The role of red clover—a forage legume, in mitigating nitrous oxide (N_2O) emissions from a perennial grassland

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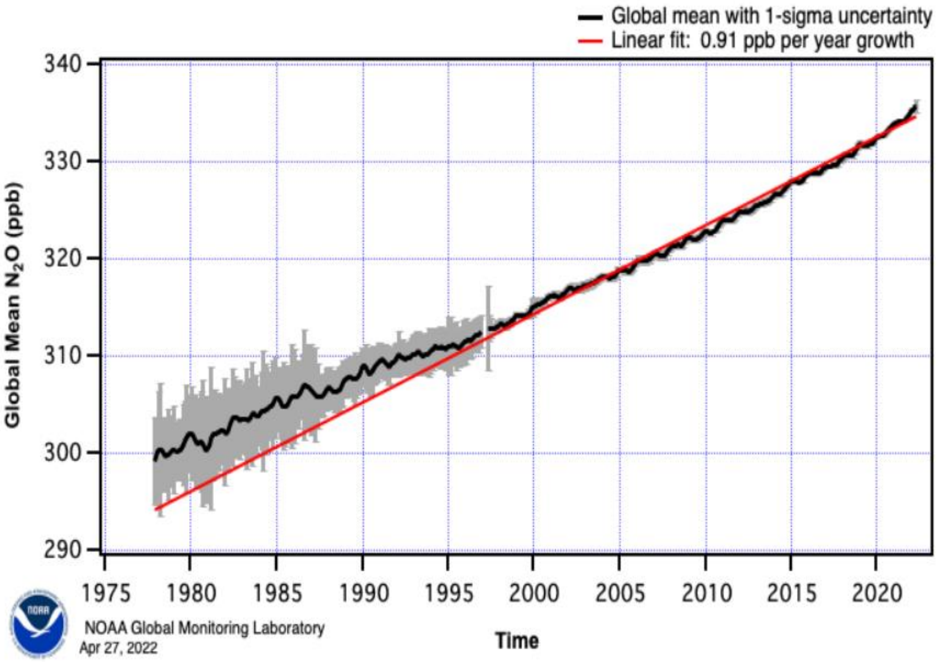
Background—Soil as a N_2O sink, it’s importance and mechanism

