Depth differential colonization and biodiversity of mycorrhizal fungi in four prairie grass species

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Abstract

The biodiversity of AMF at different soil depths was studied in pure stands of the grasses crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), switchgrass (*Panicum virgatum* L.), green needlegrass (*Nassella viridula* Trin.) and western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve), growing in southwest Saskatchewan. The biodiversity of AMF was described in roots from 3 to 15, and 30 to 45 cm depth sampled in 2006 using phylogenetic and molecular tools. Soil depth reduced root colonization and influenced AMF community composition, which was dominated by six AMF phylotypes of the genus *Glomus*. Three AMF phylotypes were common colonizers and three were preferentially associated with some grasses. AMF communities at different depths differed from each other in all plant stands, and diversity and richness of AMF phylotypes was higher at shallow depth, except in *N. viridula* which showed higher richness of AMF in deeper root samples. We conclude that although some AMF are general colonizers, some AMF have a strong host preference. Our results also indicate that soil depth is a important driver of AMF phylotype distribution, and suggest the existence of niche specialization in AMF along the soil profile, which is influenced by the host plant.

Introduction

The symbiosis with AMF is ubiquitous in terrestrial ecosystems and usually provides plants with nutritional benefits and community stability. Despite the cosmopolitan distribution of some species (Sanders, 2004), a high diversity and specialization towards specific environments (Appoloni et al., 2008) and plant hosts (Gollotte et al., 2004) may exist in AMF.

The diversity of AMF has been linked to plant productivity and ecosystem stability (van der Heijden et al., 1988). Most knowledge on the diversity and relevance of these plant symbionts has been gained by identifying spores from soil or stained roots obtained from the top 10 or 15 cm soil layer. This cytological approach provided most of the current knowledge about the importance and ecology of AMF in soil plant ecosystems. But it has limitations. It can overlook AMF taxa with sparse or

periodical spore production (Rosendahl, 2008) or that might be adapted to deeper soil layers (Oehl et al., 2005). In addition, differences may exist within isolates of the same species (Koch et al., 2004), that can not be assessed by microscopic observations. Specific genes can be used to fingerprint species or isolates and the use of DNA based methods were proven useful for AMF identification and diversity assessment (Rosendahl, 2008).

Some prairie grasses posses a surprisingly deep root system that crosses different soil horizons, therefore contrasting soil environments exist within a same plant root. Since AMF in roots can be affected by surrounding soil conditions and some degree of specificity exists between plants and AMF species, it is possible that different AMF communities associate with prairie grasses at different soil depths.

The diversity of these AMF communities that might develop in different plant species or soil depths is important since different taxa may contribute differently to plant fitness or respond differently to environmental changes. The Canadian mixed grass prairie ecozone shows an ever changing soil environment driven by contrasting changes in vegetation and climate along a single growing season, which might promote highly diverse AMF communities. However, little is known about the patterns of AMF root colonization and diversity in this environment, or how such diversity might be distributed across different plant species or soil depths.

In this research we combined molecular and phylogenetic approaches to describe the community composition of AMF in roots of prairie grasses at different soil depths. Specifically we wanted to know if AMF community composition differs in four grass common grass species of the mixed grass prairie ecozone, and if it varies across soil depths.

Materials and methods

The colonization and community composition of mycorrhizal fungi at different soil depths was studied in pure stands of the grasses switchgrass (*Panicum virgatum* L.), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), green needlegrass (*Nassella viridula* Trin.) and western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve), growing in southwest Saskatchewan. The long term (8 years) experimental plots are located at the Semiarid Prairie Agricultural Research Centre (SPARC), latitude 50° 18’ N; longitude 107° 41’ W, near Swift Current, SK, Canada.

The region receives an average of 361.4 mm of precipitation per year and has a yearly mean temperature of 3.6°C (54 years average). About 50% of annual precipitation occurs between 1 May and 30 September and about 30% falls as snow in winter. Mean air temperature difference between July and January is about 33°C. The experiment was set in 2001 on a slight sloping (<3%) on an Orthic Brown Chernozem (Ayres et al., 1985), with pH of 6.51 and 6.59 (saturated soil paste) and a silty loam and loamy texture in its A and B horizons respectively. The experimental site was cropped with barley during four years before the establishment of the experiment, which was planted with the grasses in 2001 in a complete randomized block design with four replicates. Each plot is 6 by 4 m in area.

