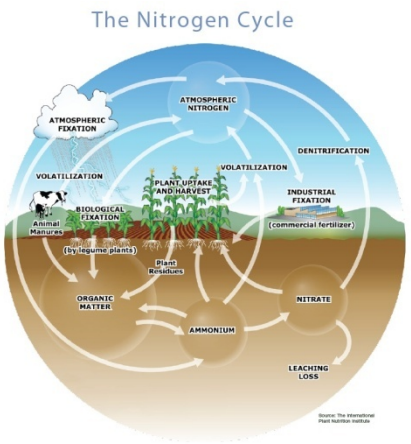
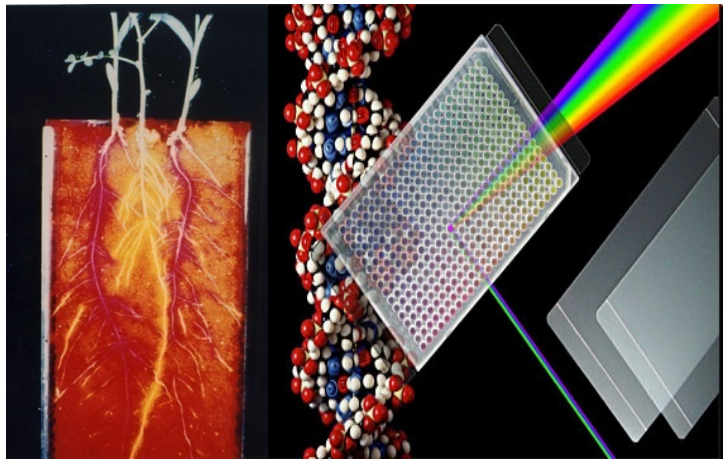
Response of rhizosphere microbial communities to legume inoculation

Katarina Huić Babić, Sanja Sikora, Kristina Kleineidam, Michael Schloter





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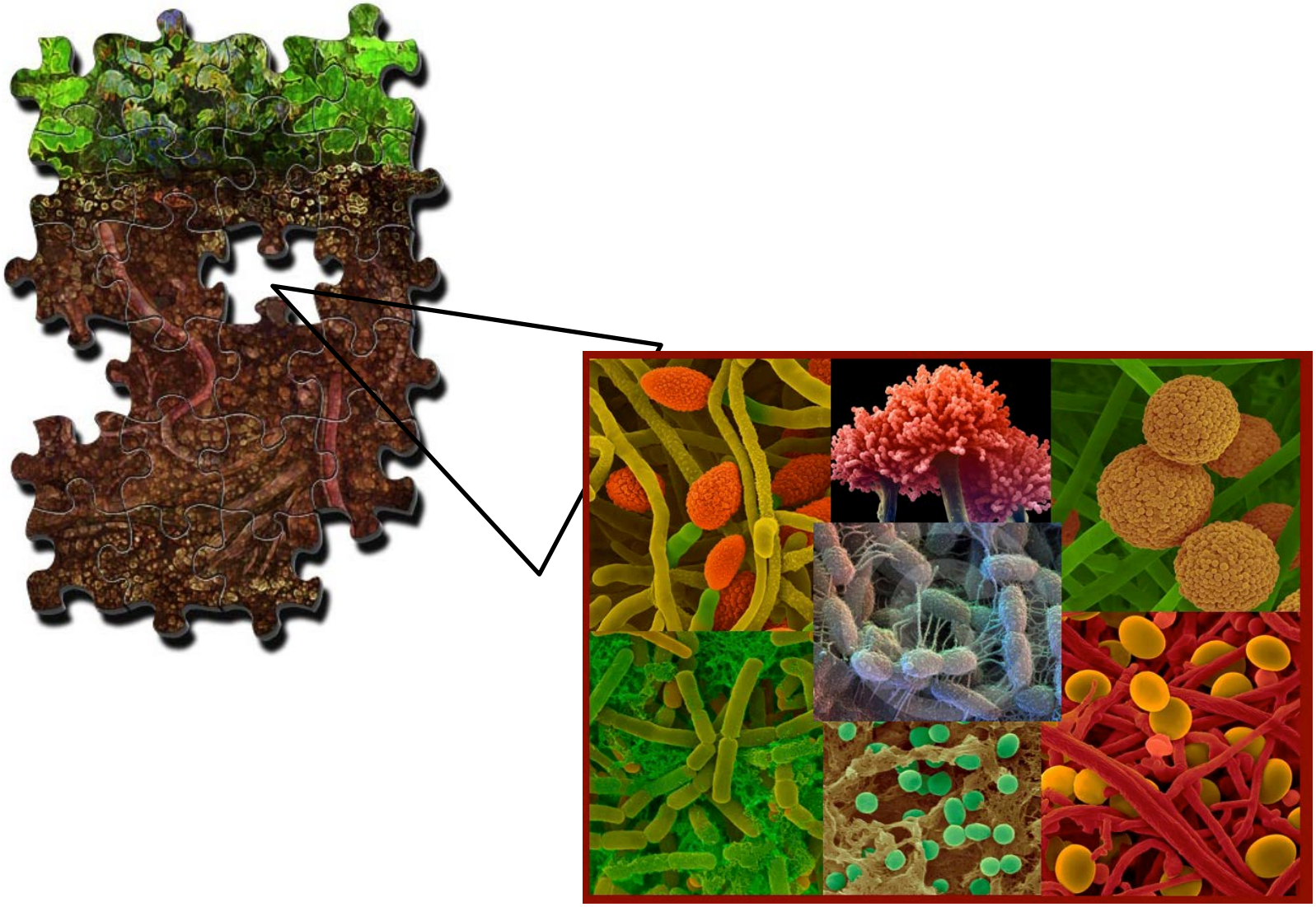


