Review of Aanderud et al.: Fungal loop transfer of N depends on biocrust constituents and N form

In their research article "Fungal loop transfer of N depends on biocrust constituents and N form" Aanderud and co-authors investigate the potential presence of a fungal connection, which may facilitate the transport of nutrients and carbohydrates between dryland vegetation constituents. This is a highly interesting research question and there have been several publications on this research hypothesis during the last years. While in some studies the rapid movement of $^{15}NO_3^-$ in a root-free environment could be shown, other studies are by far less clear and demonstrate the necessity of further research activities.

In the current study, $^{15}NH_4^+$ and $^{15}NO_3^-$ were applied to two different types of biocrusts and the occurrence of ^{15}N in the biocrust and grasses at varying distances away from this spot were subsequently analyzed. Unfortunately, there are some major drawbacks in this approach.

1. There are no controls, where unlabeled NH_4^+ and NO_3^- were applied and analyzed.
2. The number of true replicates per crust type is only 3 and thus rather small. The authors calculate with an n of 14 and 15 for cyanobacteria- and moss-dominated biocrusts; however, most of the samples are not independent (since related to the same source, where ^{15}N was applied). Thus, calculations have to be conducted accordingly, distinguishing between pseudo- and true replicates!
3. A 2.5 mm rainfall equivalent was only applied to the central 5 cm diameter circle, whereas the area outside remained dry. As grasses and crusts at a distance of 29-120 cm and 22-97 cm, respectively, were collected, the amount of water may have been not sufficient to activate such a large area around the central biocrust. The result, that a transport was observed in cyanobacteria- but not in moss-dominated biocrusts, supports this assumption, as moss-dominated biocrusts are known to need by far more water to be activated than cyanobacteria-dominated biocrusts.

Other major comments:

1. The authors show that in cyanobacteria crusts, the gene copy number of Ascomycota decreases parallel to the $\delta^{15}N$ from $^{15}NO_3^-$. However, this does not proof that there is a functional relation between both parameters. In moss-dominated biocrusts, gene copy numbers of Ascomycota are similarly high, but no relationship to $\delta^{15}N$ was found. In the discussion (line 303ff.) it is suggested, that fungi, likely dark septate endophytes, rapidly translocate N at a rate of 40 mm h-1. I did not find any proof for this assumption and thus it needs to be phrased VERY carefully, showing that this is mainly speculation. There are fungi within the biocrust, but a proof for their functional role was not given here. How can one be sure that fungi and not e.g. bacteria or just diffusion are relevant for the transport of nutrients in this experiment?
2. In line 333 f. it is stated that "mosses may be effective scavengers for N and outcompete fungal endophytes for newly fixed N". This indeed is very speculative, as there have been several publications on mosses, showing that N compounds are strongly leached during

major rainfall events (Coxson, 1991; Coxson et al., 1992). In addition, mosses are frequently associated with cyanobacteria, which also fix and deliver nitrogen (Rousk et al., 2013, 2017). Thus, it is hard to believe that the mosses actively scavenge and hold the N compounds. I consider it as much more likely, that the amounts of water/liquid were not enough to activate the moss-dominated biocrusts at a large enough diameter.

3. Line 350 f.: A dominance of dark septate fungi does not allow any conclusion on their role in transport processes. This needs to be shown in an experimental approach or phrased much more carefully.
4. Line 376: The theory that Pleosporales are the most likely conduits is based on theory and speculation, as their functional role has not been tested. This needs to be made clear.
5. In the manuscript, gene copy numbers are seen equivalent to biomass. This is not really correct. By means of qPCR one can get an idea regarding the relevance of the different organism groups but this information is by no means equal to biomass. For example, qPCR also does not distinguish between genetic material of living and dead organisms. Thus, it is much more appropriate to speak of gene copy numbers.

As there are several major drawbacks in this experimental approach, the manuscript cannot be published in its present form. The experiments need to be analyzed in an adequate form (considering 3 replicates per crust type). It also is necessary to reassure that the different results obtained for different biocrusts are caused by functional differences and not by water limitation. The presence of organisms (determined by qPCR) does not allow conclusions on their functional roles.

Minor comments:

Line 64: *Bouteloua* sp. (instead of *Bouteloua* species)

Line 80 ff.: "In such loops, minor rainfall events may stimulate N2 fixation by free or lichen-associated cyanobacteria (Belnap et al. 2003), N mineralization by bacteria and fungi (Cable and Huxman 2004, Yahdjian and Sala 2010) and nitrification and possibly denitrification (Wang et al. 2014) all increasing the levels of NH_4^+ or NO_3^-": I think one cannot say exclusively that levels of NH_4^+ and NO_3^- will increase during all of these processes. During nitrification for example, NO_3^- is expected to decrease, but NH_4^+ will decrease and during denitrification NO_3^- amounts are expected to decrease. Thus, I think one can say that that NH_4^+ and NO_3^- levels will be affected by these processes, but the overall direction of change depends on the relevance of the different processes involved.

Line 329 ff.: The authors state that "When *S. caninervis* was lost from this system, a dramatic increase in NH_4^+, which ultimately nitrifies to NO_3^-, was observed". I had a look in the publication of Reed et al. (2012), and there a decrease of NH_4^+ and an increase of NO_3^- was reported.

Line 336: There are no "stem cells" in bryophytes as they are thallophytes and not cormophytes!

Literature cited:

Coxson DS (1991) Nutrient Release from Epiphytic Bryophytes in Tropical Montane Rain-Forest (Guadeloupe). Canadian Journal of Botany-Revue Canadienne De Botanique 69: 2122-2129.

Coxson DS, McIntyre DD, Vogel HJ (1992) Pulse release of sugars and polyols from canopy bryophytes in tropical mountain rain forest (Guadeloupe, French West Indies). Biotropica 24(2a): 121-133.

Rousk K, Jones DL, DeLuca TH (2013) Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems. Frontiers in Microbiology. Doi: 10.3389/fmicb.2013.00150.

Rousk K, Laerkedal Sorensen P, Michelsen A (2017) Nitrogen fixation in the High Arctic: a source of 'new' nitrogen? Biogeochemistry 136: 213-222.