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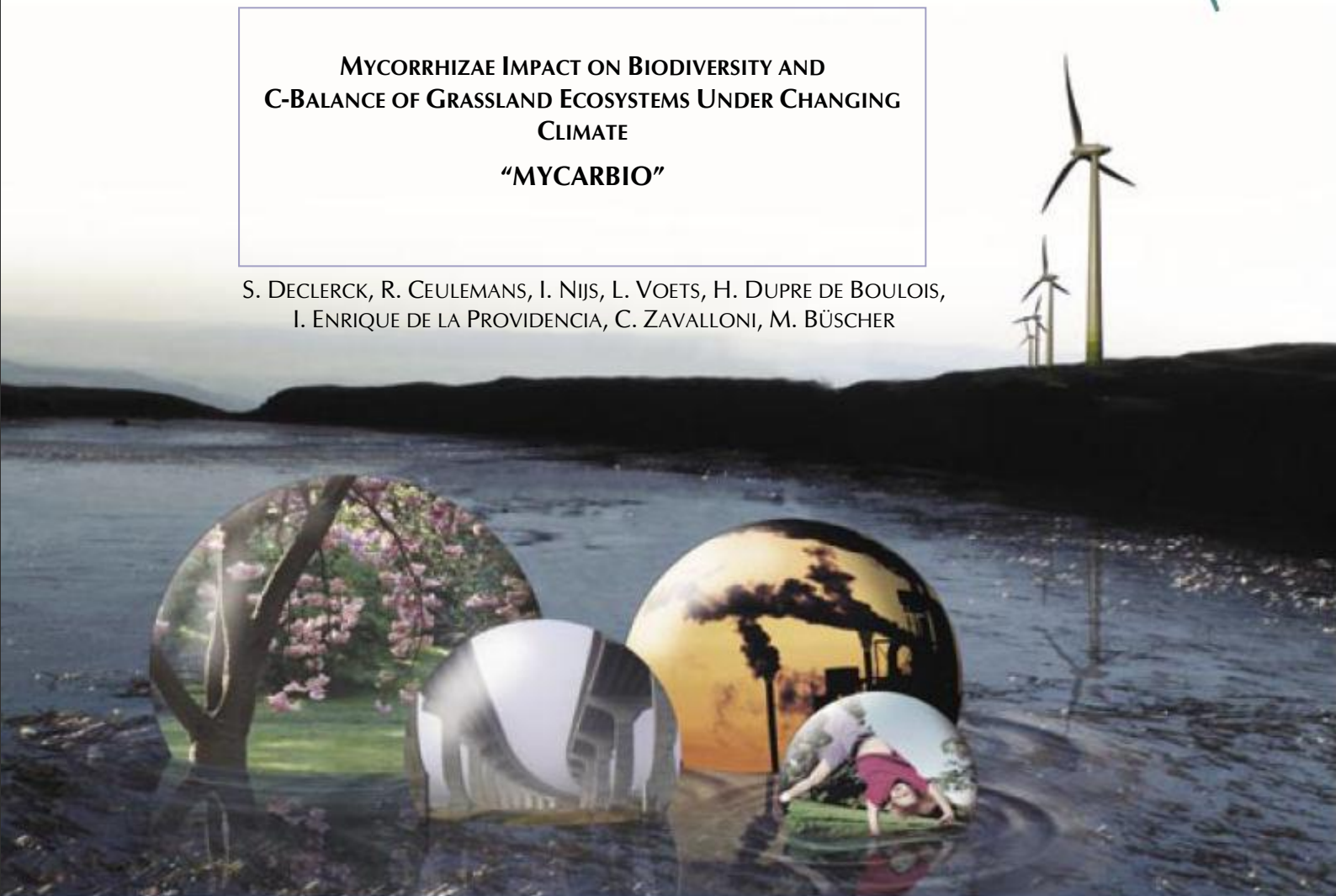
SCIENCE FOR A SUSTAINABLE DEVELOPMENT



**MYCORRHIZAE IMPACT ON BIODIVERSITY AND  
C-BALANCE OF GRASSLAND ECOSYSTEMS UNDER CHANGING  
CLIMATE**

**“MYCARBIO”**

S. DECLERCK, R. CEULEMANS, I. NIJS, L. VOETS, H. DUPRE DE BOULOIS,  
I. ENRIQUE DE LA PROVIDENCIA, C. ZAVALLONI, M. BÜSCHER



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CLIMATE 

**BIODIVERSITY** 

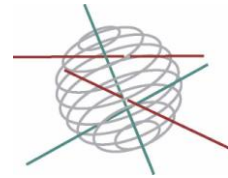


ATMOSPHERE AND TERRESTRIAL AND MARINE ECOSYSTEMS 



TRANSVERSAL ACTIONS 

SCIENCE FOR A SUSTAINABLE DEVELOPMENT  
(SSD)