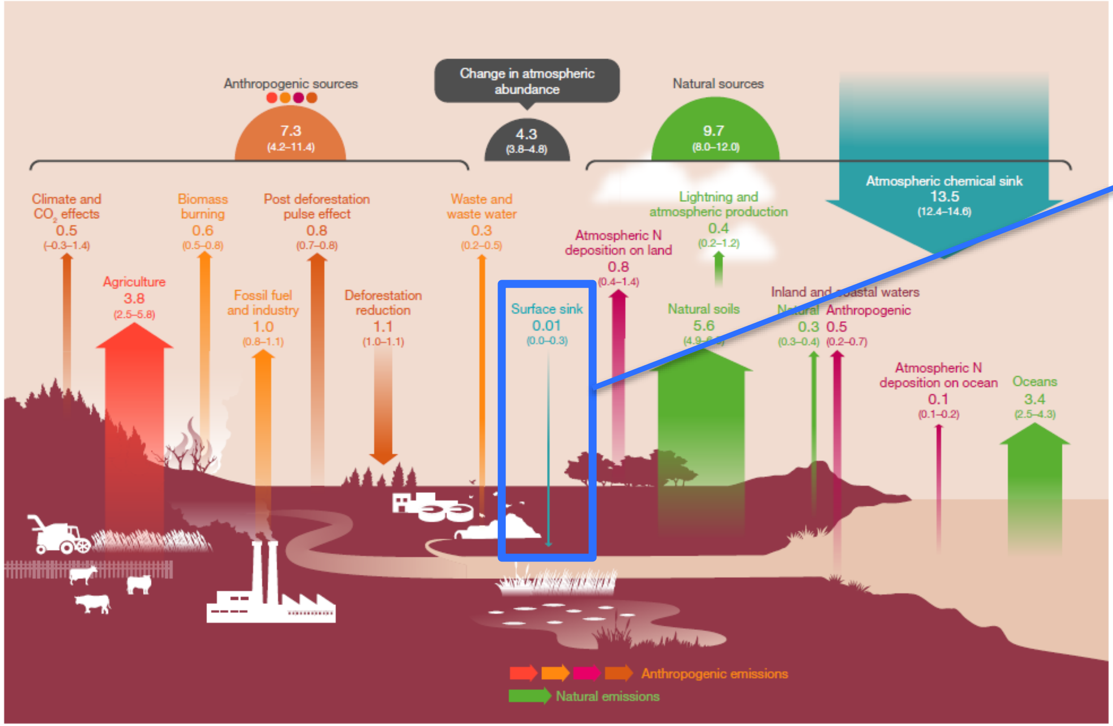


https://gml.noaa.gov/hats/combined/N2O.html

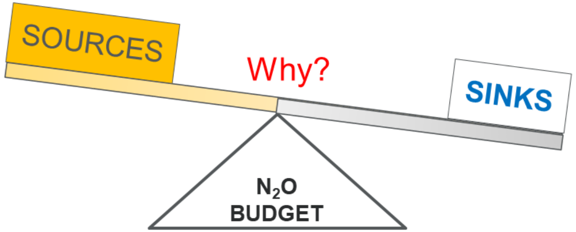
N_2O concentration is steadily increasing indicating a continous growth in emission strength

Background—Soil as a N_2O sink, it's importance and mechanism

Tian et al., 2020, *Nature*



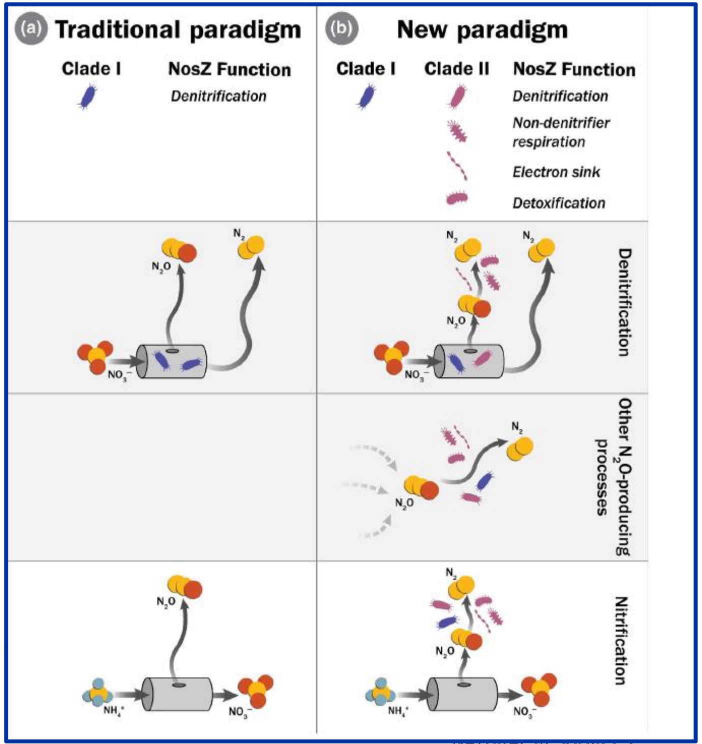
Beside a source, terrestrial surfaces act also as a sink of N_2O.



Is sink really this weak?