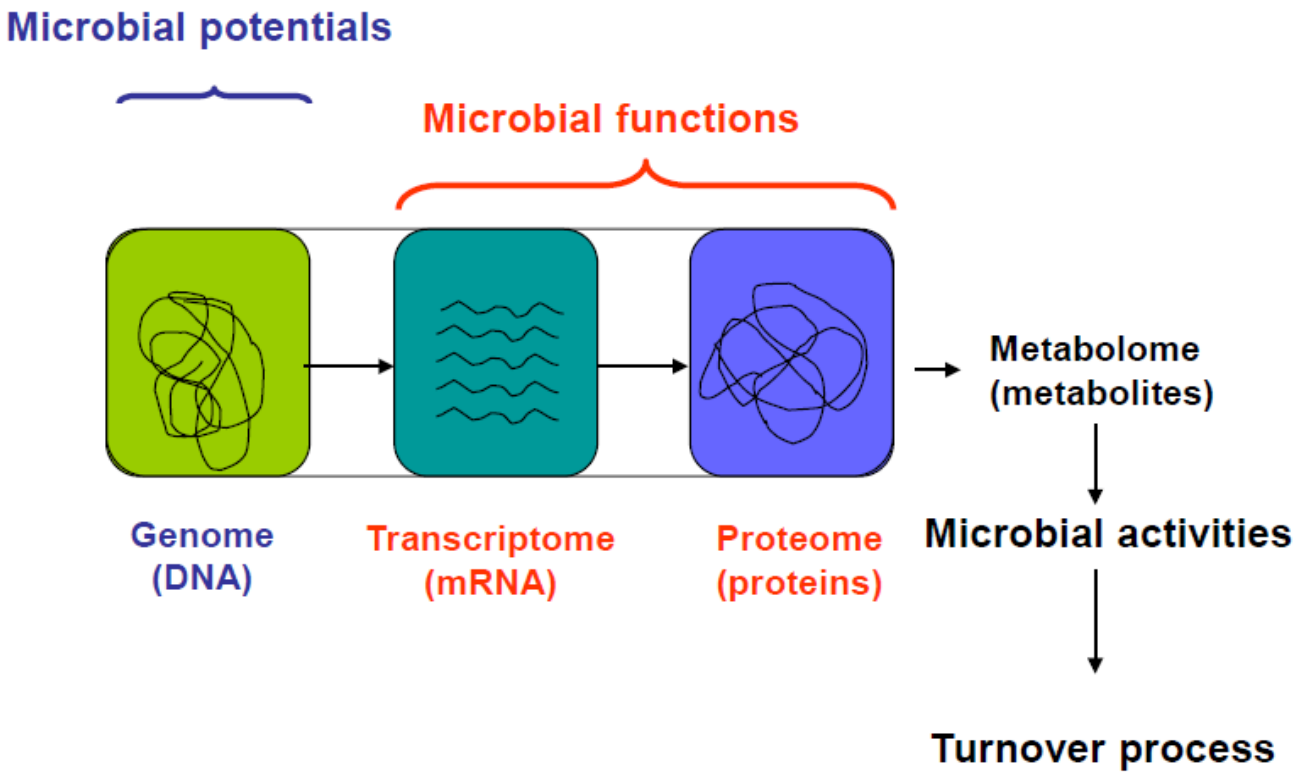
Microbes in soil

Only 1% of microbes can be easily isolated

To describe microbial communities in soils – the biggest challenge of 21st century

