

Interaction between root and soil orchid mycorrhizal communities shape the demography of a rare orchid



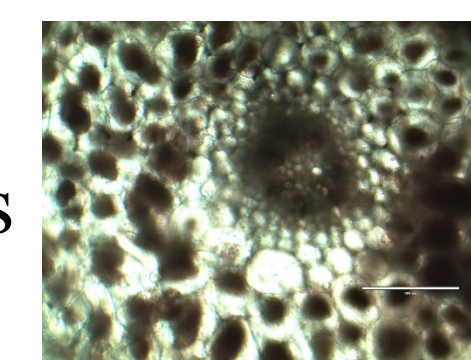
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Abstract

Untangling the direct and/or indirect effects of biotic interactions on plant populations is challenging yet necessary to understand the species' fundamental niche. The assembly of orchid mycorrhizal fungal (OMF) communities within roots likely reflects the structure of OMF in soil. We sought to reveal the interaction between root and soil OMF communities to explain the spatio-temporal variation in population size and demography of a terrestrial orchid *Platanthera cooperi*. We asked if soil OMF communities drive the assembly of root OMF communities? And if spatio-temporal differences in root OMF assemblages lead to distinct population demographic patterns? We collected *P. cooperi* roots and soil samples from six disjunct populations across three years. OMF communities were assessed by amplifying fungal internal transcribed spacer2 (ITS2) region of the nuclear ribosomal DNA followed by its sequencing. Bioinformatic data were analyzed using QIIME and VSEARCH along with biostatistical analyses in R. We identified 955 OMF operational taxonomic units (OTUs) inside roots which differed in distribution and abundance across populations and years ($P=0.001$ for both). Further, 30 most abundant root-associated OTUs also had differential distribution and abundances in soil across populations and years ($P<0.05$ for both). Hierarchical clustering grouped populations with similar dynamics together for both, root and soil OMF communities. In conclusion, our data suggest that soil OMF communities influence the assembly of root OMF communities, and the fluctuations in root OMF communities in response to soil OMF likely influence the spatio-temporal population dynamics of *P. cooperi*.

Introduction

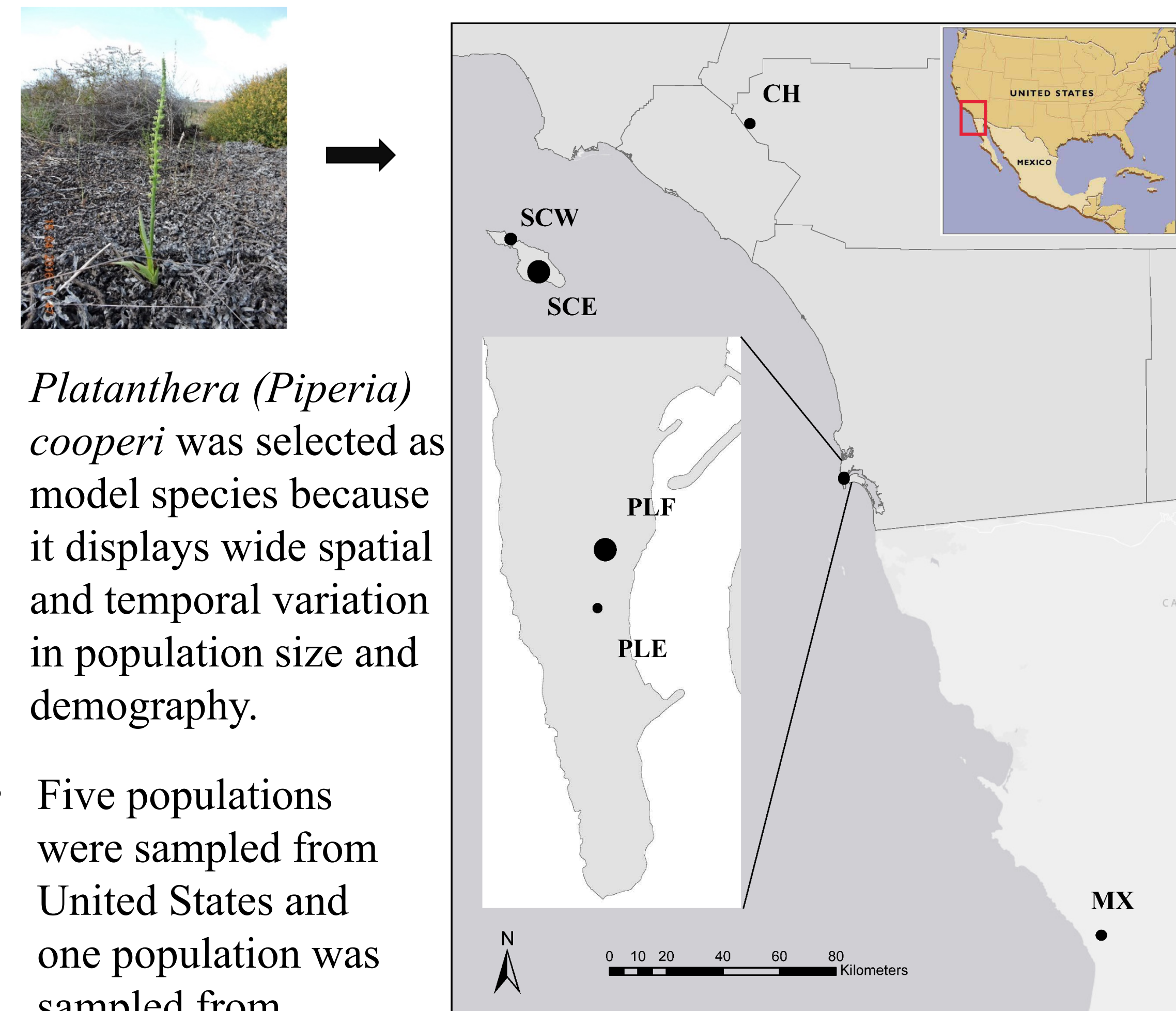
- Orchidaceae is largest and most diverse family of Angiosperms [1], along with the highest number of threatened genera than in any other plant family
- Small, mycoheterotrophic seeds
- Seeds associate with orchid mycorrhizal fungus which belongs mostly to Tulasnellaceae, Ceratobasidiaceae and Sebacinaceae fungal families
- Interaction of orchids with OMF taxa vary from generalist to specialist