***Biodiversity***



FINAL REPORT  
SUMMARY

**MYCORRHIZAE IMPACT ON BIODIVERSITY AND  
C-BALANCE OF GRASSLAND ECOSYSTEMS UNDER  
CHANGING CLIMATE**

**“MYCARBIO”**

**SD/BD/05**

Promotors

**Stéphane Declerck**

Université catholique de Louvain (UCL)

Unité de Microbiologie

Croix du Sud 3, bt 6

1348 Louvain-la-neuve

**Reinhart Ceulemans & Ivan Nijs**

Universiteit Antwerpen (UA)

Authors

**Stéphane Declerck, Liesbeth Voets, Ivan Enrique de la Providencia**

**Hervé Dupré de Boulois**

(UCL)

**Reinhart Ceulemans, Ivan Nijs, Costanza Zavalloni, Manu Büscher**

(UA)

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UCL  
Université  
catholique de  
Louvain

 Universiteit  
Antwerpen



Avenue Louise 231  
Louizalaan 231  
B-1050 Brussels  
Belgium  
Tel: +32 (0)2 238 34 11 – Fax: +32 (0)2 230 59 12  
<http://www.belspo.be>

Contact person: Aline Van Der Werf  
+32 (0)2 238 36 71

Project website : <http://emma.agro.ucl.ac.be/cesamm/projects/web/home.php?fichier=home.php>

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Stéphane Declerck, Reinhart Ceulemans, Ivan Nijs, Liesbet Voets, Hervé Dupré de Boulois, Ivan Enrique de la Providencia, Costanza Zavalloni, Manu Büscher. ***Mycorrhizae impact on biodiversity and C-balance of grassland ecosystems under changing climate “MYCARBIO”*** Final Report Summary. Brussels : Belgian Science Policy 2009 – (Research Programme Science for a Sustainable Development

Climate change has and will continue to have profound effects on the structure and function of terrestrial ecosystems. It is thus imperative to improve our understanding of the complex response of ecosystems to climate change in order to enhance the scientific basis for national and international policies regulating carbon sequestration and greenhouse gas emissions. Improved management of grassland has been identified as a potential tool to combat climate change by enhancing carbon sequestration in soils but also in vegetation, while conserving biodiversity. Several recent studies have demonstrated the fundamental role played by above- and belowground communities in controlling ecosystem processes and properties. Thus, understanding the linkages between these communities under climate change (including increasing CO<sub>2</sub> concentration) will bring new insights on how communities and biological processes will evolve under future climate scenario.

MYCARBIO aimed to investigate the impact of arbuscular mycorrhizal fungi (AMF) on biodiversity and C cycle in Belgian grassland ecosystems under changing climate conditions. To achieve this objective, five specific tasks were identified:

- (1) Evaluation of AMF biodiversity in selected Belgian grasslands,
- (2) Determination of the role of AMF for seedling establishment, plant community structure, diversity and productivity in grasslands and their feedbacks on AMF,
- (3) Understanding of the impact of elevated CO<sub>2</sub>, temperature and water availability on above- and belowground biodiversity, AMF-plant associations and C cycle,
- (4) Evaluation of the ecological significance of AMF-plant interactions on above- and below-ground biodiversity and C balance,
- (5) Modeling of the C-balance processes in grassland ecosystems.

#### **TASK 1. Evaluation of AMF biodiversity in selected Belgian grasslands**

Following the identification of representative Belgian grasslands, an assessment of the AMF biodiversity was performed from soils collected at five different sites in the Flemish and Walloon regions. Analyses showed the presence of AMF spores in each soil sample of the different sites. Twenty to fifty spores were recovered per 100 g of air-dried soil. Morphological observations revealed the presence of several strains/species of AMF at each of the five sites. Trap cultures of bulk soil were established on *Allium porrum* L (leek) and maintained under greenhouse conditions to produce AMF material (i.e. spores and intraradical fungal structures). Spores of the same morphotype were subsequently isolated and re-established on leek trap plants to produce monospecific species used for species identification and germplasm preservation. Morphological characteristics of the spores allowed identifying the AMF at the genus level. All the strains collected belonged to the *Glomeraceae* and *Diversisporaceae* families.

Further taxonomic and phylogenetic analyses will be performed on the AMF beyond the time frame of the project using morphological and molecular tools. Finally, long-term preservation of the *in vitro* cultures will be initiated. These cultures will be deposited into the Glomeromycota *In vitro* Collection (GINCO).