One gram of soil harbors more than 100 000 different species

From potentials to functions

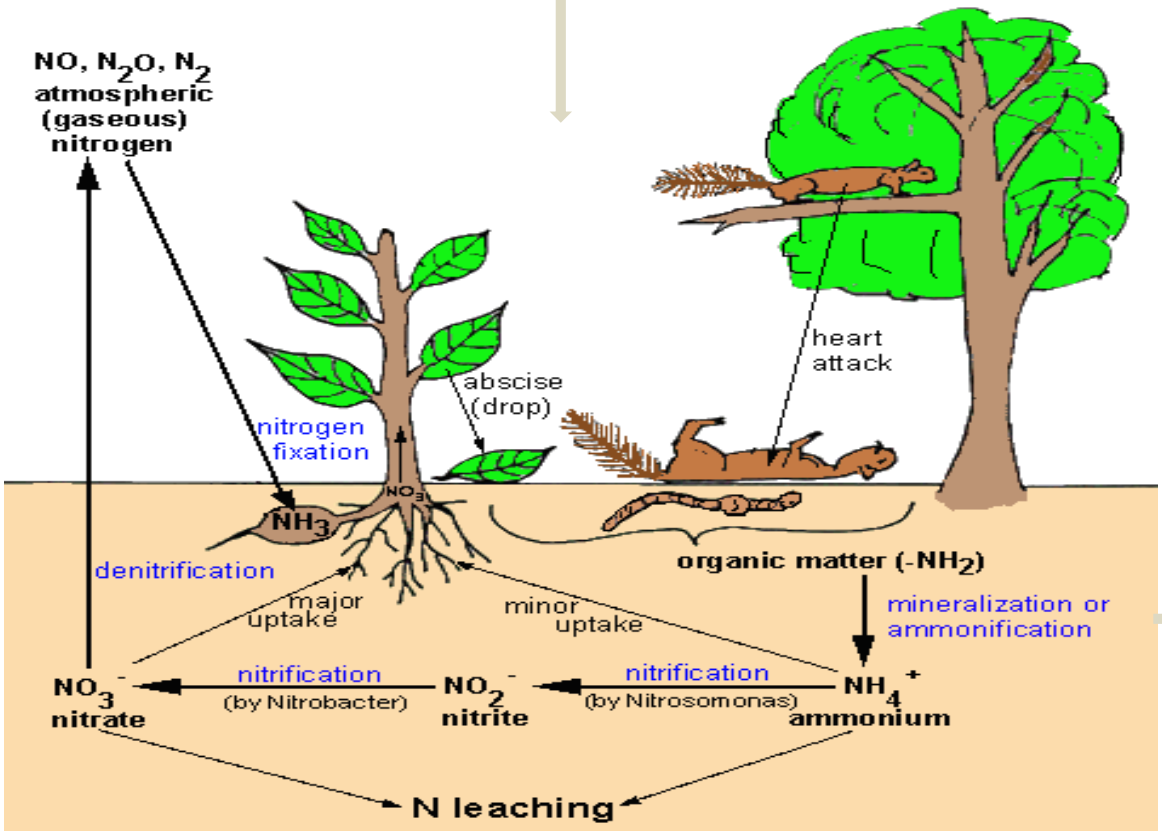


Schloter et al., (2008) BS

Nitrogen cycle

-responsible N use in agriculture is key to sustainability
-economic and environmental considerations dictate that we must exploit biological alternatives
-proportion of biologically fixed N can be significantly enhanced by legume inoculation with efficient rhizobial strains

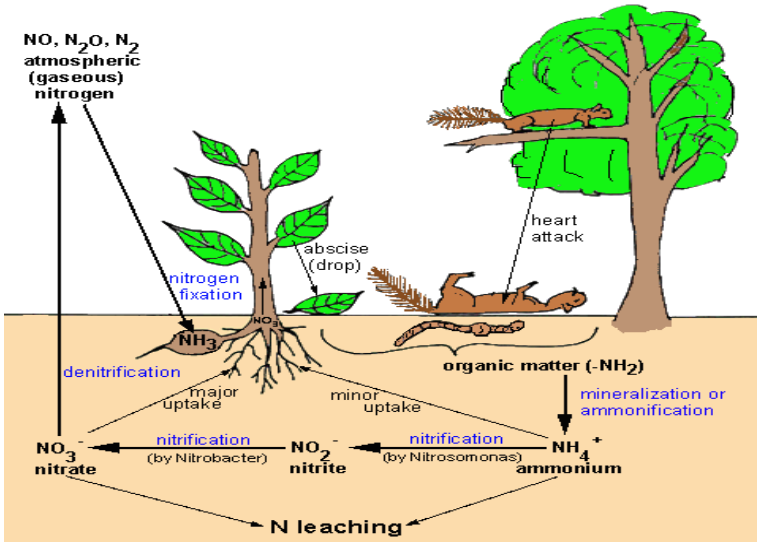
Fertilization
Plant exudates
Deposition



- improve this ratio
- understand the N cycle processes

55% Plant uptake
16% Leaching
15% Soil erosion
14% Gaseous emission

The effect of *Sinorhizobium meliloti* efficiency on nitrogen transformations in soil and plant




Rhizobial strains
Propotion of biologically fixed N
Challenge
High competition in the soil
Plant

Objective

- to evaluate the influence of inoculation with different indigenous *S. meliloti* strains on N cycle processes in the rhizosphere of alfalfa and on plant growth promotion, as well as their competitive abilities in the soil