Questions

- Do spatio-temporal differences in root OMF assemblages lead to distinct population size and demographic patterns?
- Do soil OMF communities drive the assembly of root OMF communities?

Materials and Methods

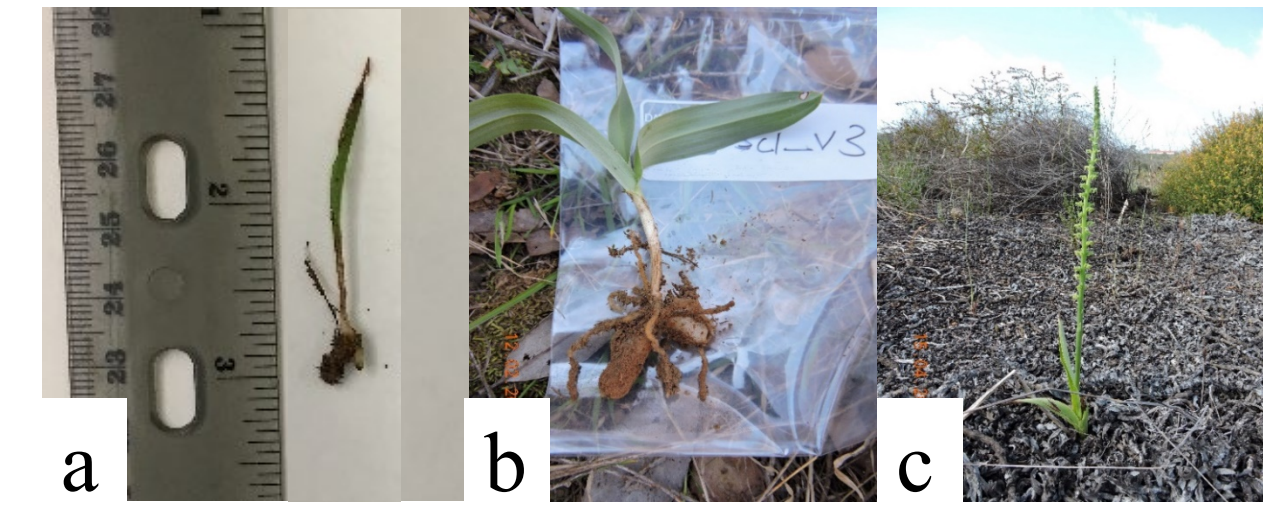


- Platanthera (Piperia) cooperi* was selected as model species because it displays wide spatial and temporal variation in population size and demography.
- Five populations were sampled from United States and one population was sampled from Mexico (Fig. 1)