Background—Soil as a N_2O sink, it's importance and mechanism

Shan et al., 2021, *Golb Change Biol.*



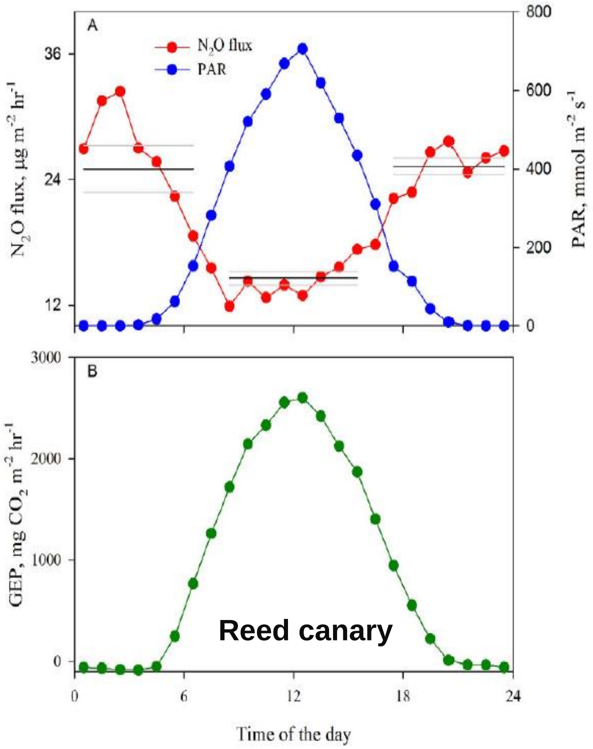
Reasons could be,

1. Terrestrial sinks are weak.
2. Research focus has remained mainly towards emissions
3. Flux measurement uncertainities and doubts
   - *observed negative fluxes are discarded*
4. Soil complexity and it's heterogenity: negative N_2O fluxes has been observed in soils with,
   - *Both, high and low N content*
   - *Both, very high (> 80% WFPS) and very low (~5% WFPS) moisture*
   - *Both, plus and minus temperatures*
   - *Both, with and without plant cover*

Higher functional diveristy of N_2O reducers; microbes that contain nitrous oxide reductase enzyme (nosZ gene)—the only biological mechanism known in the biosphere to contribute to the soil N_2O sink.

Project ENSINK: motivations behind it

Shurpali et al., 2016, *Sci. Rep.*



Motivation 1: Dirunal pattern of N_2O emissions at ecosystem scale with low N_2O emissions when there is high CO_2 uptake.

Motivation 2: In 2017, we observed a clear and sustained N_2O uptake when the site was under legume-grass cultivation. N_2O uptake occured mainly during day time when fertilization effect was diminshed. (unpublished yet)

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ENSINK—Mechanisms for N_2O uptake in cropping systems in different climate zones

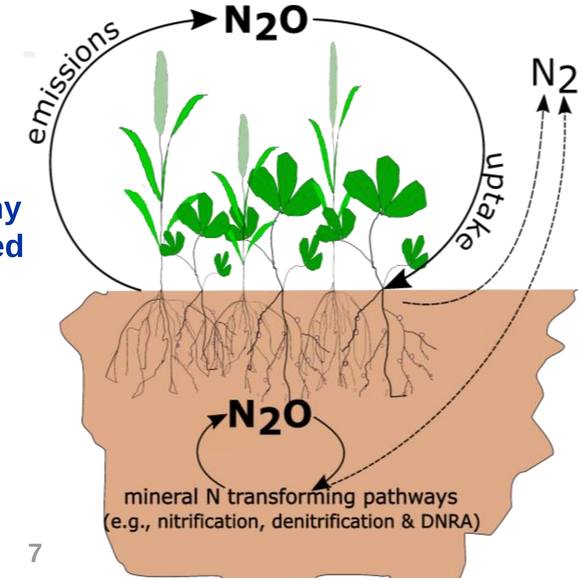
Main Hypothesis
How the above and below ground microbial processes drive atmospheric N_2O exchange in legume based agricultural ecosystems of diverse climatic zones?

Project ENSINK: our current focus

We seek answers for Who, Where, When and Why.

A legume grassland on a mineral soil—ecosystem of interest becuase legumes serve as a source of N to an ecosystem via biological N fixation, thus reduces the demand of synthetic N for biomass production. So, it could be an additional benefit if they can support the reduction of N_2O.

Timothy and Red clover



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NOTES

Nitrous Oxide Reduction in Nodules: Denitrification or N_2 Fixation?

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Published: 11 November 2012

Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation

Manabu Itakura, Yoshitaka Uchida, Hiroko Akiyama, Yuko Takada Hoshino, Yumi Shimomura, Sho Morimoto, Kanako Tago, Yong Wang, Chihiro Hayakawa, Yusuke Uetake, Cristina Sánchez, Shima Eda, Masahito Hayatsu & Kiwamu Minamisawa