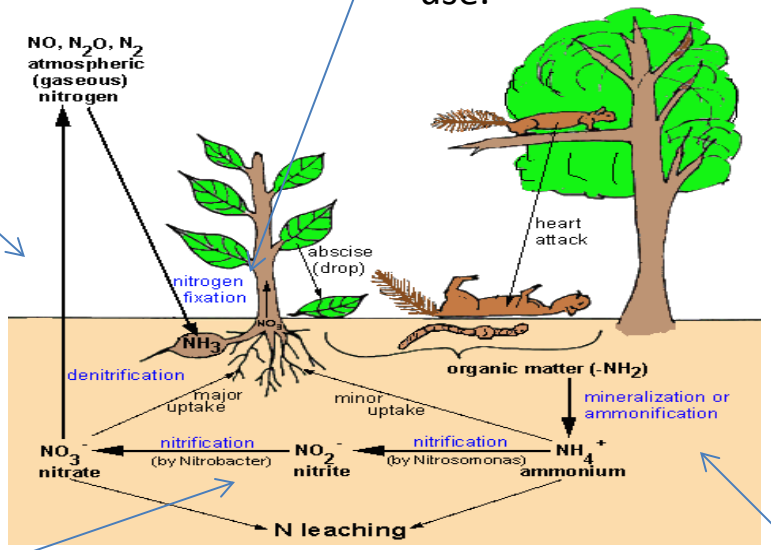
- a pot experiment was carried out under controlled conditions using two factors *(3 x 3)* on the basis of a completely randomized block design with four replications

- the factors in the experiment were :
    1. indigenous *S. meliloti* strains exhibiting different efficiency
    2. three different alfalfa development stages

3	2	8	6	5	1	7	4	9
6	4	1	9	8	3	7	2	5
4	8	6	3	9	2	1	5	7
9	7	4	6	3	5	1	8	2

Denitrification
Nitrates are reduced to nitrogen gas, returning nitrogen to the air and completing the cycle.

Nitrogen fixation
The first step in the synthesis of virtually all nitrogenous compounds.
Nitrogen gas is fixed into forms other organisms can use.



Nitrification
Nitrification is a two-step process. Ammonia or ammonium ions are oxidized first to nitrites and then to nitrates, which is the form most usable by plants.

Ammonification
The decomposers, certain soil bacteria and fungi, break down proteins in dead organisms and animal wastes releasing ammonium ions which can be converted to other nitrogen compounds.

Rhizosphere Samples (roots + adhering soil)

↓

DNAextraction

Griffiths et al., 2000

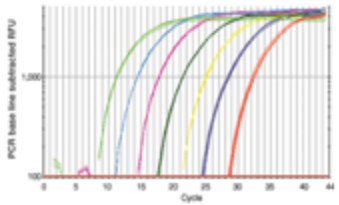
↓

DNA

↓

Quantification of functional genes involved in nitrogen cycling using real-time PCR

↓



- **Nitrogen fixation**
  nitrogenase reduction
  nifH
- **Nitrification**
  ammonia oxidation
  amoA (AOB + AOA)
- **Denitrification**
  nitrite reduction
  nirS + *nirK*

  nitrous oxide reduction
  nosZ

- except…

functional genes encoding the enzymes catalyzing processes in the nitrogen cycle (nitrification, denitrification and nitrogen fixation) in the rhizosphere

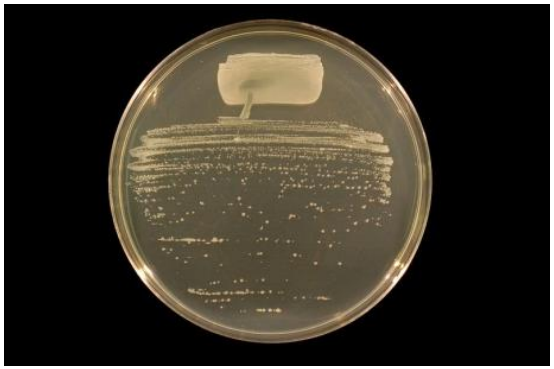
For each sampling the following traits were measured:

- NH_4^+ and NO_3^- conc. in rhizosphere
- N and C content – plant
- Chlorophyll content in plant
- dry matter and green mass yield -plant
- N/C content – microbial biomass
- Nmin – soil
- N/C content – soil (DON/DOC)

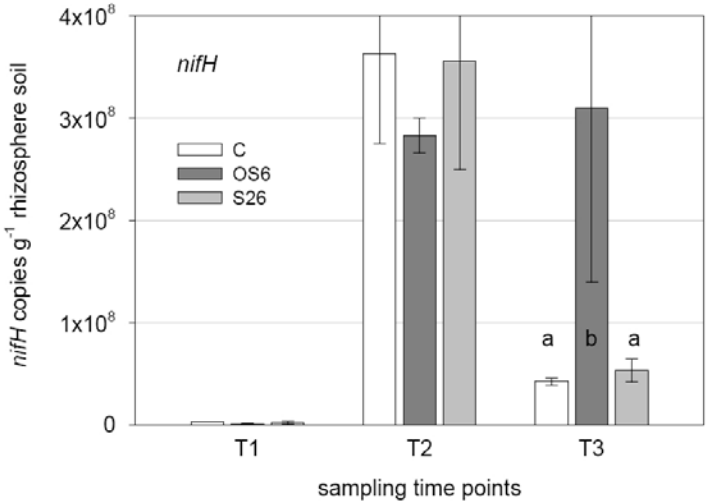
- except…

functional genes encoding the enzymes catalyzing processes in the nitrogen cycle (nitrification, denitrification and nitrogen fixation) in the rhizosphere

