the increase of stem N concentrations contributed to the observed increase of N accumulation in stem biomass in drought treatments with respect to control plots (8 kg N ha(-1)). Neither warming nor drought changed NUE in the period 1999-2005. Warming increased soil organic C relative to drought. The effects of warming and drought on C and N concentrations, on N accumulation and on leaf/stem N distribution were not the result of dilution or concentration effects produced by changes in biomass accumulation. Other factors such as the changes in soil N availability, photosynthetic capacity, and plant internal C and N remobilization must be involved. These changes which differed depending on the

species and the plant tissue show that the climate change projected for the coming decades will have significant effects on the C and N cycle and stoichiometry, with probable implications for ecosystem structure and function, such as changes in plant-herbivore relationships, decomposition rates or community species composition.

Sardans, J., J. Penuelas, et al. (2008). "Changes in Ca, Fe, Mg, Mo, Na, and S content in a Mediterranean shrubland under warming and drought." Journal of Geophysical Research-Biogeosciences **113**(G3).

In an evergreen Mediterranean shrubland we conducted a 6-year field experiment simulating the warming and drought projected by general circulation models and ecophysiological models for the next decades: 20% reduction of soil moisture and 1 degrees C of temperature increase. We tested whether warming and drought have effects on Ca, Fe, Mg, Mo, Na and S availability, concentrations and accumulation patterns in the three dominant plant species and in soil. Warming increased concentrations of Ca and Mo in leaves in Erica multiflora (42% and 65%, respectively) and in Dorycnium pentaphyllum (38% and 60%, respectively). Warming increased Mo accumulation in leaves and aboveground biomass in Globularia alypum (0.07 and 0.40 g ha(-1)) and in E. multiflora (0.12 and 0.4 g ha(-1)), and increased Fe accumulation in stem biomass of G. alypum (600 g ha(-1)), increasing the capacity to retain these nutrients in the ecosystem. The increase of Fe and Mo capture capacity under warming conditions was greater in G. alypum than in E. multiflora coinciding with its greater increases in photosynthetic capacity. Warming decreased soil total-Fe concentration by 24% and increased Mg accumulation in soil exchange complex by 19%. Drought increased Na leaf and stem concentrations (93% and 50%, respectively) and accumulation in leaf and aboveground biomass (780 and 800 g ha(-1), respectively) in G. alypum, allowing an increase of osmotic pressure which helps to prevent water losses and is related to its capacity to resist drought. Drought reduced S leaf and Mg leaf-litter concentrations of G. alypum and increased them in leaves of E. multiflora and also increased Mo and Na concentrations in leaves of D. pentaphyllum. Drought increased Fe soil solubility by 65%. The results indicate different effects of climate change on nutrient status in the ecosystem depending on whether the main change is warming or drought. The changes in concentration and biomass accumulation were different depending on the nutrient and the species, changing the stoichiometry among these nutrients and modifying the nutritional quality of plant tissues.

Sardans, J., J. Penuelas, et al. (2008). "Drought and warming induced changes in P and K concentration and accumulation in plant biomass and soil in a Mediterranean shrubland." Plant and Soil **306**(1-2): 261-271.

A field experiment involving drought and warming manipulation was conducted over a 6-year period in a Mediterranean shrubland to simulate the climate conditions projected by IPCC models for the coming decades (20% decreased soil moisture and 1 degrees C warming). We investigated P and K concentration and accumulation in the leaves and stems of the dominant species, and in soil. Drought decreased P concentration in Globularia alypum leaves (21%) and in Erica multiflora stems (30%) and decreased K concentration in the leaves of both species (20% and 29%, respectively). The general decrease of P and K concentration in drought plots was due to the reduction of soil water content, soil and root phosphatase activity and photosynthetic capacity that decreased plant uptake capacity. Warming increased P concentration in Erica multiflora leaves (42%), but decreased it in the stems and leaf litter of Erica multiflora and the leaf litter (33%) of Globularia alypum, thereby demonstrating that warming improved the P retranslocation and allocation from stem to leaves. These results correlate with the increase in photosynthetic capacity and growth of these two dominant shrub species in warming plots. Drought and warming had no significant effects on biomass P accumulation in the period 1999-2005, but drought increased K accumulation in aboveground biomass (10 kg ha(-1)) in Globularia alypum due to the increase in K concentration in stems. The stoichiometric changes produced by the different responses of the nutrients led to changes in the P/K concentration ratio in Erica multiflora leaves, stems and litter, and in Globularia alypum stems and litter. This may have implications for the nutritional value of these plant species and plant-herbivore relationships. The effects of climate change on P and K concentrations and contents in Mediterranean ecosystems will differ depending on whether the main component of change is drought or warming.