Nature Climate Change **3**, 208–212 (2013) | Cite this article

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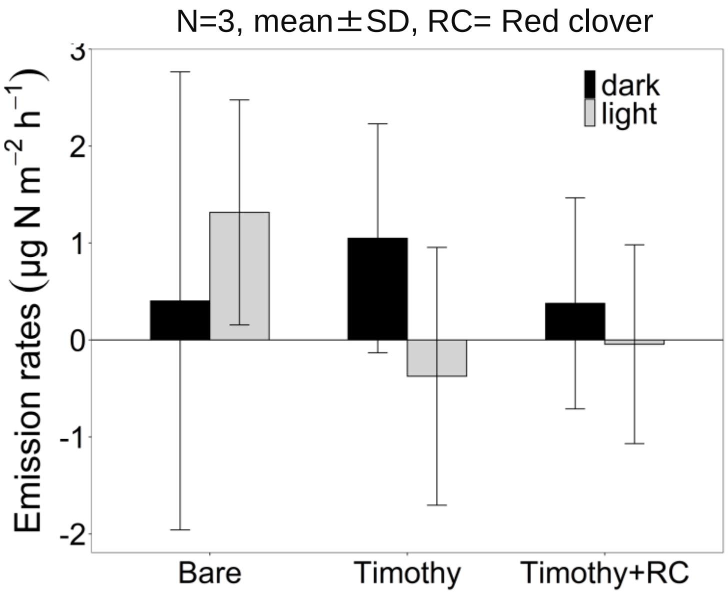
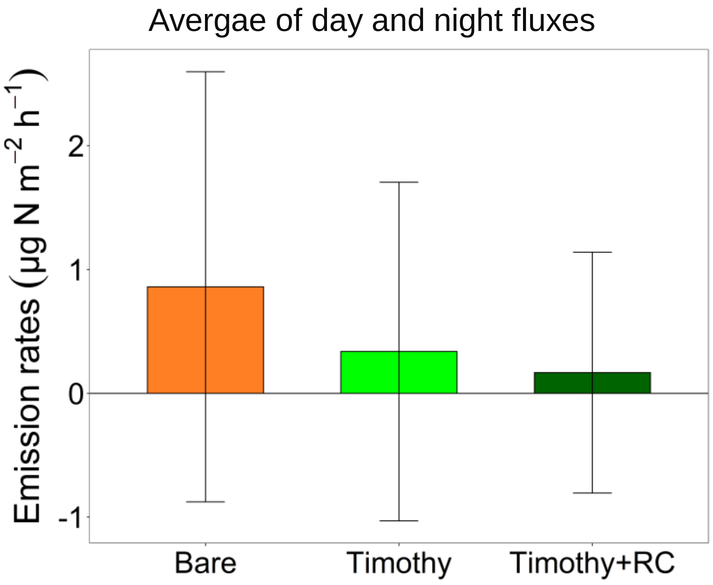
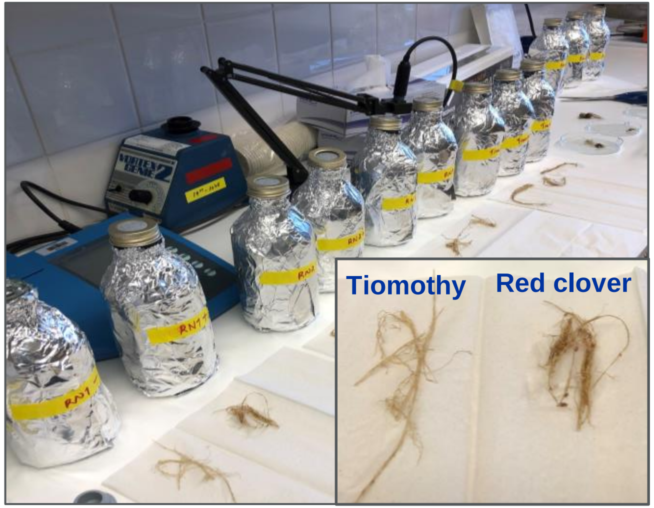
ENSINK's preliminary data : N_2O flux from mesocosms



