Implementing metabarcoding of soil microbial communities into a long-term soil monitoring network

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NABO – Swiss Soil Monitoring Network

Since 1985, the Swiss Soil Monitoring Network (NABO) assesses soil quality in Switzerland. NABO operates 111 long-term monitoring sites and, for many years, focussed on chemical parameters, e.g. pollutants. However, soil quality assessments should consider also soil biological and physical parameters (Fig. 2). Microorganisms are essential for many soil functions, such as cycling of nutrients or degradation of pollutants. Since 2012, biological parameters are analysed yearly for a sub-set of the NABO sites. The monitoring includes both 'classical' microbiological parameters (biomass, respiration) and microbial diversity.

Soil Sampling and measured parameters

Every spring, soil samples are collected at 30 NABO-sites (10 arable, 10 grassland and 10 forest sites) (Fig. 1). Three composite samples of 25 cores (0-20 cm) are taken from 10x10 m plots at each site. Measurements are soil microbial biomass, basal respiration, DNA quantity, bacterial and fungal diversities, pH, organic carbon, C/N ratio.

Specific requirements for long-term monitoring

Standardization, repeatability and a reasonable cost-benefit ratio of applied methods are crucial for long-term monitoring programs. Used methods must show temporal stability. And knowledge about the variability over the years (induced by analytical variance as well as environmental variability) is needed for correct interpretation (Fig. 2). Therefore, metabarcoding was evaluated by analysing 450 samples from 30 sites of the years 2012 - 2016.

Community structure assessment

16S V3/V4 and ITS2 were sequenced to determine soil bacterial and fungal community structures. Sequences were quality filtered and clustered by a custom pipeline largely based on USEARCH and OTUs were clustered at 97% sequence identity. Analyses of alpha- and beta-diversities were used to assess soil microbial community structures (SMCS) at the different landusetypes, and sites as well as over time.

Main Goals

- Assessment of bacterial and fungal diversities and establishment of a reference data set of site specific communities.
- Determination of main factors that shape soil microbial community structures.
- Evaluating metabarcoding for the Swiss Soil Monitoring Network.

Fig.1: The 111 long-term monitoring sites of NABO including NABObio sites.

Fig.2: NABO system – soil quality assessment

Tab. 1: Influence of landuse, site and sampling year on SMCS (PERMANOVA test).

	Bacteria			Fungi		
	F Model	R^2	p	F Model	R^2	p
Landusetype	361.61	24.8%	0.0001	179.8	17.0%	0.0001
Site	60.58	56.0%	0.0001	41.6	53.1%	0.0001
Year	7.44	1.0%	0.0001	5.7	1.1%	0.0001
Year:Landusetype	2.95	0.8%	0.0001	3.3	1.3%	0.0001
Year:Site	1.93	7.1%	0.0001	2.6	13.4%	0.0001
Residuals		10.3%			14.2%	

Results

- Alpha-diversity revealed low correlations to environmental factors, with the highest value for pH (r = 0.51).
- PERMANOVA showed significant differences among SMCS of different land-use types (arable land, grassland, forest), and among sites (Tab. 1).
- SMCS revealed high temporal stability (Tab. 2).
- Bacterial communities are more site specific, fungal communities differ especially according to the ecosystem (Tab. 2, Fig. 3).

Conclusions

- The stability of SMCS over five years allowed for the definition of site-specific baselines in the NABO system.
- Community data is much better suited for monitoring purposes then sum-parameters like DNA-biomass or alpha-diversity.
- Metabarcoding represents a robust approach for comparative analyses of microbial communities in different soils and over time.

Outlook

- Relate possible future changes of SMCS to changing environmental factors.
- Assign sensitive taxa to specific environmental stresses.
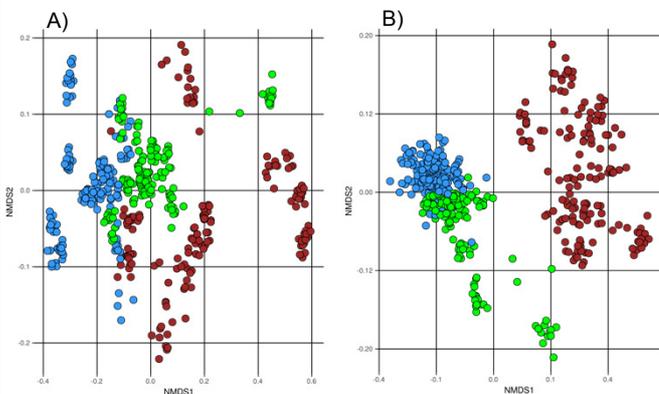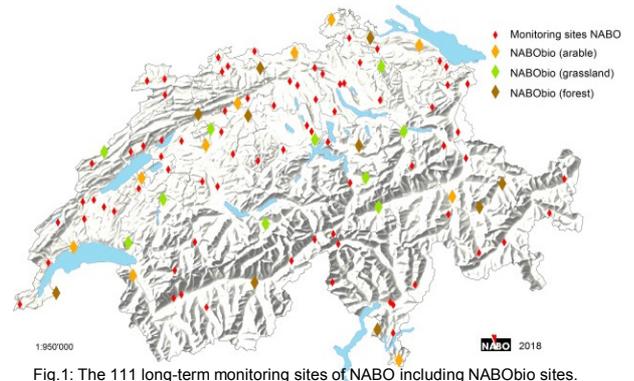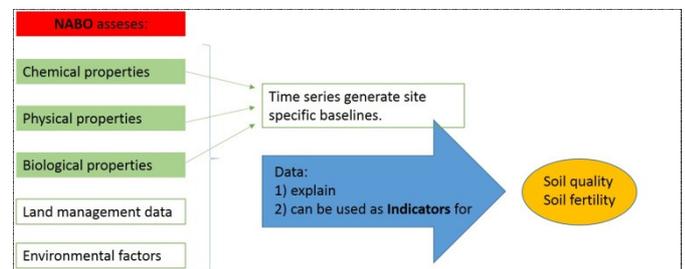- Definition and development of diagnostic indicator taxa for defined stresses as long-term objective.

Fig. 3: Ordination (NMDS, non metric multidimensional scaling) of bacterial (A) and fungal (B) community structures. Blue: arable, green: grassland, brown: forest, (n=450).

Tab.2: Reclassification success (CAP analysis)

	Reclassification to	
	Landusetpye	Site
Bacteria	100%	100%
Fungi	100%	99.60%

Agroscope gutes Essen, gesunde Umwelt