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Posudek disertační práce RNDr. Petra Kotase

Předkládaná disertační práce na téma: „Various aspect of soil microbial ecology as revealed by phospholipid fatty acids (PLFA) analysis“, se týká relevantního tématu nových metodických přístupů studia biologie. Práce má strukturu rešeršního úvodu a samotnou experimentální část tvoří 4 manuskripty, ze kterých byly 2 publikovány, 1 je v recenzím řízení po revizi a 1 je zaslán od redakce časopisu.

Téma samotné představuje moderní přístup studia mikrobiálních populací, kdy metoda PLFA umožňuje snadno velký počet analýz a v kombinaci s typem informace, kterou podává, ji nelze vhodně nahradit žádnou jinou technikou, a to včetně DNA sekvenačních metod. Její výhoda hlavně spočívá v robustnosti a spolehlivé kvantifikační informaci několika skupin mikroorganismů bez nutnosti kultivačních kroků. Další výhodou je určitá flexibilita ve využívání markerových mastných kyselin vzhledem k charakteru vzorku. Tyto a další informace využitelnosti metody PLFA jsou velmi zdařile shrnuty v úvodní části na zhruba 30-ti stranách s více než 120 citacemi. Tato úvodní část je sepsána kultivovanou angličtinou bez zbytečných překlepů a očividných chyb. Struktura této část i je logická a domnívám se, že kandidát by se mohl pokusit tuto část opublikovat, coby samostatný rešeršní článek.

Jak bylo zmíněno, v práci následují 4 manuskripty, kdy 2 z nich již prošli náročným recenzním řízením v kvalitních časopisech. První 2 články jsou klasickou aplikací PLFA metody pro popis mikrobiálních populací za různých environmentálních podmínek. Třetí článek představuje využití PLFA metody v aplikaci ^{13}C -PLFA-SIP, která je rovněž robustní metodou pro popis mikrobiálních procesů a mnohdy je mnohem vhodnější než méně citlivý DNA-SIP. Velmi zajímavou a inovativní aplikaci představuje 4. článek, který se snaží za pomoci PLFA popsat

kritické limitující koncentrace fosforu pro mikroorganismy. Snad jen v tomto bodě mi přijde, že na straně 33, byly výsledky studie méně popsány, než u ostatních článků.

Obecně mohu konstatovat, že práce je kvalitní a že kandidát projevil svojí disertační prací dostatečnou erudici, a proto doporučuji jeho práci k obhajobě

Dotazy k obhajobě:

1. V úvodní práci zdařile popisujete úskalí jednotlivých mastných kyselin pro využití metody PLFA. Vyvětlete, proč v každém manuskriptu využíváte trochu jiné zástupce mastných kyselin pro popis jednotlivých mikrobiálních populací.
2. Jakým způsobem jste získal data zobrazená na obrázku 4a str. 93?



V Praze 13.3.2018

Prof. RNDr. Tomáš Cajthaml, Ph.D.

Reviewer's report on Ph.D. thesis entitled

„Various aspects of soil microbial ecology as revealed by phospholipid fatty acids (PLFA) analysis“

elaborated by RNDr. Petr Kotas

The reviewed thesis consists of four scientific papers which have been published in international scientific journals and a general introduction that gives comprehensive overview of theory and methodology of the use of phospholipid fatty acids (PLFA).

General introduction is divided into 7 subsections dealing with sample preparation, limitations of PLFA's use in characterization of biomass of different components of soil microbial communities, precision of the conversion of the PLFA's content to biomass, the use of PLFA's as markers of physiological state of microbial biomass, the use of ^{13}C labelling in tracing of carbon fluxes in microbial communities and in ecosystems and with reliability of PLFA profiling. The detailed information contained in the text shows the depth of the author's insight into the topic, which fully corresponds to recent state of the knowledge.

Particularly important and useful is the subsection 1.3 that provides the list of PLFA's specific for different groups of organisms, including bacteria, archaea, microscopic algae, fungi, protozoan groups and plants. Regarding this section, I would like to pose my first question (to be answered during the thesis defence) that is connected with PLFA markers of Protozoa. According to the recent taxonomic system, the group ("kingdom") Protozoa is an artificial conglomerate of high level taxa (kingdoms – Amoebozoa, Excavata, Rhizaria, Chromalveolata, some Plantae and some Opisthokonta). Is this taxonomic notion reflected in presence of some specific markers in representatives of these high level taxa? Is there some research reported in the literature that is focused on the inconsistency between high-level taxonomy of eukaryotes and markers that are in use? In fact, the important sub-subsection 1.3.5 that deals with Protozoa is the shortest one within the General introduction. Does this mean that protozoans are of marginal interest in the studies involving analysis of PLFA markers?