Root sampling and processing

One or two soil cores 5 cm wide and 45 cm long were extracted in May and August of 2006 from each of four replicated plots. The first three cm layer of soil and any external root in the core were removed and discarded before cutting in segments from 3 to 15 and 30 to 45 cm depth, and hand extracted inner roots from a same plot and depth were pooled and gently washed over a 100 mesh sieve under running tap water. Then roots wrapped in a nylon mesh and attached to a Styrofoam floater were placed during 5 minutes in an ultrasonic cleaner (FS 30, Fisher scientific, Pittsburgh), dried with sterile towel paper and finally cut into 1 cm pieces with sterile scalpels. Root samples were cleaned within 48 hours after harvest, and stored at –12 °C until analysis.

Community composition of AMF at two depths

The community composition of AMF in each plant stand was obtained from 32 composite samples (4 plant stands x 2 depths x 4 replicates per depth) obtained by pooling roots sampled in May and August of 2006, and further cloning and sequencing of the 5' end of the long subunit of ribosomal genes (LSU rDNA).

Fifty mg of roots from each composite sample were freeze dried and milled with a tungsten bead in a microcentrifuge tube, by vigorously shaking for three minutes in a paint shaker. The DNA was extracted using the DNAeasy plant mini kit (Qiagen Inc.), according to the manufacturer's instructions, and stored at -20°C until use.

Amplification of DNA followed a nested approach. A first PCR reaction with primers LR1 [5'GCATATCAATAAGCGGAGGA3'] (Trouvelot et al., 1999) and FLR2 [5'GTCGTTTAAAGCCATTACGTC3'] (Van Tuinen et al., 1998) amplified the 5' end of the LSU rDNA of fungi in general. Ten μL of PCR cocktail contained 5 μL of AmpliTaq Gold PCR master mix (Applied Biosystems), 3.8 μL of ultrapure water, 0.1 μL of a 50 μM solution of each primer and 1 μL of template DNA. The amplification included one step at 95°C for 10 min, followed by 35 cycles of 1 min at 93°C, 1 min at 60°C, 1 min at 72°C and a final step of 10 min at 72°C. The second PCR reaction with primers LR1 and FLR4 [5' TACGTCAACATCCTTAACGAA 3'] (Gollotte et al., 2004) amplified the 5' end of the LSU rDNA of AMF. Amplification conditions were as described above, except that 1 μL of a 1/500 dilution of PCR product from the first reaction was used as a DNA template. PCR products were analysed by agarose gel electrophoresis for the presence of an ≈ 800 bp band of DNA. After the second PCR cycle, one composite sample of PCR products was made from the four replicates of each depth, and 1.2 μg of this DNA was cloned using a Topo TA cloning kit for sequencing (Invitrogen Corp) resulting in one clone library for each depth in a given plant species. This pooling procedure does not reduce the detection of AMF (Renker et al., 2006), but reduced to ¼ the number of cloning reactions. Positive transformants containing a fragment of DNA of about 800 bp were screened by PCR with primers LR1-FLR2, and 345 plasmids were sequenced with primers T3-T7 at the Plant Biotechnology Institute in Saskatoon (National Research Council, Saskatoon, SK, Canada). The rDNA sequences presented in this research were deposited in Genbank under codes EU379972 to EU380188.

Alignment and check for chimera rDNA sequences

The sequences were aligned in Clustal W with gap opening and extension penalties of 6 and 3 respectively. Chimera sequences formed during the PCR amplification (Wang and Wang, 1997) were removed by re-submission of the alignment to the software Bellerophon (Huber et al., 2004), until no chimera sequences were detected.