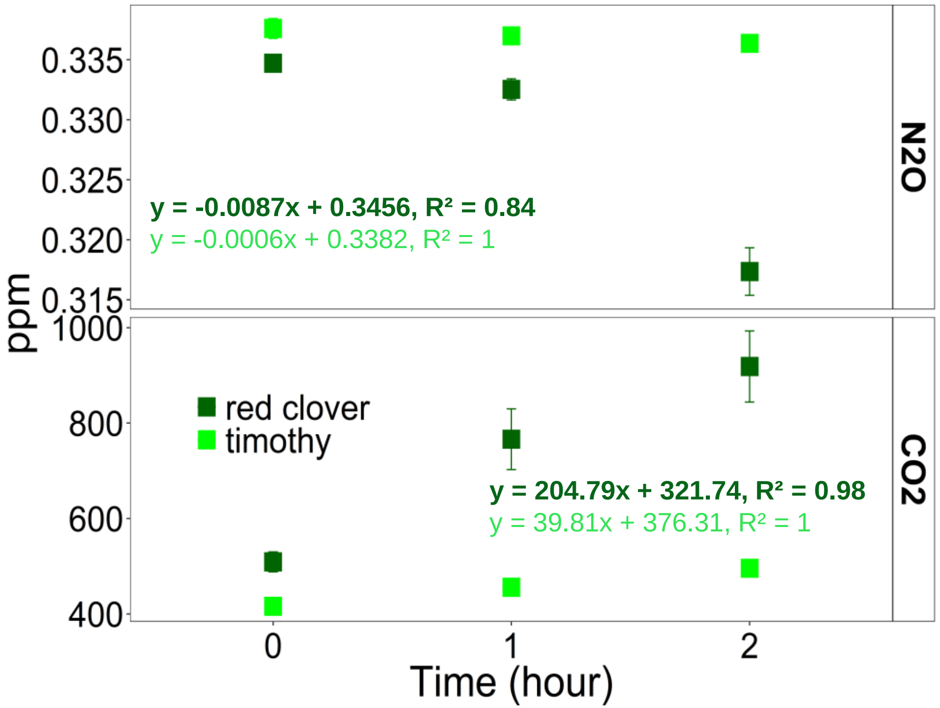


- **Soil**: mineral, pH: 6.4,
- **Mesocosms**: 58 days old greenhouse grown. No N fertilization. 45% to 55% WFPS and BD: 1.0 g cm^{-3}
- **Flux measurement**: inside a temperature controlled growth chamber using static chamber technique

ENSINK's preliminary data : assessment of roots from mesocosms



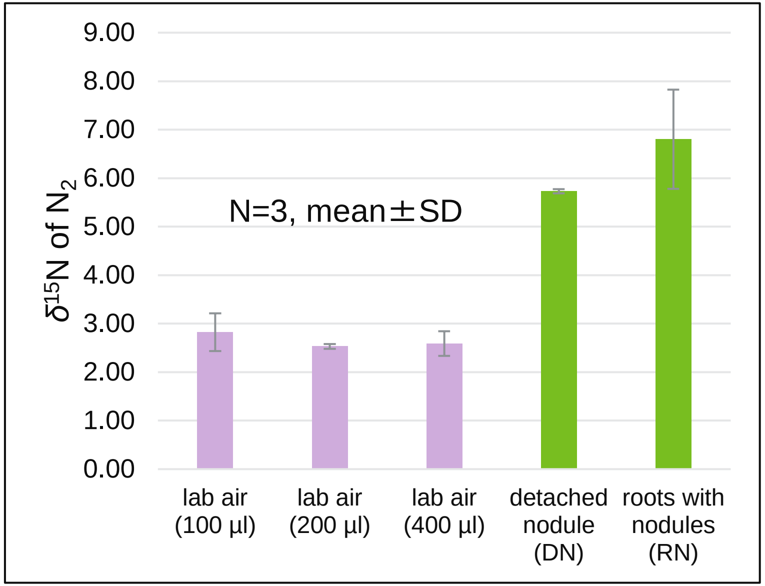
- Roots samples were collected destructively from flux measured mesocosms
- Tap water washed fresh roots (~2-3g) were incubated in 500 ml incubation bottles for 2 hours under ambient conditions.
- 25 ml of headspace gas samples were taken with sryinges to measure ghg concentrations



ENSINK's preliminary data : assessment of roots from field

- Sample collected in August 2021.
- Water washed detached nodules and roots with nodules (~0.1 g fw) were transferred into 12 ml Exetainer vials and flushed with pure He for 10 mins to make anoxic headsapce.
- 416 ppb $^{15}N_2O$ (98 atom%) in the headspace
- Exetainer vials were then directly measured (without gas subsampling) for $^{15}N_2$ within 52 hours after injecting $^{15}N_2O$ with isoprime100.