#### **TASK 2. Role of AMF for seedling establishment, plant community structure, diversity and productivity in grasslands and their feedbacks on AMF**

AMF are able to interconnect different plants through a common mycorrhizal network (CMN). It favors the transport of soil-derived nutrients (e.g. phosphorus) and plant-derived carbon within the network and possibly between plants. It was observed that seedlings established more easily within an existing mycorrhizal network, most likely because they have a rapid and direct access to a large pool of soil nutrients through the mycorrhizal network or even directly from other plants. Moreover, because the mycorrhizal network is already connected to the surrounding vegetation, seedlings could access carbon from the established plants through this CMN. This could thus imply that the carbon cost of the seedling to the mycorrhizal network is strongly reduced or even inexistent, therefore favoring the seedling establishment. It can also be hypothesized that in a plant community, carbon could circulate from plants to plants through a CMN depending on source/sink relationships.

The transfer of carbon through a CMN, from a *Medicago truncatula* Gaertn. donor plant to a receiver plant, was investigated under highly controlled *in vitro* conditions. Two scenarios were considered:

- The receiver plant was growing under decreased light conditions,
- The receiver plant was a seedling.

Following labeling of the donor plant with  $^{13}\text{CO}_2$ ,  $^{13}\text{C}$  was detected in the donor plant shoots and roots, in the extraradical mycelium and in the receiver plant roots. Fatty acid analysis of the receiver's roots showed a  $^{13}\text{C}$  enrichment in the AMF-specific lipids, while no significant  $^{13}\text{C}$  enrichment was detected in the plant-specific lipids.

We concluded that:

- (1) Carbon could be transferred from a donor to a receiver plant via a CMN, but remained within the intraradical AMF structures of the receiver's roots and was not transferred to the receiver's plant tissues.
- (2) CMN do not sustain seedling establishment through the transfer of carbon,

### **TASK 3. Impact of elevated $\text{CO}_2$ , temperature and water availability on above- and belowground biodiversity, AMF-plant associations and C cycle.**

Due to their key position at the soil-root interface, it is of critical importance to include AMF in studies on the impact of global change on plant communities. Consequently, any climate change affecting plants is likely to affect AMF and plant-AMF interactions. Elevated temperatures or changes in precipitation rates may have direct impact on both the AMF and their host plants, while elevated  $\text{CO}_2$  can directly impact plant C fixation and indirectly AMF by an alteration of below-ground C allocation. AMF are known to contribute to the C sequestration into the soil and therefore it is essential to elucidate their role in the C cycle under changing climate.

Two microcosm experiments conducted under *in vitro* conditions were designed to investigate the effect of elevated  $\text{CO}_2$  and temperature on:

- AMF growth,
- N and P transport by AMF.

*In vitro* culture systems were placed in either ambient (22/18°C day/night and  $380 \pm 15$  ppm  $\text{CO}_2$ ) or elevated  $\text{CO}_2$  (e $\text{CO}_2$ :  $600 \pm 15$  ppm  $\text{CO}_2$ ) and temperature (e°T: ambient +3°C) conditions (same photoperiod 16/8h and relative humidity of 70%). Shoot length, number of leaves, root length, root and shoot fresh and dry weight, number of spores, extraradical hyphae length and intraradical root colonization were estimated.

A short-term dynamic (16 days) study of root colonization of *M. truncatula* plantlets and a long-term dynamic (8 weeks) study of spore production and extraradical mycelium development from *M. truncatula* plantlets grown in a mycelium network were conducted. For the short-term dynamic study, the plants grew significantly as shown by all plant parameters measured, except for the shoot length. No differences were observed between plants exposed to either ambient or e $\text{CO}_2$  and e°T conditions at the different time of observations (i.e. 4, 8, 12 and 16 days after the introduction of the plantlets into the mycelium network), except at day 16 where shoot dry weight of plants exposed to elevated  $\text{CO}_2$  and temperature was significantly higher as compared to the plants in the ambient conditions. The number of spores and hyphal length did not differ at any date between the two climate conditions, except at day 8 where the number of spores was higher in the systems exposed to elevated  $\text{CO}_2$  concentration and temperature as compared to the systems exposed to ambient conditions. Concerning the root colonisation, no difference was observed at any date between the two climate conditions. For the long-term dynamic study, results on spore production and extraradical hyphae length showed no clear tendency between the two climatic conditions. However, at week 2, 6 and 8, hyphal length in the systems exposed to elevated  $\text{CO}_2$  and temperature conditions was higher as compared to the systems exposed to ambient conditions.

hyphale était plus élevée aux semaines 2, 6 et 8 de cette expérience lorsque les cultures se trouvaient en condition de CO<sub>2</sub> et de température élevées.