Overall structure, phylogenetic identification and distribution of AMF phylotypes across plant stands and depths

The overall structure of the AMF community was obtained from the computation of similarity values between all non-chimera rDNA sequences, and further visualization by multidimensional scaling (MDS). The procedure followed the computation of a matrix containing square pairwise differences [D]. Then a proximities or similarity matrix [S] to use in the MDS analysis was obtained by subtracting [D] from its highest value of dissimilarity (d*max*) according to: [S] = d*max* – [D] (Legendre and Legendre, 1998). [D] and [S] were computed in the software Arlequin 3.11 (Excoffier et al., 2005) and MS Excel® respectively. The MDS configuration was obtained from a Guttman loss function in the software Systat 12.

Identification of AMF was obtained by Bayesian phylogenetic analysis using sample sequences from each cluster obtained by MDS along with 32 sequences representing all families of known glomeromycota and two sequences of the genus *Mortierella* included as an out group, and aligned as described above. The consensus phylogenetic tree was obtained from two independent runs of mrBayes 3.11 (Huelsenbeck and Ronquist, 2001) using a GTR + G model, 1,000,000 generations, 35% burnin time and tree sampling each 500. Additional confidence was obtained from bootstrap analysis of a Neighbour-Joining tree built with JC genetic distances and gamma rates of nucleotide substitution in the software MEGA4 (Tamura et al., 2007). Models of nucleotide substitution for both trees were selected using the software PhyML (Guindon and Gascuel, 2003). Support for the branches is presented as posterior probabilities in mrBayes or bootstrap values from 1000 iterations in the Neighbor Joining tree. Values lower than 0.95 using Bayesian posterior probabilities (Larget and Simon, 1999) or 70% in bootstrap analysis (Hillis et al., 1993) are considered non-significant. Since no formal definition of species exists for phylogenetic identified AMF individuals based on LSU rDNA analysis, AMF phylotypes are presented here as aligned sequences sharing more than 81.6% similarity estimated from pairwise nucleotide differences, which clustered together in significantly supported clades or within known species of AMF in the phylogenetic analysis. Further detailed distribution of AMF species was attained by partitioning according to plant stands and depths, the matrix of similarities used above for MDS analysis.

Assessment of treatment effects

Differences in composition of AMF communities between depths in each plant stand were tested by a permutation approach. Briefly, matrixes containing JC genetic distances were calculated with rDNA sequences from each depth in the program DNAdist in Phylip, and loaded in the software $\int$-LIBSHUFF (Singleton et al., 2001). A *P* value was obtained from 1000 permutations, Bonferroni corrected according to the number of comparisons, and then used to test significant differences between AMF communities at each depth in a given plant stand. This test is asymmetrical, therefore

the AMF population at two soil depths A and B can be significantly different, while B versus A is not; this would indicate that the species composition of population B is likely to represent a subset of population A (Singleton et al., 2001).

Results

Community composition of AMF in different grass stands and root sampling depths

The distribution of AMF species across plant stands and soil depths was obtained from 217 usable sequences after removing 128 suspicious chimera sequences. The MDS analysis grouped the sequences in six clusters (Figure 1a), which were well supported as six AMF phylotypes of the genus *Glomus* (Figure 1b). These AMF species are referred later as *Glomus intraradices*, *Glomus clarum* or Glo3 to Glo6.

The overall community was dominated by Glo5 and *G. intraradices*, which accounted for 59 and 22% of the rDNA sequences respectively, while *G. clarum*, Glo3, Glo4 and Glo6 were less frequent and accounted for the remaining 19% of the sequences (Figure 2).

Each plant species showed a characteristic community of AMF, despite the presence of some general colonizers. Furthermore, in all plant species the AMF communities at any depth were significantly different from each other ($P < 0.025$) and the recovery of some AMF species at a given depth depended on the plant species analyzed. For instance; Glo5 or *G. intraradices* were common colonizers in all plant species (Figure 2), but they were absent from shallow roots of *N. viridula* or deeper root samples of *P. virgatum* respectively (Figure 3). In addition, Glo4 was restricted exclusively to shallow roots of *P. virgatum* and deeper roots of *N. viridula* (Figure 3). The diversity and richness of AMF species in roots was generally higher at shallow depth, except in *N. viridula*, which showed higher richness of AMF at deeper depth (Figure 3).