- the ability of both *S. meliloti* strains used for nodulation to compete with indigenous population in the soil was determined by checking their presence in the nodules of alfalfa



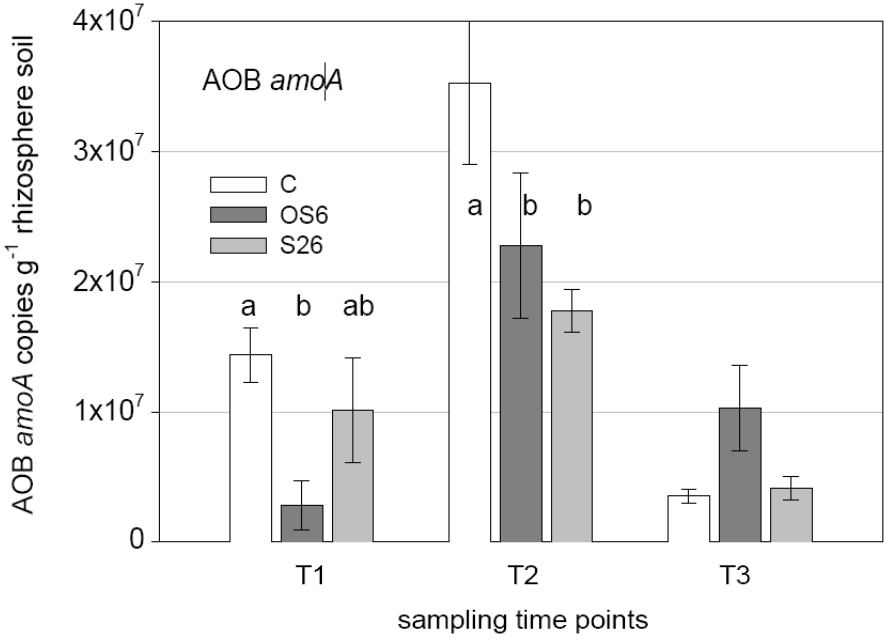
Gene abundances of nitrogen fixers

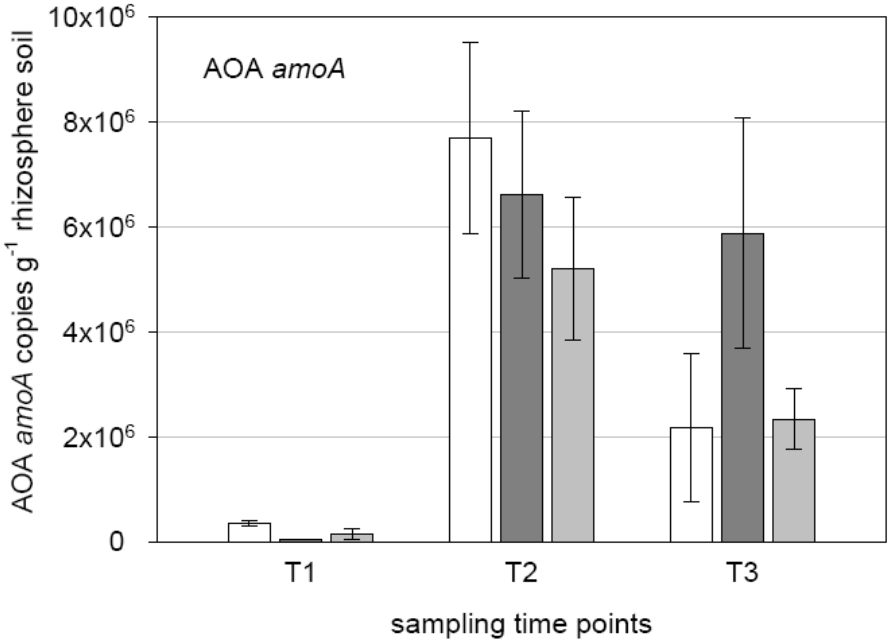


- abundance of *nifH* genes increased in all treatments towards T2 and amounted up to 3.7 ¥ 108 copies g-1 dw soil

- At T3, the effect of the more efficient OS6 strain was very prominent, revealing a significantly higher abundance of *nifH* genes in the rhizosphere compared with S26 and C

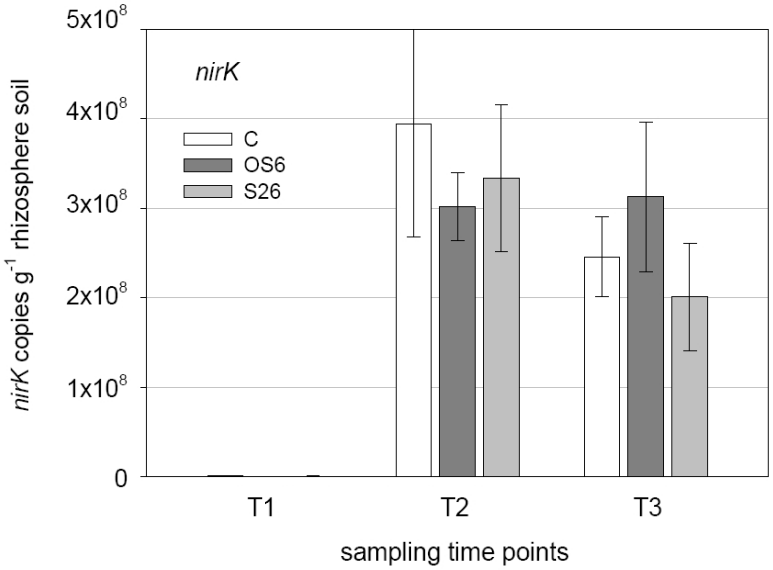
Gene abundances of ammonia oxidizers (AOA, AOB)