Schmidt, I. K., A. Tietema, et al. (2004). "Soil solution chemistry and element fluxes in three European heathlands and their responses to warming and drought." Ecosystems **7**(6): 638-649.

Soil water chemistry and element budgets were studied at three northwestern European Calluna vulgaris heathland sites in Denmark (DK), The Netherlands (NL), and Wales (UK). Responses to experimental nighttime warming and early summer drought were followed during a two-year period. Soil solution chemistry measured below the organic soil layer and below the rooting zone and water fluxes

estimated with hydrological models were combined to calculate element budgets. Remarkably high N leaching was observed at the NL heath with 18 and 6.4 kg N ha(-1) year(-1) of NO3-N and NH4-N leached from the control plots, respectively, indicating that this site is nitrogen saturated. increased soil temperature of +0.5degreesC in the heated plots almost doubled the concentrations and losses of NO3-N and DON at this site. Temperature also increased mobilization of N in the O horizon at the UK and DK heaths in the first year, but, because of high retention of N in the vegetation or mineral soil, there were no significant effects of warming on seepage water NO3-N and NH4-N. Retention of P was high at all three sites. In several cases, drought increased concentrations of elements momentarily, but element fluxes decreased because of a lower flux of water. Seepage water DOC and DON was highly significantly correlated at the UK site where losses of N were low, whereas losses of C and N were uncoupled at the NL site where atmospheric N input was greatest. Based on N budgets, calculations of the net change in the C sink or source strength in response to warming suggest no change or an increase in the C sink strength during these early years.

Sowerby, A., B. Emmett, et al. (2005). "Microbial community changes in heathland soil communities along a geographical gradient: interaction with climate change manipulations." Soil Biology & Biochemistry **37**(10): 1805-1813.

Climate change constitutes a serious threat for European heathlands as unlike other sources of damage, such as over-grazing, local remediation is not a possibility. Within the large pan-European projects, CLIMOOR and VULCAN, the effect of periodic drought and increased temperature were investigated in four heathland ecosystems along a geographical and climatic gradient across Europe. Fluorogenically labelled substrates for four enzymes (glucosidase, sulphatase, phosphatase, leucine amino peptidase) were used to measure extra-cellular enzyme activity in soil samples from each of the CLIMOOR sites. Microbial extra-cellular enzyme production is linked to microbial activity as well as soil physico-chemical properties, making soil enzymes one of the more reactive components of terrestrial ecosystems and potentially excellent indicators of soil microbial functional status and diversity. Across all sites and over all the substrates, organic matter content was exponentially, inversely related to enzyme activity. Although the increase in temperature produced by the CLIMOOR roofs was small (on average 0.9 degrees C), this was sufficient to increase enzyme activity in all sites (on average by 45%). The increase was within the range of seasonal variability at each of the sites. The effect of drought on enzyme activity was more pronounced in the Northern European sites than the southern European, and most moisture limited, site. This suggests that the effect of temperature increases may be observed across all regions; however, the soils of northern Europe may be more sensitive to changes in rainfall patterns than more moisture limited Southern European soils.

Sowerby, A., B. A. Emmett, et al. (2008). "Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils." Global Change Biology **14**(10): 2388-2404.

Current predictions of climate change include altered rainfall patterns throughout Europe, continental USA and areas such as the Amazon. The effect of this on soil carbon efflux remains unclear although several modelling studies have highlighted the potential importance of drought for carbon storage. To test the importance of drought, and more importantly repeated drought year-on-year, we used automated retractable curtains to exclude rain and produce repeated summer drought in three heathlands at varying moisture conditions. This included a hydric system limited by water-excess (in the UK) and two mesic systems with seasonal water limitation in Denmark (DK) and the Netherlands (NL). The experimental rainfall reductions were set to reflect single year droughts observed in the last decade with exclusion of rain for 2-3 months of the year resulting in a 20-26% reduction in annual rainfall and 23-38% reduction in mean soil moisture during the drought period. Unexpectedly, sustained reduction in soil moisture over winter (between drought periods) was also observed at all three sites, along with a reduction in the maximum water-holding capacity attained. Three hypotheses are discussed which may have contributed to this lack of recovery in soil moisture: hydrophobicity of soil organic matter, increased water use by plants and increased cracking of the soil. The responses of soil respiration to this change in soil moisture varied among the sites: decreased rates were observed at the water-limited NL and DK sites whilst they increased at the UK site. Reduced sensitivity of soil respiration to soil temperature was observed at soil moisture contents above 55% at the UK site and below 20% and 13% at the NL and DK sites, respectively. Soil respiration rates recovered to predrought levels in the NL and DK sites during the winter re-wetting period that indicates any change in soil C storage due to changes in soil C efflux may be short lived in these mesic systems. In contrast, in the hydric UK site after 2 years of drought treatment, the persistent reduction in soil moisture throughout the year resulted in a year-round increase in soil respiration flux, a response that accelerated over time to 40% above control levels. These findings suggest that carbon-rich soils with high organic matter content may act as a