Discussion

We found that soil depth strongly influenced AMF community composition in all grass species studied. We also found that the recovery of some AMF phylotypes at a given depth depended on the host plant species analyzed. There were depth specific assemblages of AMF communities.

The effect of soil depth on AMF community composition as assessed by molecular tools suggest that at least some members of the AMF community found at deeper soil depth differed from that existing at shallow depth. Soil depth effects in AMF at this molecular level were not presented before, and it might be important to understand the ecology of these fungi. Previous reports consistently indicated the presence of different types of AMF mycelia in grasses (Dickson, 2004), and a negative impact of soil depth on AMF root colonization (Abbott and Robson, 1991; Jakobsen and Nielsen, 1983). Our results indicate that diversity of AMF at several taxonomical levels may be also influenced by plant species. For instance: in *P. virgatum* differences between AMF communities across depths were induced by apparent differences between isolates of a same AMF phylotype, since rDNA sequences of Glo5 in deeper root samples were unrelated to those on shallow root samples ($P = 0.012$, Figure 3), which included also Glo5. However, in *N. viridula* and *P. smithii* differences between shallow and deeper AMF communities were induced by a shift in AMF species (Figure 3).

Since levels of AMF diversity or genetic variability were previously related to plant productivity or ecosystem stability (van der Heijden et al., 1988), the differences in AMF community composition found in this research, suggest that each grass species may have access to different resources along the soil profile. For instance, intra species variants may show high variability in mycelia length and improvement in plant P nutrition, with less impact on plant growth (Munkvold et al., 2004), while a number of distantly related AMF species are more important in providing stability to plant productivity than the same number of closely related species (Maherali and Klironomos, 2007). The differences in AMF diversity found in this research may be related to different functional strategies in arbuscular mycorhizal plants, which in turn would affect plant fitness under different environmental conditions.

Taken together, the results of molecular and cytological analysis suggest a high degree of specialization in AMF according to soil depths, which is influenced by the host plant. This might indicate differences in symbiotic access to soil resources and ultimately would explain relative plant fitness in this environment.