Second section is dedicated to the formulation of aims and objectives. As expected, the working hypotheses have been emerged in this section. The aims involve a loose aggregate of ideas dealing with effects of soil fertilization, soil organic matter, phosphorus availability and other factors on soil microbial communities, the analysis of PLFA's being the unifying moment here. The defendant extensively participated in the reported works not only as a provider of PLFA analysis but significantly contributed to devising the studies and writing the publications. This is reflected in the fact that he appears as first author of the two out of the four publications constituting the thesis.

The publications themselves are of high scientific value, present the reliably performed works and have passed through stringent peer-review process. This makes my work as a thesis reviewer easier and superfluous in some aspects. However, one question appears regarding overall concept not only of the thesis but also of the general defendant's scientific orientation. He uses PLFA markers to characterize microbial communities but somehow neglects the use of other advanced methodology: next generation sequencing. Out of the four publications constituting the thesis, the sequencing approach has been involved only in a single text (Šantrůčková et al., accepted to SBB). Does this mean that the defendant relies for some reason to PLFA analysis and takes other methods as

auxiliary at best? If so, why? It is extremely important to me to combine data obtained using different independent approaches to obtain a picture of the reality that corresponds to really existing phenomena. Unfortunately, the article accepted to SBB does not attempt to compare the descriptions of the microbial communities simultaneously obtained by PLFA and sequencing approaches, which would be very interesting. For example, it might be possible to compare the relative abundances of G+, G- and Actinobacteria measured by the two different approaches.

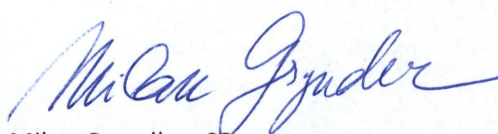
In the Study I, Kotas et al. (2016) came to the conclusion that NPK fertilization decreases soil pH and CEC. The study was performed on the oligotrophic mowed meadow with removal of the harvested plant biomass. The above negative effects of fertilization might be attributed to unbalanced supply of mineral ions and accompanying ions (sulphate). It would be very interesting in this case to evaluate the mineral nutrients balance, i. e. were „harvested“ minerals just replaced by fertilization or the mineral fertilization outweighed the output? It seems that the latter is true but what would happen if the nutrients supply and output would be balanced? Is it possible that under balanced mineral nutrition the adverse effects of fertilization will be eliminated?

Study III presents a pioneering work on dark heterotrophic CO₂ fixation in soils. In this study, the authors concluded that dark CO₂ fixation is detectable and corresponds up to approx. 16% of the net respiration rate. The authors conclude that the fixation processes „may refix CO₂ that has been respired for energy production“ (pg. 137). O. K. but anaplerotic reactions themselves (see for example formation of oxalacetate by carboxylation of pyruvate) demand energy produced by oxidation of organic compounds. Then, if interpreted as a process of replenishment of organics lost by respiration, the formation of organic compounds from heterotrophically fixed CO₂ would represent a kind of „Perpetuum mobile“ from the viewpoint of the energetical metabolism, mainly in situations when „organic carbon is limiting“. I would thus prefer other interpretations of heterotrophic CO₂ fixation presented in Study III: as a source of carbon nutrition in chemoautotrophs (pg. 138) and photoautotrophs or as a source of carbon in synthesis of fatty acids (pg. 137).

The reviewed thesis provides many interesting examples of the use of PLFA analysis in the research of soil microbial communities. The defendant is well acquainted with problems of current soil microbiology, is able to formulate relevant scientific hypotheses as well as to design the experiments/observations and is prepared to provide up-to-date analytical support for the research team. He is undoubtedly able to process and interpret experimental data and publish results in respected international journals.

On the basis of the above mentioned facts, I am persuaded that the defendant is prepared for thriving scientific career. As he meets all the criteria known to me, I can recommend to award him the Ph. D. degree after a successful thesis defence.

Reviewer:



Doc. RNDr. Milan Gryndler, CSc.

In Ústí nad Labem, 15/03/2018

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