Is the composition of fungal populations in surface waters an appropriate parameter for the risk assessment of multiple pesticide loads from fruit cultivation?

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Introduction

So-called 'application sequences' in apple cultivations consist of many pesticides, which are repeatedly applied in short time intervals one after the other. The risk assessment of pesticides, however, includes only the evaluation of single substances so far [1]. Pesticide loads reach surface waters through several pathways such as e.g. spray drift and runoff (Fig. 1). This raises the question, whether continuous contamination of surface waters with pesticides over time may lead to negative effects on aquatic ecosystems although the individual substances should not have effects on aquatic organisms (Fig. 1).

Aquatic fungi are important for surface water ecosystems because they supply energy through litter decomposition [2]. In ecotoxicological risk assessments aquatic fungi are not yet included as standard organisms although 'application sequences' comprise especially fungicides [3].

In a mesocosm study the effect of an 'application sequence' scenario on the diversity of aquatic fungi was examined through fingerprinting of fungal communities colonizing *Alnus glutinosa* leaves in order to find out if this is a good parameter for the risk assessment of pesticides.

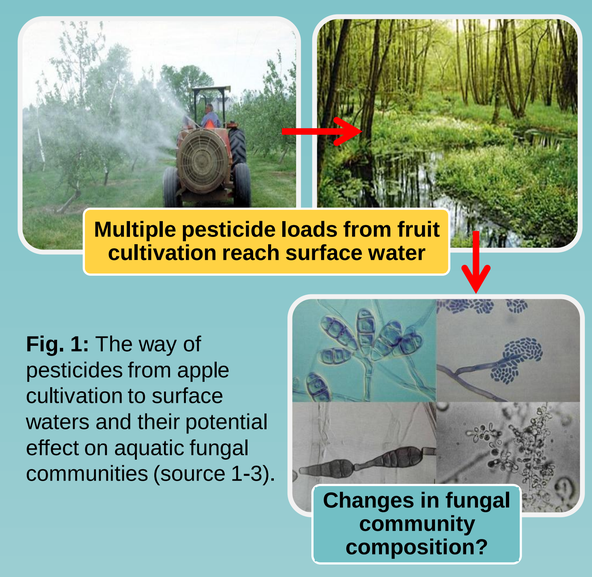


Fig. 1: The way of pesticides from apple cultivation to surface waters and their potential effect on aquatic fungal communities (source 1-3).

Material and Methods

Mesocosm study

Preparing leaf litter bags

Immersing the litter bags into the mesocosm ponds

Sampling the litter bags

Litter bags:
7g litter material of *A. glutinosa* per litter bag (measuring 20 cm in length).
6 replicates per pond and exposure time (= 24 bags per pond), successively sampled.

Mesocosms:
4 ponds with pesticide loads, 4 control ponds (6.9 x 3.2 x 2.5 m) (Fig. 2).

Application:
Spring scenario of multiple pesticide usage as in apple cultivations (April to June).

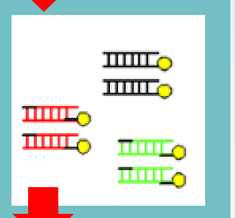
Active substances:
5 fungicides,
3 herbicides, and
3 insecticides

Regulatory acceptable concentration (RAC):
RAC is the 'environmental concentration of an active substance expected to have no unacceptable adverse effects on the environment' [4]. RAC was used for each substance.

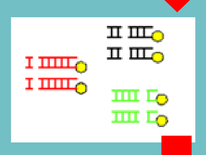
TRFLP analysis

Fungal DNA extract-ion

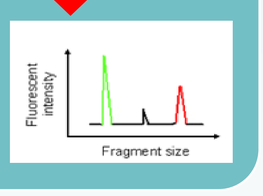
Amplification of fungal ITS region through PCR with labeled primers



Digestion with restriction enzyme



Analysis



Molecular analysis:
Fingerprinting of the fungal community composition was done with terminal restriction fragment length polymorphism (TRFLP) technique (Fig. 2).
454 pyrosequencing analysis is in progress.