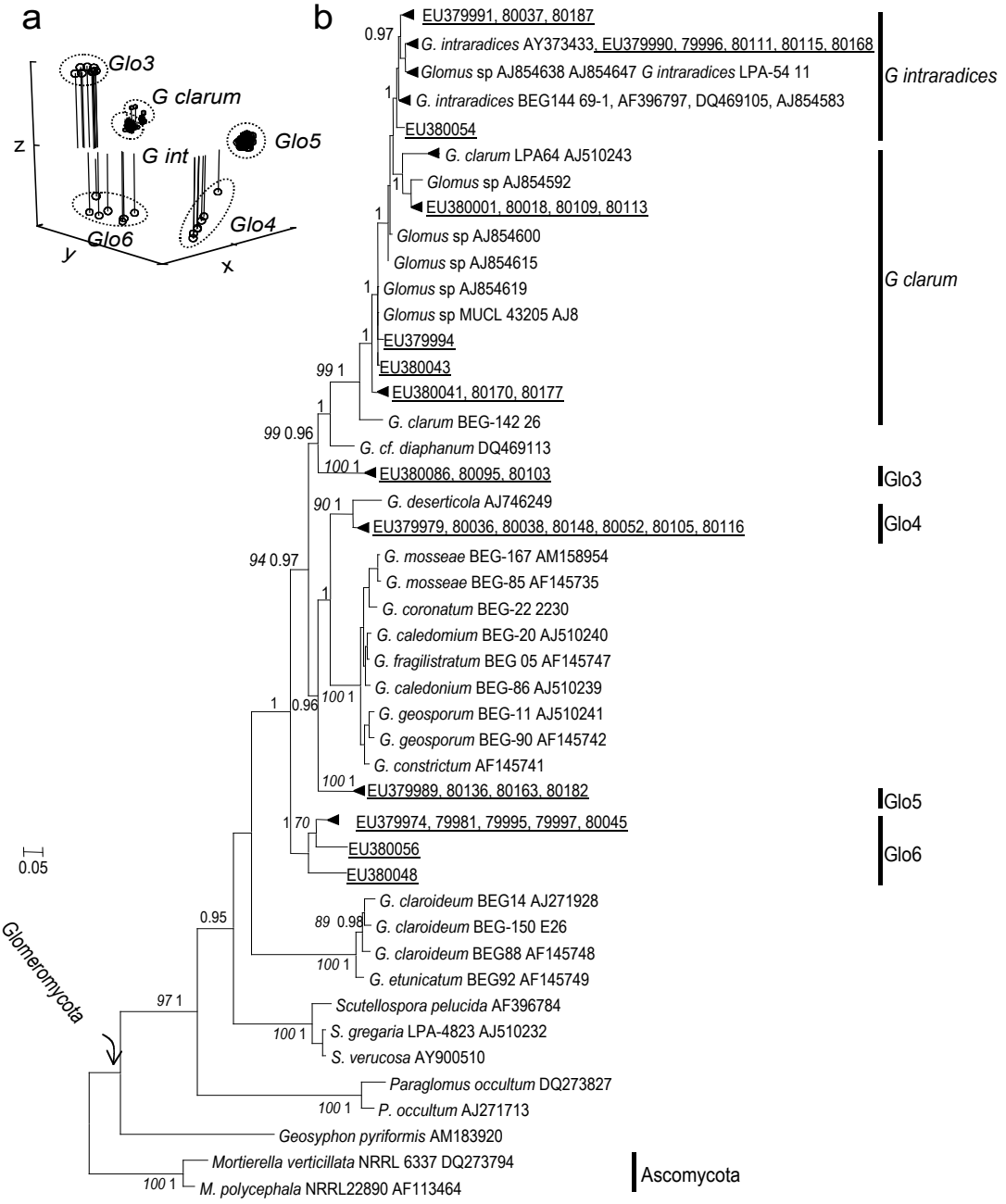

Figure 1 a) Overall AMF community structure revealed by multidimensional scaling analysis of all LSU rDNA sequences. Each circle in the scatter plot represents a sequence of rDNA and circles closer to each other represent more similar rDNA sequences (Stress = 0.041). **b)** Phylogenetic analysis of AMF rDNA sequences from this research (In bold) or downloaded from GenBank. Non-italicized numbers in the branches are posterior probabilities from the Bayesian tree. Numbers in italics are bootstrap values from 1000 iterations in a neighbor joining analysis. Values higher than 0.95 or 70 for Bayesian or neighbor-joining analysis respectively, are significant.