Fig. 1. Distribution and study populations of *P. cooperi*

Materials and Methods

Root sampling from seedling (a), vegetative (b) and Reproductive (c) phenological Stages



Soil sampling

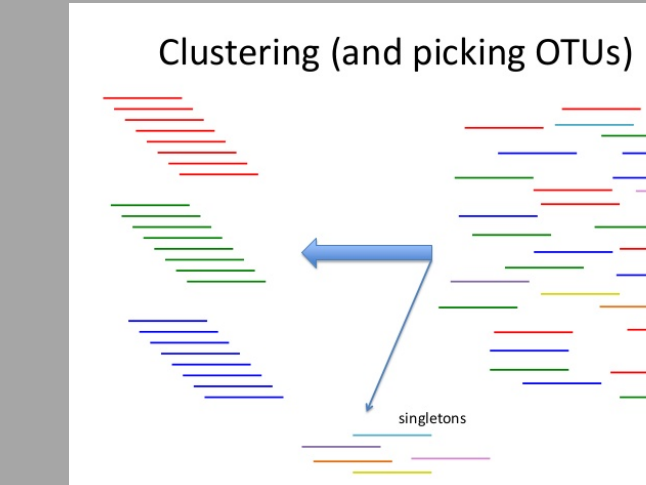
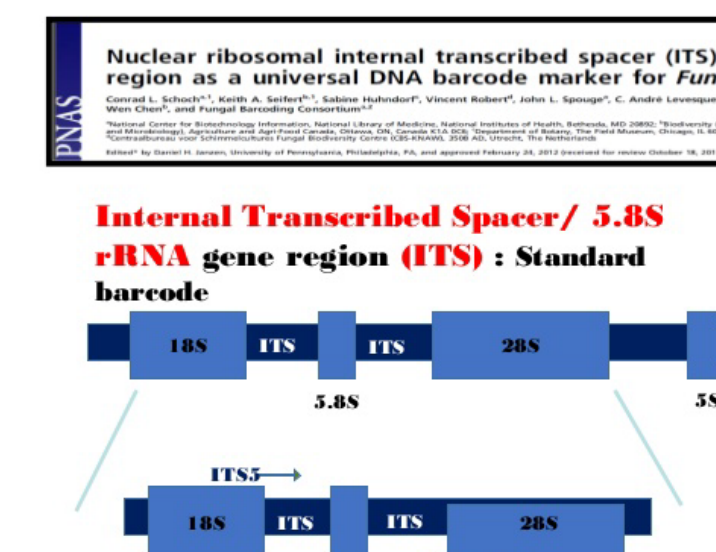


DNA extraction and fungal DNA barcoding with nuclear ribosomal ITS2 (nrITS) locus

Illumina MiSeq sequencing, bioinformatic analysis with QIIME, USEARCH and VSEARCH and biostatistical analysis in R

Species concept in microbes?

Operational Taxonomic Units (OTUs)



Results

- We identified 955 OMF operational taxonomic units (OTUs) after clustering ITS2 sequences from *P. cooperi* roots at 97% similarity threshold. The 25 most abundant OTUs contributed to 75-95% of abundances across sampling events. The most abundant OTU in roots belonged to Tulasnellaceae and the second most to Ceratobasidiaceae.
- Hierarchical clustering grouped populations with similar size and demographic patterns (Fig. 2).
- Permutational analyses of variance (PERMANOVA) revealed the differences in distribution and abundance of root OTUs across populations and years ($P=0.001$ for both), whereas OMF communities were similar across three phenological stages.
- Similarity percentage (SIMPER) analyses revealed ***Tulasnellaceae* otu10325 as the most important OTU** which distinguishes between large and small populations, where it showed higher abundances in roots of individuals collected from large populations..
- The most abundant 25 OTUs inside roots also had differential abundances in soil across populations and years ($P=0.001$ for both).