significant source of CO2 to the atmosphere following repeated summer drought. Nonrecovery of soil moisture and a persistent increase in soil respiration may be the primary mechanism underlying the reported substantial losses of soil carbon from UK organic soils over the last 20 years. These findings indicate that the water status of an ecosystem will be a critical factor to consider in determining the impact of drought on the soil carbon fluxes and storage.

Tietema, A., G. E. M. van der Lee, et al. (2007). "The production of N2O in Douglas fir litter as affected by anoxic conditions within litter particles and pores." Soil Biology & Biochemistry **39**(1): 239-248.

The development of anoxic conditions in forest litter and the relation with nitrous oxide (N2O) production and emission rates are not completely understood. Water content is an important factor in the regulation of N2O production due to its effect on the development of anoxic conditions. A combination of simulation modeling and incubation experiments was used to study (1) O-2 concentrations in water and organic matter at various water saturation fractions of inter-particle pores in Douglas fir litter (F2 horizon), (2) the relationship between N2O production and moisture content of litter and (3) to test whether diffusion constraints of nitrate (NO3-) could have explained measured N2O production rates within litter fragments. Model simulations showed that the occurrence of high N2O production rates in samples with extremely high water contents coincided with the development of anoxic conditions in water-filled inter-particle pores. Measured N2O production rates started to increase exponentially after 1-2 days in glucose-amended samples, during which substantial microbial growth was established. For these latter samples model simulations showed that the increase in O-2 consumption due to microbial growth lead to anoxic conditions in water-filled pores at locations which were far from the O-2 saturated air-filled pores. It was concluded that anoxic conditions in water-filled pores was the crucial factor for the development of high N2O production rates. Diffusion limitation of NO3- and glucose were estimated to be negligible in the highly fragmented litter material used. The occurrence of diffusion limitation depended on litter particle size, the NO3- reduction potential and the NO3- concentration. Therefore, diffusion limitation together with N2O production in litter cannot be neglected under field conditions with a low NO3- concentration or a high NO3- reduction potential.

Toberman, H., C. Freeman, et al. (2008). "Summer drought decreases soil fungal diversity and associated phenol oxidase activity in upland Calluna heathland soil." Fems Microbiology Ecology **66**(2): 426-436.

Natural moisture limitation during summer drought can constitute a stress for microbial communities in soil. Given globally predicted increases in drought frequency, there is an urgent need for a greater understanding of the effects of drought events on soil microbial processes. Using a long-term field-scale drought manipulation experiment at Clocaenog, Wales, UK, we analysed fungal community dynamics, using internal transcribed spacer-denaturing gradient gel electrophoresis (DGGE), over a 1-year period in the 6th year of drought manipulation. Ambient seasonality was found to be the dominant factor driving variation in fungal community dynamics. The summer drought manipulation resulted in a significant decline in the abundance of dominant fungal species, both independently of, and in interaction with, this seasonal variation. Furthermore, soil moisture was significantly correlated with the changes in fungal diversity over the drought manipulation period. While the relationship between species diversity and functional diversity remains equivocal, phenol oxidase activity was decreased by the summer drought conditions and there was a significant correlation with the decline of DGGE band richness among the most dominant fungal species during the drought season. Climatically driven events such as droughts may have significant implications for fungal community diversity and therefore, have the potential to interfere with crucial ecosystem processes, such as organic matter decomposition.

Van Meeteren, M. J. M., A. Tietema, et al. (2007). "Regulation of microbial carbon, nitrogen, and phosphorus transformations by temperature and moisture during decomposition of Calluna vulgaris litter." Biology and Fertility of Soils **44**(1): 103-112.