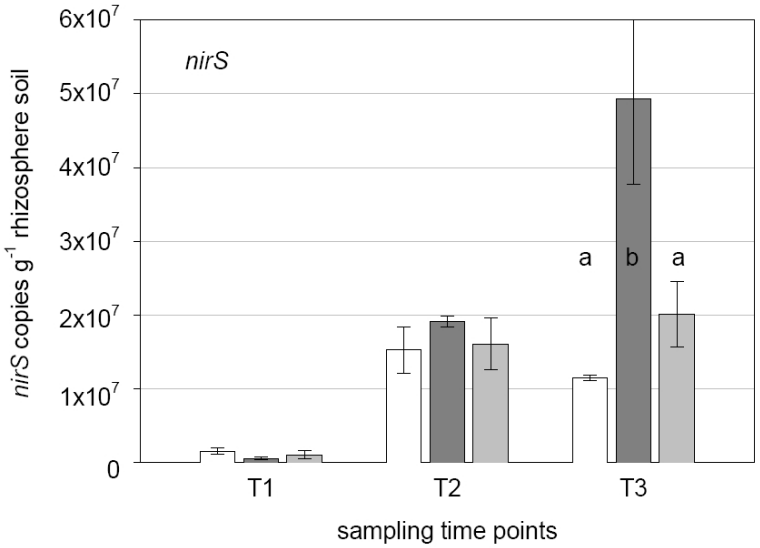




- oxidation of the ammonia to nitrite is obviously mainly performed due to bacterial activity
- in T3 the maximum values were obtained from treatments inoculated with strain *S. meliloti* OS6

Gene abundances of nitrite reducers

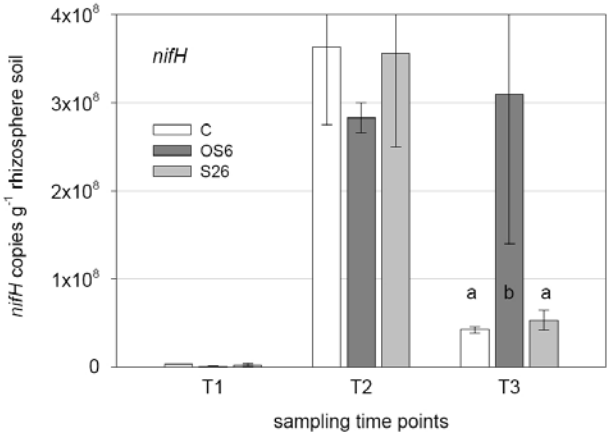


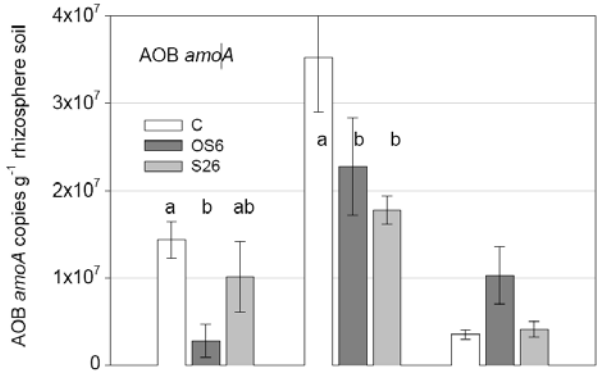


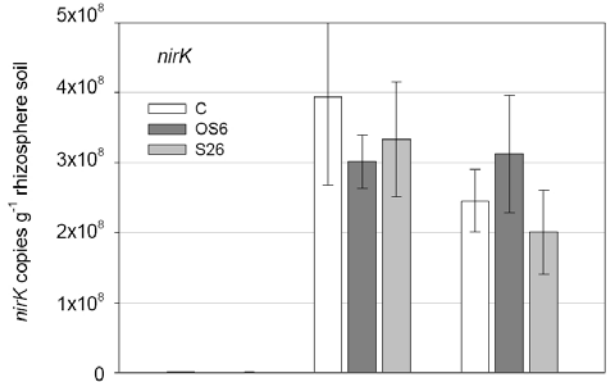
- the *nirK* copy numbers dominated over *nirS* by factor 10 in all treatments at T2 and T3, except treatment OS6 at the late flowering stage