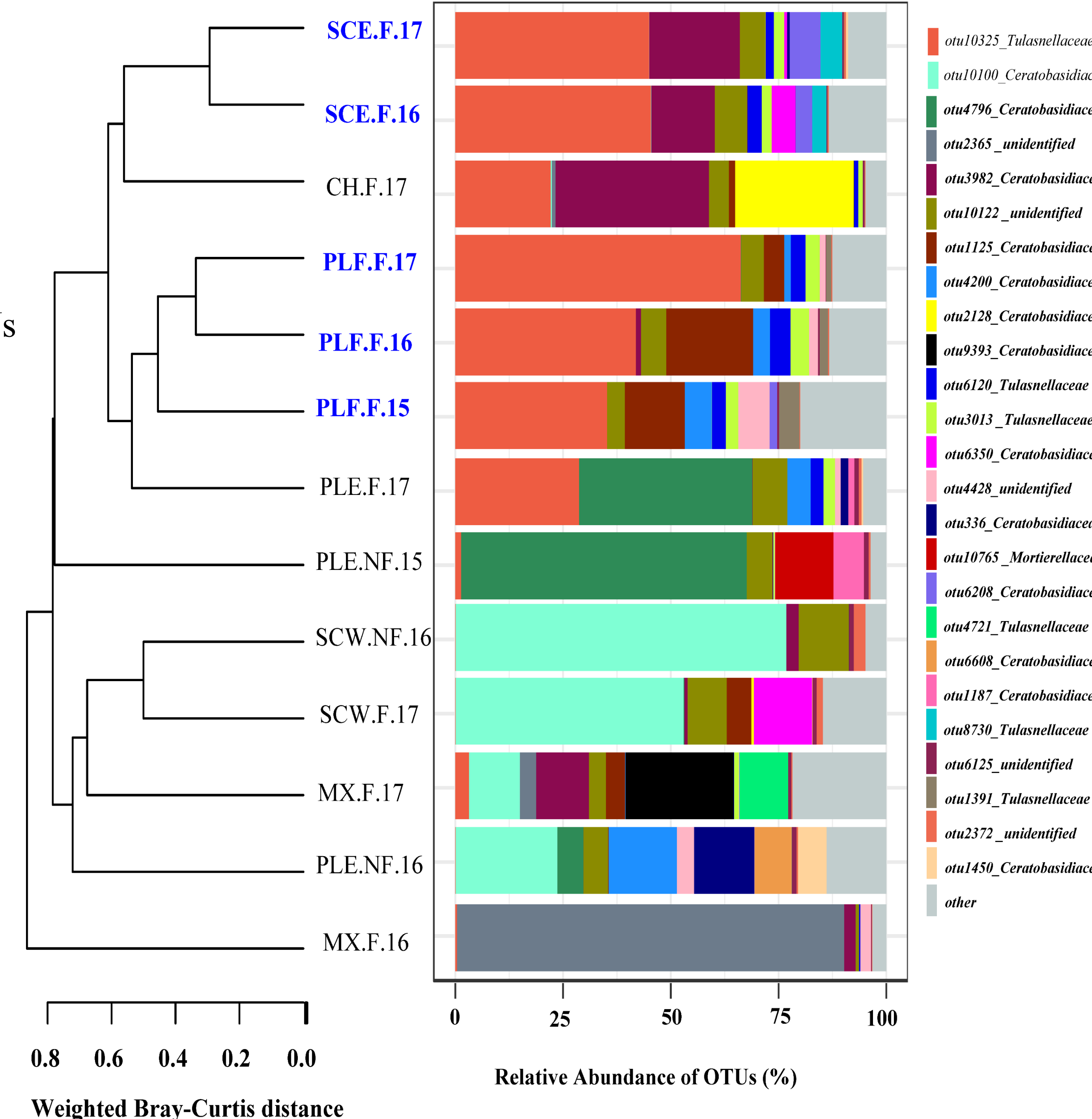


Fig. 2. Hierarchical clustering of *P. cooperi* populations based on root OMF community dissimilarities, and the barplot corresponding to each node shows the relative abundances of 25 most abundant OTUs inside roots. The blue color font represents large populations and black color font represents small populations. The letters F and NF followed by population names represents the presence (F) and absence (NF) of flowering individuals in a population. The digits 15, 16 and 17 represent sampling year 2015, 2016 and 2017, respectively.