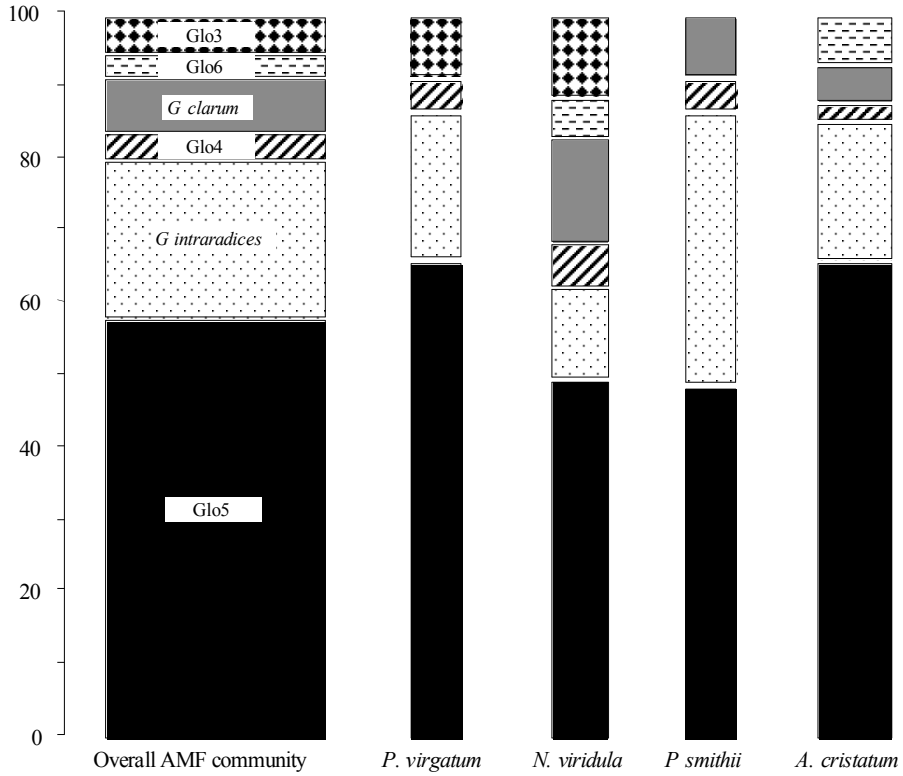

Figure 2 Overall compositions of the AMF community and AMF communities detected in each grass stand. Labels for compartments in each plant species are as in the widest bar. The width of a bar is proportional to the size of the clone library constructed ($N = 217$ for the overall AMF community).

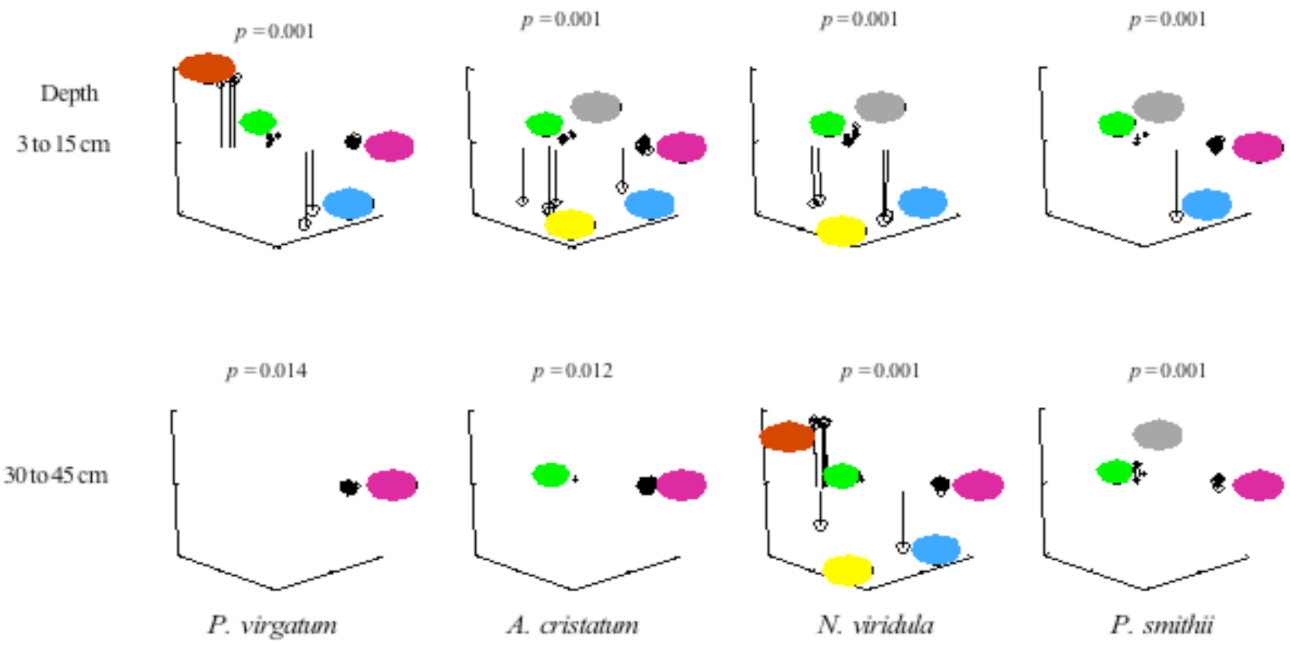

Figure 3 Composition of the AMF community at two soil depths in roots of different prairie grass species. Each circle in a scatter plot represent a sequence of AMF rDNA, and the distance between circles is proportional to the degree of similarity between the sequences that they represent. Labels indicate AMF phylotypes. Values of *P* above a scatterplot compare one AMF community to its counterpart in the other depth and they are significant if $P < 0.025$ (Bonferroni correction).

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