Statisticial analysis:
PERMANOVA was done in R.

Fig. 2: Mesocosm study in the artificial pond and stream system (FSA) of the German Federal Environment Agency at the field station in Berlin-Marienfelde (source 4) and the steps of the molecular TRFLP fingerprinting method used at Helmholtz Zentrum München (changed according to source 5).

Results and Discussion

Fingerprinting of fungal communities

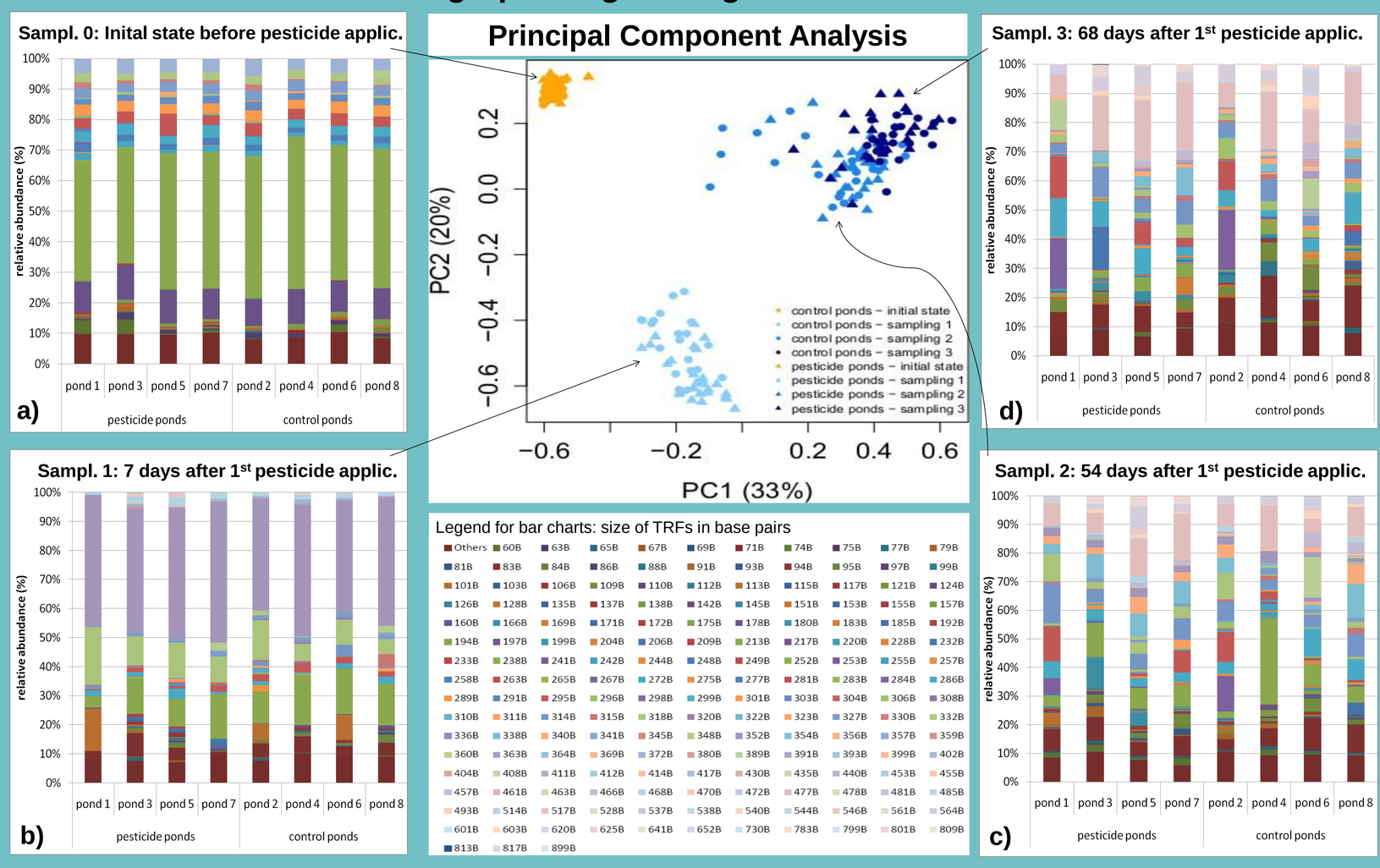


Fig. 3: PCA shows the variance in the composition of the TRFs (relative abundance) between control ponds and pesticide ponds for 4 sampling times. Bar charts a) – d) show the relative abundance (%) of all TRFs of one sampling time as an average of all litter bag replicates in one pond. ‚Others' = all TRFs < 1%.

Physico-chemical Parameters

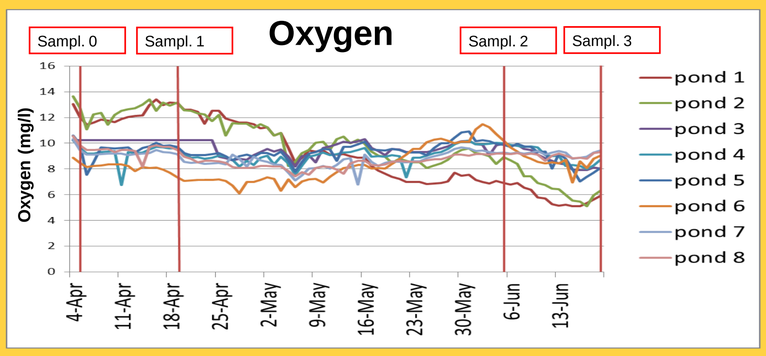


Fig. 5: Oxygen (mg/l) in the water was measured every day (8:30 a.m.).

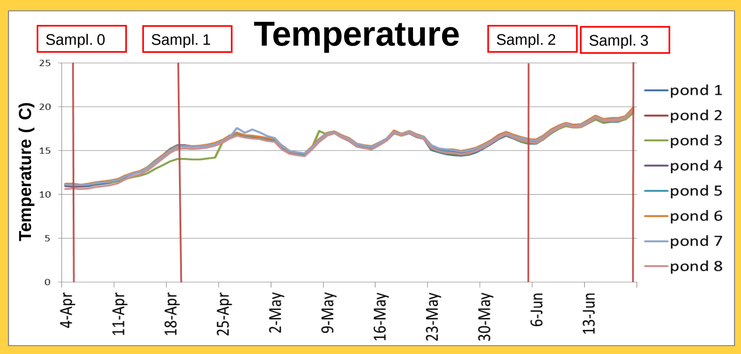


Fig. 4: Water temperature was measured every day (8:30 a.m.).

- **Principal component analysis (PCA) of the composition of aquatic fungal communities based on the relative abundance of terminal restriction fragments (TRFs) showed significant differences regarding the time course ($p < 0.001$) (Fig. 3).**
- **In comparison to the strong effect of time, the effect of the multiple pesticide loads on fungal community composition was negligible.**
- **For sampling dates 2 and 3 a tendency to differentiation between control ponds and pesticide treated ponds can be seen (PCA in Fig. 3).**
- **Physico-chemical parameters such as e.g. oxygen or temperature may have had a high effect on the aquatic fungal communities in this study (Fig. 4 und 5).**

Conclusions

- **In general fingerprinting is a promising tool to examine aquatic fungal communities since the temporal succession in fungal communities could be displayed.**
- **However, fingerprinting alone may not be sensitive enough for the detection of minor effects originating from multiple pesticide loads.**
- **Sequencing may allow for a deeper look into the fungal communities.**

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References:
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S 3: http://quizlet.com/21411279/mycology-flash-cards/
S 4: Federal Environment Agency S 5: http://www.e-cew.co.jp/Microbe-contents/21trflp.html