Raw data from isoprime



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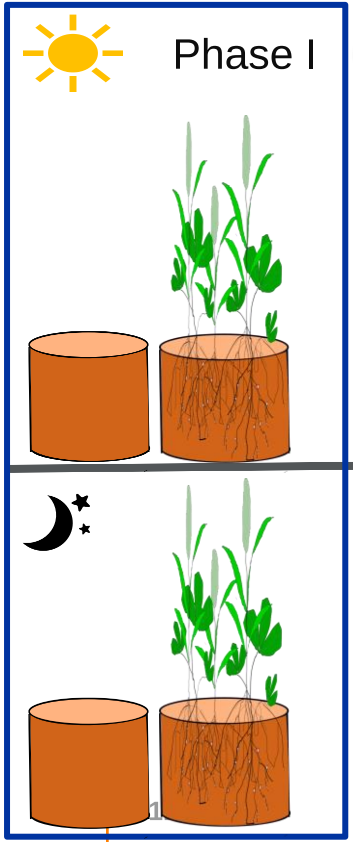
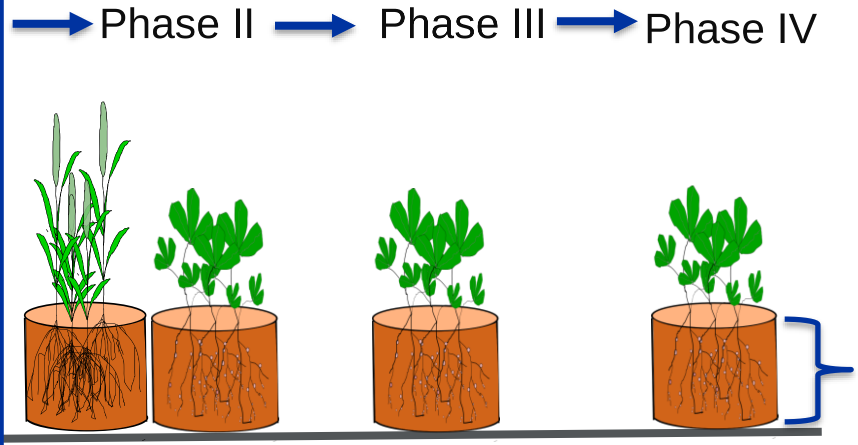
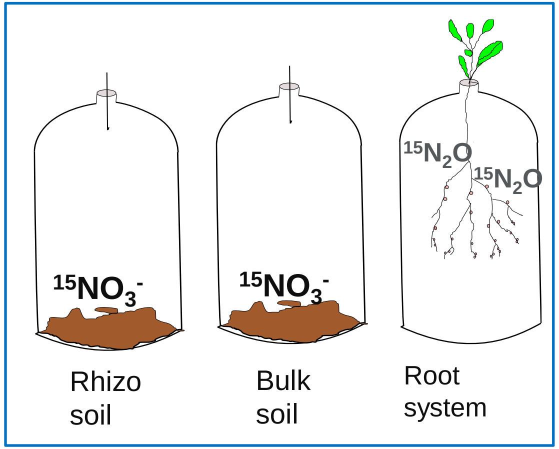
Ongoing ENSINK's work and future plans: a mesocosm and field study





