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Referee's evaluation of the PhD thesis
"**Wood ants of genus Formica as important ecosystem engineers**"
submitted by Mgr. Veronika Jílková

Wood ants are among the few insect taxa that evolved an eusocial lifestyle. Wood ants rear their brood in cooperation, and the ability to communicate, forage in groups, and work in parallel allowed these insects to exploit and dominate resources over vast areas. The workers are closer related to their sisters than to their offspring and this kin selection allows them to be much more agressive than solitary insects, and to sacrify themselves during defence of their nest mounds or during attack of potential prey.
The nests of wood ants are impressive large structures that can become several decades old and with time accumulate food and continuously deplete the surrounding woodlands of prey and forage. Next to protein-rich prey for the brood, ants gain energy in form of simple sugars through foraging for aphid honeydew in tree crowns, which is subsequently transported in great amounts into the ant nests. In order to speed up the larval development, ants also keep an elevated and more stable temperature in their nests compared to the surrounding forest soil. The enhanced temperature and accumulation of nutrient-rich organic matter and simple sugars also might provide ideal conditions for soil microorganisms, which play important functional roles in the ecosystem through the re-mineralisation of nutrients and the emission of greenhouse gases, such as CO_2 and CH_4. Ant mounds thus create hot-spots of nutrients in the often acidic soils of nutrient-poor forest ecosystems. Due to the quantity and mangnitude of ecosystem processes by which ants influence the life of other organisms in the forest, including microbes in soil, ant prey, but also the surrounding forest trees, and even the tree's insect parasites, they must be regarded as important ecosystem engineers.
On this scientific background Ms Jílková's PhD thesis aimed to quantify the roles of wood ants in these important ecosystem processes.
The thesis is composed of an Abstract, a General Introduction, Major Hypotheses, Major Findings, an impressive List of Publications published by Ms Jílková during her thesis, and of

five scientific articles in manuscript form: one book chapter and four manuscripts published in refereed and well-known soil science journals, that form the core of the submitted thesis. Overall, the PhD thesis is well structured and written in excellent English.

The Abstract is well-focused and clear. The General Introduction of the thesis gives a thorough review of the ecology of wood ants and the relevant scientific literature, and it highlights the special importance of wood ants as ecosystem engineers. It suffers some repetitions due to the need to address the different, but partly overlapping topics in the separate manuscripts. The Major Hypotheses are clearly stated and confirm a structured scientific approach to the topic. The Major Findings and Major Contributions of the thesis clearly summarize the results and set the published manuscripts in context to the hypotheses.

Manuscript 1, the book chapter with Ms Jílková as second author, is a thorough review on the different and complex roles of wood ants in nutrient cycling and ecosystem function. It provides an up to date overview on the relevant literature, and, most importantly, new data on element accumulation, and new insights on the interplay of territory-related nutrient effects (i.e. enrichment vs depletion) and energy inputs via aphid tending.

Of all subsequent manuscripts, Ms Jílková is first author.

In Manuscript 2 Ms Jílková investigates the contribution of wood ants to the production of green-house gases over a whole year. In particular, she quantified the contribution of wood ants (*Formica aquilonia*) to CO_2 and CH_4-fluxes from soil in a well-designed field study. A few typos with unit µg instead of g (CH_4 and N) should be corrected; but she clearly shows that nests of wood ants are important sources of CO_2 production, but that the production of the green-house gas methane was strongly suppressed in ant nests compared to the forest floor.

With another ant species (*F. polyctena*), Ms Jílková investigates in Manuscript 3 the contribution of wood ants to the production of the green-house gases CO_2 and CH_4 in greater detail. She separately quantified the mineralization capacity and green-house gas productions of 'dry' and 'wet' ant nests over the seasons in a field study, and she separated the contributions of ant-, litter- and ant-plus-litter respiration in laboratory experiments. Her results clearly show that two functional classes of ant nests, i.e. 'dry' and 'wet', must be distinguished when measuring the contribution of wood ants to mineralisation and matter fluxes in forests. Interestingly, decomposition was only slightly increased in wet, but strongly suppressed in dry nests. The laboratory experiments clearly show that respiration of wood ants is relatively low compared to microbial respiration in nest litter material, but that ants indirectly stimulate microbial respiration geatly, evidenced by the strongly increased respiration rates in the ant-plus-litter treatment.

Manuscript 4 again deals with the contribution of wood ants (*Formica aquilonia*) to the respiration of CO_2, but from an energetic point of view. In field and laboratory studies Ms Jílková tests the hypothesis that wood ants at higher altitudes, where colder climatic conditions prevail, must invest more energy in the maintenance of heat to support brood development, and consequently should respire relatively more CO_2 from their nests and individually than ants from lower altitudes. By combining an impressive array of different techniques Ms Jílková was able to disect the different energetic and foraging strategies of ants in this study under different climatic settings. The isotopic enrichment of ants with ^{15}N

at higher altitudes showed that these ants were foraging for more prey with higher protein content. Differences in foraging strategies and climate were also reflected in differing seasonal microbial community successions, but CO2-fluxes in spring were not higher at high elevations, and apparently forced by energy limitation at high altitudes. A few typos with unit µg instead of g (CO_2) should be corrected, but overall this is a really excellent study providing novel and significant insights in the ecology of wood ants by combining elements of climate change with energy limitation and nutrient stoichiometry.

In Manuscript 5 Ms Jílková investigated in eastern Finland the composition of wood ant nests in great detail in order to quantify the contribution of the different materials to overall nest respiration in comparison to the surrounding soil and litter material. In order to separate the effects of deciduous vs. coniferous forests on the CO_2-fluxes, she compared ant nest materials from birch and pine forests. She could show that respiration was mostly related to moisture, carbon content and pH of the mound and litter materials of the specific forest types.

Overall, I have been very much impressed by the work of Ms Jílková. By combining an impressive array of techniques in field and laboratory experiments, and by thorough publications of these findings in well known and peer-reviewed journals, she has presented new and deep mechanistic insights into the complexity of the interplay of climatic conditions, microbial composition and ant activity, litter quality, and nutrient and energy limitation for greenhouse gas emissions from wood ant nests to the scientific community.

Without reservation I consider the thesis suitable for the defense by Ms Jílková.
The quality of Ms Jílková's PhD thesis clearly fulfills the criteria necessary for obtaining a Ph.D. degree by the candidate.

(Prof. Dr. Michael Bonkowski)