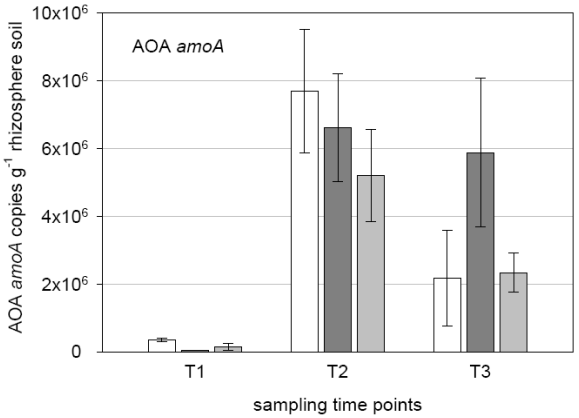
Green mass yield –plant ($m_{6plants}$ / g)

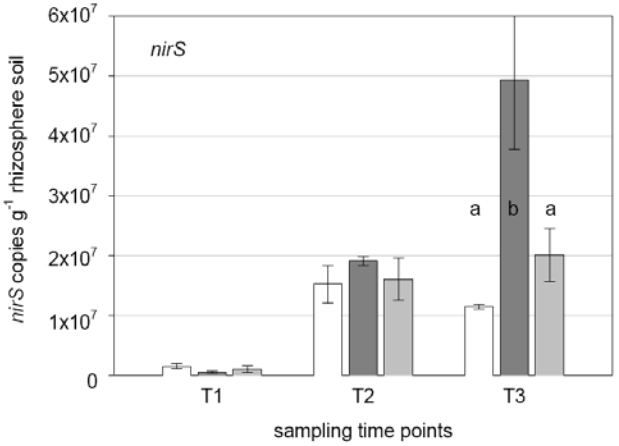
	C	OS6	S26	x
T1	0.9375 d	1.202 d	1.085 d	**1.075 c**
T2	13.67 c	14.65 c	13.78 c	**14.03 b**
T3	42.67 b	56.43 a	43.76 b	**47.62 a**
x	**19.09 b**	**24.09 a**	**19.54 b**	


nifH
C
OS6
S26
a
b
a
T1
T2
T3
sampling time points
nifH copies g-1 rhizosphere soil
4x10^8
3x10^8
2x10^8
1x10^8
0

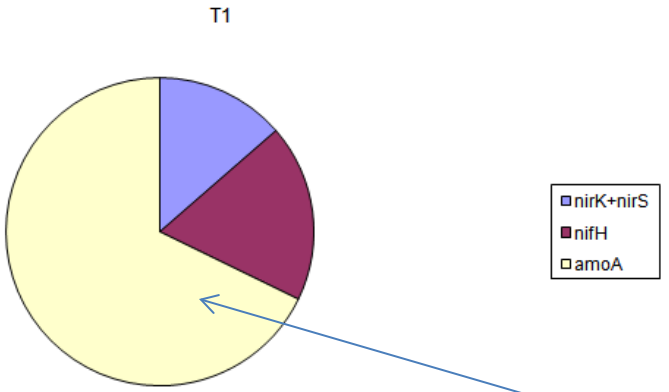

AOB amoA
C
OS6
S26
a
b
ab
a
b
b
AOB amoA copies g-1 rhizosphere soil
4x10^7
3x10^7
2x10^7
1x10^7
0


nirK
C
OS6
S26
nirK copies g-1 rhizosphere soil
5x10^8
4x10^8
3x10^8
2x10^8
1x10^8
0


AOA amoA
AOA amoA copies g-1 rhizosphere soil
10x10^6
8x10^6
6x10^6
4x10^6
2x10^6
0
T1
T2
T3
sampling time points


nirS
a
b
a
nirS copies g-1 rhizosphere soil
6x10^7
5x10^7
4x10^7
3x10^7
2x10^7
1x10^7
0
T1
T2
T3
sampling time points

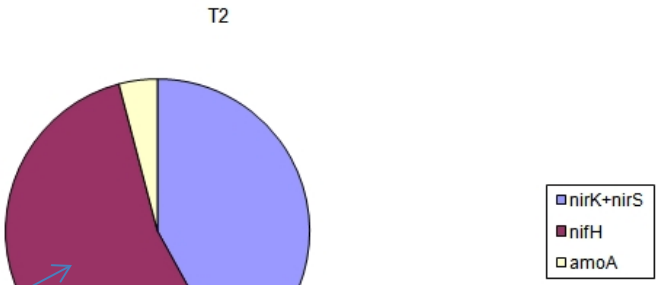
Proportion of microbial populations involved in the N cycle



Nitrification

Autorophic process

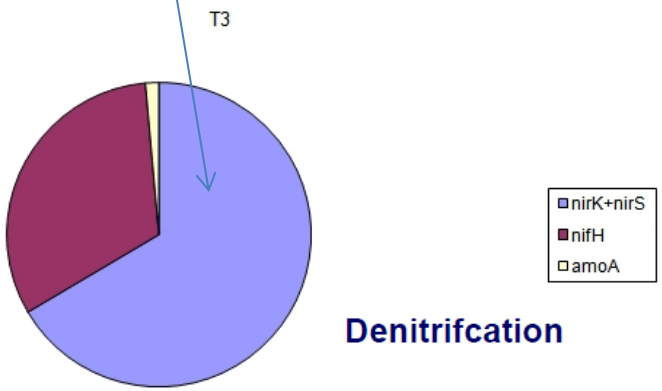
Low number of functional guilds (AOA and AOB)



N – fixation

Anoxic conditions; mostly in close assotiation with plants (requires large amounts of energy)

Comparable low number of functional guilds, but well distrubuted among procaryotes



Denitrifcation

Redox process

High number of functional guilds, well distributed amongst bacteria and (archaea)

Paper was published in Environmental Microbiology……..