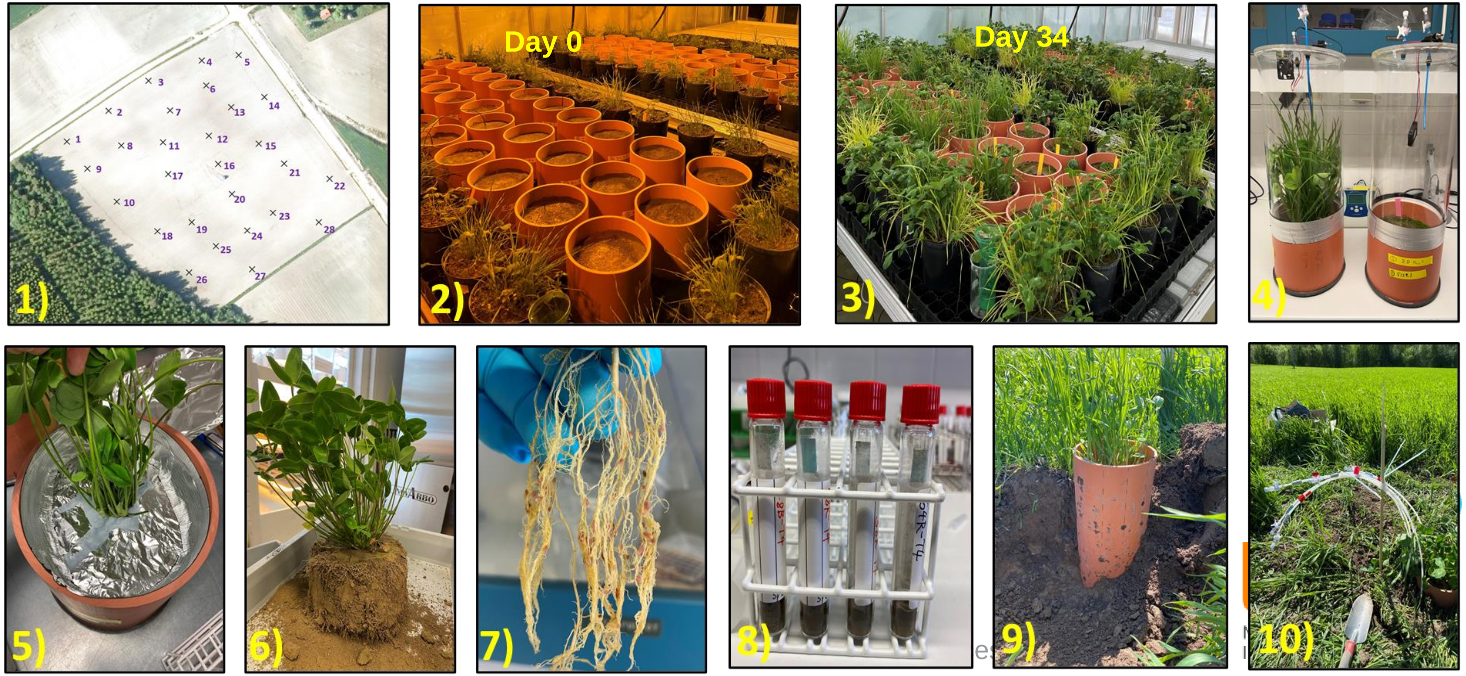


	Hypothesis w.r.t N_2O uptake rates
Phase I	Legume grass cover > Bare surfaces; Day > night
Phase II	Red clover > Timothy
Phase III	Belowground > aboveground
Phase IV	Nodules > Rhizo soil > Bulk soil

Method: $^{15}N_2$OPD (15 at%) and ^{15}N tracer (98 at%)
Gas collection intervals: 1h, 2h and 3h
Measured gases: All ghg, $^{15}N_2O$, and $^{15}N_2$
Soil analyses: pH, EC, mineral N, microbial C and N, DOC, gross N transformation rates

Ongoing ENSINK's work and future plans: study in practice

1) study site, **2-3)** greenhouse grown mesocosms, **4)** $^{15}N_2OPD$ setup, **5)** a mesocosm with separated above and below-ground red clover's compartments, **6)** intact red clover removed from a mesocosm, **7)** sterilized red clover's root and nodules (tiny pink structures) **8)** extenainer vials with bulk and rhizospheric soil from diffferent treatments ammended with $^{15}NO_3^-$ tracer in anoxia **9)** 40cm PVC core installed in the field and **10)** teflon tubes emerging from five different soil depths for N_2O gas collection.



Plans ahead

1. **Processing ^{15}N gas flux raw data for gross rate calculation.**
2. **Nucleic acid extractions, qPCR and sequencing**
3. **Manuscript writing**

Expected outcomes from the ENSINK

- By conducting experiments at laboratory and *in-situ* conditions using stable isotope ($^{15}N_2$OPD and ^{15}N tarcer) and soil microbiology tenchinques, we will better understand whether a red clover based grassland ecosystem can support/improve soil biogeochemistry associated with N_2O reduction/uptake or not.

- Further, our understanding about *processes and drivers* **(Who, e.g., clade I or Clade II),** *environmental and soil conditions* (**When and where**) and *ecological benefits* (**Why**) associated with the N_2O producers and reducers in a red clover based grassland ecosystem will improve.

Thank you!

Acknowledgement

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Ministry of Agriculture and Forestry of Finland

Luke's field and lab personnel

Luke

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