To evaluate the effect of climate change on ecosystem functioning, the temperature and moisture response of microbial C, N, and P transformations during decomposition of Calluna vulgaris (L.) Hull. litter was studied in a laboratory incubation experiment. The litter originated from a dry heathland in the Netherlands where P limited vegetation growth. Fresh litter was incubated at 5, 10, 15, or 20 degrees C and at a moisture content of 50, 100, or 200% in a full factorial design. Microbial nutrient transformations and activity were evaluated during two successive periods: an initial period of 48 days characterized by microbial growth and a second period from 48 to 206 days in which microbial growth declined significantly. Temperature and moisture response of respiration rate, the metabolic quotient (qCO(2)), C, N, and P immobilization, net N and P mineralization and nitrification rates were evaluated by performing linear

regressions. Microbial nutrient transformations and microbial activity depended both on temperature and moisture. In the first period, the respiration rate, qCO(2), microbial C and N immobilization, net P mineralization, net N mineralization and net nitrification rates were more strongly affected by temperature, while the microbial P immobilization rate was more strongly affected by moisture. The respiration rate, qCO(2), P immobilization rate, net P and N mineralization rate, and nitrification rate increased with temperature and moisture, while the C and N immobilization rate decreased with increasing temperature and increased with moisture. In the second period, C, N, and P immobilization and net N and P mineralization rates were significantly lower. The respiration rate and qCO(2) continued to increase with temperature and moisture, but C and N immobilization rates increased with temperature and declined with increasing moisture. Net P mineralization rate decreased at higher temperature and moisture, and nitrification rate declined with increasing temperature and increased with moisture. It was concluded that plant growth in these P-limited systems is very sensitive to climate change as it strongly relies on the competition for P with microbes, and temperature and moisture have a large effect on the immobilization rate of available P.

van Meeteren, M. M., A. Tietema, et al. (2008). "Microbial dynamics and litter decomposition under a changed climate in a Dutch heathland." Applied Soil Ecology **38**(2): 119-127.

Climate change scenarios predict changes in temperature and precipitation. The effect of a modest temperature increase and repeated summer droughts on the rate of litter decomposition and microbial biomass dynamics was studied by a field scale manipulation experiment at a phosphorus (P) deficient dry heathland ecosystem in the Netherlands. Retractable covers were used to create artificial nighttime warming or prolonged summer drought in the experimental plots. The warming treatment initially enhanced litter mass loss and two consecutive years of summer drought retarded litter decomposition rate. Microbial carbon (C), nitrogen (N) and P immobilization was affected by the warming treatment as well as by the drought treatment. Enhanced temperatures resulted in increased microbial biomass C during the first half year of incubation, whereas the first drought treatment significantly retarded microbial N and P immobilization. The delayed net microbial N and P immobilization in the drought plots prevented net N and P mineralization. After 1 year microbial biomass C, N and P were significantly higher in the drought plots, probably as a result of availability of new substrate caused by the drying and rewetting process. Although microbial biomass was higher in the drought plots, the microbial C/N ratio was equal to the control and varied between 6 and 8. This suggested that in both the control and drought plots, the microbial community was dominated by bacteria at the longer term. Both treatments reduced net P mineralization and together with decreased foliar P concentrations this indicated the progressive importance of P limitation in restraining plant growth in this N saturated ecosystem.

Wessel, W. W., A. Tietema, et al. (2004). "A qualitative ecosystem assessment for different shrublands in western Europe under impact of climate change." Ecosystems **7**(6): 662-671.

Climate change may affect the dynamics of ecosystems and the goods and services they provide. To investigate the consequences of warming and drought for the goods and services provided by different shrublands in various western European countries, an assessment was carried out using results of field manipulation experiments of the CLIMOOR and VULCAN projects. Goods and services of these shrublands mainly encompass biodiversity, various forms of recreation, conservation of culturally and historically important landscapes, groundwater as a drinking water source, and carbon sequestration. Warming of dry lowland heathlands in The Netherlands and Denmark increases nutrient availability, which may lead to grass encroachment reducing biodiversity and decreasing recreational values. Drought may reduce the chances of grass encroachment but increase the chances of disturbances to heather vegetation. Similarly, warming increases and drought decreases the chances of nitrate pollution to the groundwater, which is often used as a drinking water source. Warming of the upland heathland in the UK increases its productivity, which might enable higher grazing densities leading to improved agricultural production. However, complex interactions between heather and invading species may be affected. Furthermore, nitrate production is increased, which may lead to groundwater pollution. Under drought conditions, productivity decreases and agricultural production capacity drops. in the Mediterranean shrubland in Spain, both warming and drought led to a shift in the species composition of seedlings and recruitment, which might lead to a change in the plant community and a reduction in biodiversity. In the drought treatment, a decreasing soil carbon content may lead to a loss of biodiversity, recreational possibilities, and an increased threat of wildfires and erosion.