Environmental Microbiology (2008) 10(11), 2922–2930 doi:10.1111/j.1462-2920.2008.01782.x

Influence of different *Sinorhizobium meliloti* inocula on abundance of genes involved in nitrogen transformations in the rhizosphere of alfalfa (*Medicago sativa* L.)

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Summary

Inoculation of leguminous seeds with selected rhizobial strains is practised in agriculture to ameliorate the plant yield by enhanced root nodulation and nitrogen uptake of the plant. However, effective symbiosis between legumes and rhizobia does not only depend on the capacity of nitrogen fixation but also on the entire nitrogen turnover in the rhizosphere. We investigated the influence of seed inoculation with two indigenous *Sinorhizobium meliloti* strains exhibiting different efficiency concerning plant growth promotion on nitrogen turnover processes in the rhizosphere during the growth of alfalfa. Quantification of six target genes (bacterial *amoA*, *nirK*, *nirS*, *nosZ*, *nifH* and archaeal *amoA*) within the nitrogen cycle was performed in rhizosphere samples before nodule formation, at bud development and at the late flowering stage. The results clearly demonstrated that effectiveness of rhizobial inocula is related to abundance of *nifH* genes in the late flowering phase of alfalfa. Moreover, other genes involved in nitrogen turnover had been affected by the inocula, e.g. higher numbers of *amoA* copies were observed during flowering when the more effective strain had been inoculated. However, the respective gene abundances differed overall to a greater extent between the three plant development stages than between the inoculation variants.

Received 31 May 2008; accepted 13 August, 2008. *For correspondence. E-mail khulic@agr.hr; Tel. (+385) 12 394 034; Fax (+385) 12 393 881.

Introduction

In sustainable agriculture, biological N_2 fixation is an important pathway of nitrogen input into agricultural soils besides the application of organic and mineral fertilizers (Sharma *et al.*, 2005a; Rosenblueth and Martínez-Romero, 2006). In this respect, cultivation of legumes is of great importance due to the symbiosis with nitrogen-fixing bacteria. The perennial legume *Medicago sativa* (alfalfa), a major herbal protein source for livestock, is frequently grown in ecologically based agriculture (Bradić *et al.*, 2003) being able to fix up to 180 kg N ha^{-1} per season (Vance, 1998). Moreover, cultivation of alfalfa may improve the soil quality by increasing the organic matter content, porosity, structure, water holding capacity, recycling nutrients, and thus preventing soil erosion, leaching of nutrients and breaking disease build-up and weed problems of grass-type crops (Bruulsema and Christie, 1987; Campbel *et al.*, 1994; Ominski *et al.*, 1994; Entz *et al.*, 1995; Sharma *et al.*, 2005a). The soil bacterium *Sinorhizobium meliloti* (also known as *Ensifer meliloti*) is able to live in symbiosis with alfalfa in its intracellular space forming root nodules (van Rhijn and Vanderleyden, 1995; Gordon *et al.*, 2003).

Inoculation of legume crops with rhizobia has been widely used in agricultural systems to improve legume productivity in the field. However, commercially available rhizobial inoculants often fail to become established in soils with indigenous rhizobial populations (Hartmann *et al.*, 1998). In this respect, the selection of more effective and highly competitive *S. meliloti* strains for alfalfa inoculation is necessary (Sikora *et al.*, 1997). It is generally accepted that indigenous populations are better adapted to their environment and thus capable of forming a more effective symbiosis than commercial inoculants isolated from a distant or unrelated soil type (Gandee *et al.*, 1999; Bradić *et al.*, 2003). Bradić and colleagues (2003), for example, investigated the symbiotic effectiveness of 12 indigenous Croatian *S. meliloti* strains, i.e. their ability to improve the N supply of the plant and hence to

Conclusions

- According to our hypothesis, the inoculation of alfalfa seeds with different *S. meliloti* strains altered not only the abundance of *nifH* genes in the rhizosphere, but also the abundance of further functional genes of microbes involved in nitrogen cycling

- Similarly, the positive effect of inoculation with a highly effective strain was confirmed in most of the investigated parameters related to the plant

- The plant development stages had, as aspected, a great impact on the gene abundance patterns as well as on the studied parameters of the plant itself

Conclusions

- **increasing awareness of the importance of soil microbiology in agriculture will assure that microbiological parameters become a vital component of future soil analysis and diagnosis**


Thanks for your attention
Vitamin
Minerals
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+ ion
- ion
DNA
PESTCID