These results tended to indicate that eCO<sub>2</sub> and e°T could influence:

- (1) AMF soil exploration and resources foraging,

The fact that spores production was not affected by eCO<sub>2</sub> and e°T, but that hyphal length increased, showed that the capacity of AMF to explore and exploit new environments as well as colonize new plants could be higher due to:

- (1) Increased dispersion of spores,
- (2) Higher probability of colonization of surrounding plants.

Three mesocosms studies were conducted.

**Study 1** investigated:

- (1) How climatic change and the presence of AMF affected grassland communities' CO<sub>2</sub> fluxes, above- and belowground biomass, and leaf nitrogen (N) and phosphorus (P) relations,
- (2) Whether interaction effects of climatic factors and presence of AMF were playing a significant role on CO<sub>2</sub> fluxes, biomass and leaf nutrient content in the AMF-plant system.

**Study 2** was performed on a soil collected from a Belgian grassland field taking advantage of the richness of AMF that the soil contained. The main objective of this study was to investigate the effect of climate change on AMF root colonization, communities' CO<sub>2</sub> fluxes, biomass and above-ground N and P relations.

**Study 3** was conducted to determine the influence of an AMF community on carbon and nitrogen allocation productivity and community structure of grassland communities, both under the current climate and a future climate scenario.

For these studies, growth chambers were exposed to ambient air temperature (T<sub>air</sub>) and 380 ppm of CO<sub>2</sub> (Amb), while others were continuously warmed at 3°C above T<sub>air</sub> and exposed to 610 ppm CO<sub>2</sub> concentration (future climate, T+CO<sub>2</sub>). Plant communities were assembled using six perennial species, chosen for their co-occurrence in Belgian grasslands, selected from three functional groups that were equally represented in each community: two grasses (*Poa pratensis* L., *Lolium perenne* L.), two N-fixing dicots (*Medicago lupulina* L., *Lotus corniculatus* L.), and two non-N-fixing dicots (*Rumex acetosa* L., *Plantago lanceolata* L.).

For **study 1**, the soil was pasteurized with two 8-hours cycles at 90 °C and communities were planted in soil that was either (i) pasteurized (non mycorrhizal – NM) or (ii) pasteurized and subsequently inoculated with AMF (AMF). Each inoculated community received 100 g of inoculum containing two AMF taxa, *Gigaspora margarita* and *Glomus intraradices*.

**For study 2**, communities were established in containers filled with un-manipulated soil (natural soil, non-treated with pasteurization) collected from the grassland field.

In **study 3** pasteurized or pasteurized and subsequently inoculated soil was used. The inoculum contained two AMF taxa: *Glomus intraradices* and *Glomus fasciculatum*. At three different times throughout the growing season ecosystems were labeled with <sup>13</sup>C and <sup>15</sup>N. In this way more detailed information on C and N allocation in AMF and the different plant compartments could be obtained.

**Conclusions for study 1:**

- (1) CO<sub>2</sub> fluxes of newly established grassland communities were not significantly affected by AMF in the two climate scenarios considered, although AMF root colonization was higher under combined increased temperatures and CO<sub>2</sub> concentrations,
- (2) Under the future climate scenario gross primary productivity (GPP) was slightly higher, determining increasing root biomass only in the absence of AMF suggesting different strategies of below-ground C allocation in the presence of AMF,
- (3) On the long term the probable allocation of C to the AMF pool instead of to the root biomass observed in this study could affect below-ground processes such as respiration and, consequently, grassland community CO<sub>2</sub> fluxes.

**Conclusions for study 2:**

- (1) Newly established grasslands could benefit from the future climate scenario in terms of total growth,
- (2) Carbon was allocated mainly to above-ground plant parts, whereas roots and AMF did not increase under the future climate scenario. Together with the increased soil respiration ( $R_{soil}$ ) in  $T+CO_2$  as compared to  $Amb$ , this points towards a negative effect on soil C sequestration.

**Conclusions for study 3:**

The results of this study are currently processed. Only above-ground biomass has been analyzed so far.

- (1) Above-ground biomass was positively influenced by the future climate scenario,
- (2) At the end of the growing season AMF had a positive influence on the above-ground biomass under both climate scenarios.

Further conclusions will be formulated beyond the time frame of the project.

**TASK 4. Evaluation of the ecological significance of AMF-plant interactions on above- and below-ground biodiversity and C balance.**

This task was planned for the second phase of the project.

**TASK 5. Modeling of the C-balance processes in grassland ecosystems**

The ANAFORE model (ANALysis of FORest Ecosystems) was modified to simulate grassland ecosystems in order to evaluate the impact of climate and AMF on the C cycle. ANAFORE includes the effects of additional factors affecting growth such as elevated atmospheric CO<sub>2</sub>, fertilization, drought, ozone, and temperature extremes. The data collected during 2007 and 2008 will be implemented into the ANAFORE model in 2009.