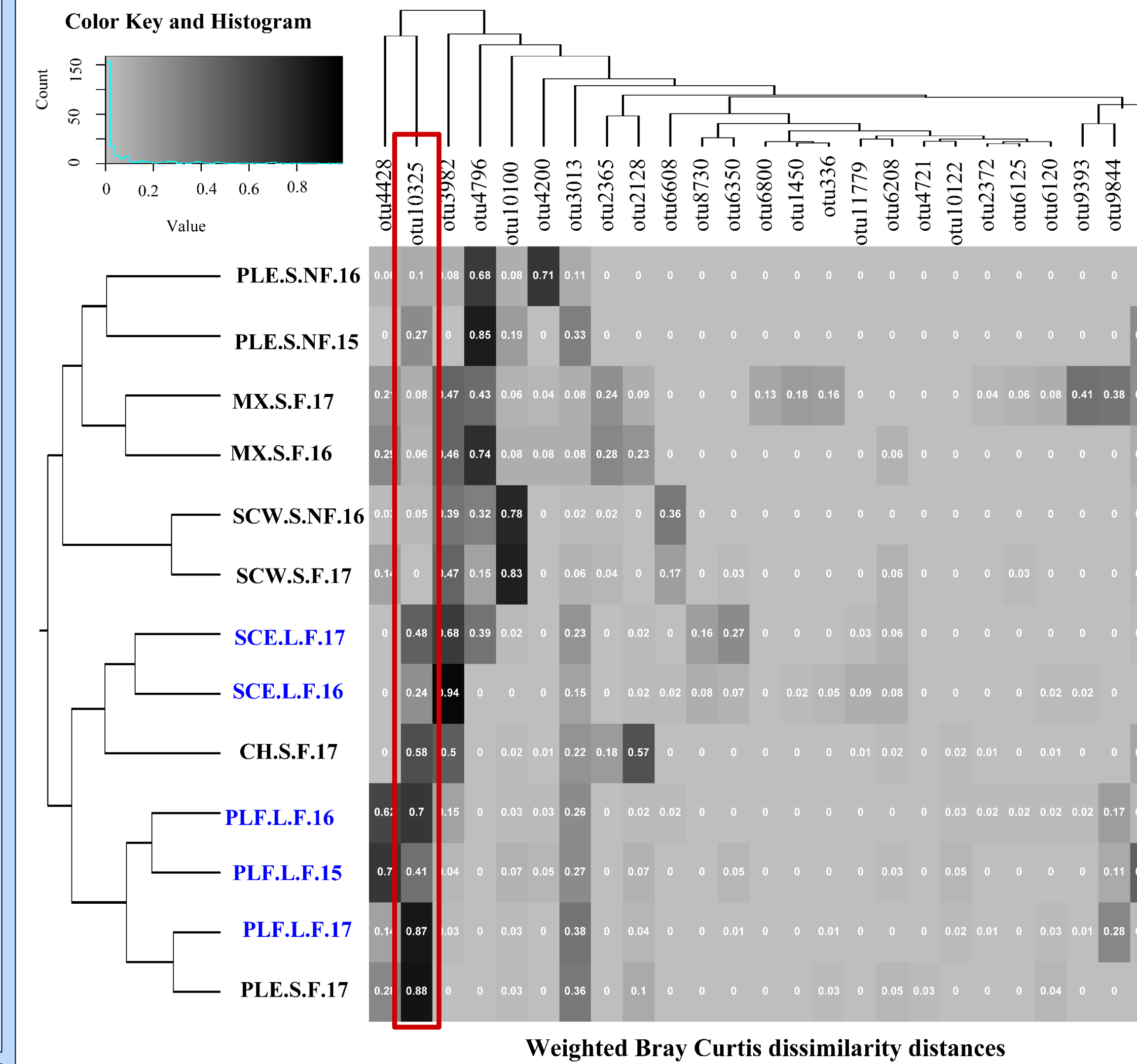


Fig. 3. Hierarchical clustering of *P. cooperi* populations based on relative abundances of OTUs in soil. The values inside matrix cells provides Hellinger transformed relative abundance value. The blue color font represents large populations and black color font represents small populations. The letters F and NF followed by population names represents the presence (F) and absence (NF) of flowering individuals in a population. The digits 15, 16 and 17 represent sampling year 2015, 2016 and 2017, respectively.

- Large populations (PLF and SCE) had higher abundance of otu10325 in soil (Fig. 3) in comparison to small populations.
- Multiple regression based on root and soil Bray Curtis dissimilarity matrices revealed high correlation between soil and root OMF communities ($r^2 = 0.32$, $P= 0.00$).

Conclusions

- Our data suggest that soil OMF communities influence the assembly of root OMF communities, and the spatial and temporal fluctuations in root OMF communities in response to soil OMF likely influence the spatial and temporal populations dynamics of *P. cooperi*.

Acknowledgements

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Literature Cited

1. Rasmussen, H. N. (1995). Terrestrial orchids: from seed to mycotrophic plant. Cambridge University Press.