Characterizing the interactions of plants and the soil microbiome at the James River Park in Richmond, VA

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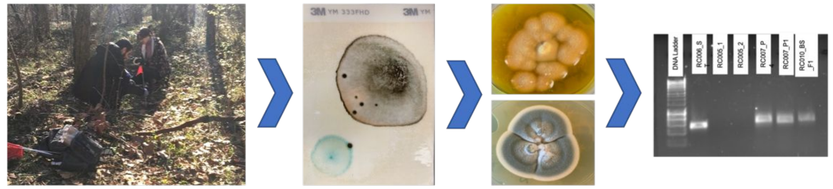
Abstract

The soil microbiome plays a vital role in the species composition and richness of plant communities in various ecosystems. (Van der Putten, 2017). A small amount of soil may contain thousands of microbial species that can drive plant community diversity via plant-microbial interactions (Bardgett and Van der Putten, 2014; O' Brien et al., 2005). A Course-Based Research instructional design was implemented to characterize the fungal community from samples obtained at the James River State Park System (JRPS) in Richmond, VA. This course is an introductory laboratory class for first-semester transfer students. One of our goals was to determine the feasibility of implementing a reliable molecular approach to identify fungi using DNA barcoding using the ITS rRNA region (O'Brien et al., 2005). In addition to student generated data, preliminary data was collected to assess the knowledge and technical skills that students gained during the course. Data that students generated, data on student knowledge and skills gains and potential research questions that students can generate based on this instructional design will be discussed.

Background

The James River is Virginia's largest river and its largest tributary to the Chesapeake Bay. Within the city of Richmond, the James River Park System (JRPS) extends along the fall line of the river and includes 14 sections of shorelines and islands. The park is a living laboratory that includes wilderness areas such as meadows, forests, rapids, and rocks. The close proximity of VCU to the park system provides an opportunity to for VCU students to study the rich diversity of plants, animals, and microbial communities in different habitats present.

Our project focuses on establishing a series of research modules that are integrated into a course that designed for first-year transfer students in our department. The research focus is the impact of the soil microbiome on native and invasive plants in the park and the potential impacts of climate change on these interactions. Students engaged in research to identify the microbial composition of soil samples from areas inhabited by native PawPaw (*Asiminia triloba*) trees using DNA barcoding. Student perceptions of knowledge and skills gains was also assessed.

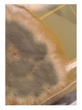


RC009_ST_F2 (Reedy Creek JRPS, top 4" soil core, fungal isolate 2)

Description	Total Score	Query Cover	E Value	% Identity	Accession
***Cladosporium cladosporioides* isolate UASWS2026** (ITS1, partial sequence; 5.8S rRNA gene, ITS 2)	963	94%	0.0	99.81%	MN833925.1
Dothideomycetes sp. isolate FT1O-2 (ITS1, partial sequence; 5.8S rRNA gene, ITS 2)	963	94%	0.0	99.81%	KX641948.1
***Cladosporium cladosporioides* isolate RP128_2_4 2** (ITS1, partial sequence; 5.8S rRNA gene, ITS 2)	963	94%	0.0	99.81%	KX067796.1

NOTES: The genus Cladosporium is an Ascomycete that is found worldwide, indoor and outdoor and *C. cladosporioides* is a known agent of plant disease.

RC009_ST_F3 (Reedy Creek JRPS, top 4" soil core, fungal isolate 3)



Description	Total Score	Query Cover	E Value	% Identity	Accession
***Penicillium multicolor* strain XQ24** (18s rRNA gene, partial sequence; , ITS 1, 5.8S rRNA gene and ITS 2)	1022	95%	0.0	99.82%	KU216726.1
***Penicillium sclerotiorum* strain FRR 1202** (18S rRNA, partial sequence; ITS1, 5.8S rRNA gene and ITS 2)	1013	94%	0.0	99.82%	AY373931.1
***Penicillium guanacastense* strain JP-NI2** (18S rRNA, partial sequence, ITS 1, 5.8S rRNA gene and ITS 2)	1007	94%	0.0	99.64%	KF991208.1

NOTES: Penicillium species are found worldwide both indoors and outdoors. Information about these specific species is limited.

RC0003_ST_F1 (Reedy Creek JRPS, soil core top, fungal isolate 1)

Description	Total Score	Query Cover	E Value	% Identity	Accession
Umbelopsis sp. Coc4005 (ITS1, 5.8S rRNA gene, ITS2 and LSU)	1077	89%	0.0	96.81%	AB986477.1
Umbelopsis sp. OGW5 (ITS1 partial seq 5.8S rRNA gene, ITS2)	1077	89%	0.0	96.81%	JX243816.1
Umbelopsis ramanniana strain (ITS1, 5,.8S rRNA gene, ITS2)	1077	89%	0.0	96.81%	HQ608138.1

NOTES: Common and abundant soil fungus. Saprotroph in soil, leaf litter, animal dung and Ascomycete fungi spore-bodies. May be an endophyte of some conifers.

RC0005_SB_F2 (Reedy Creek JRPS, Soil Core Bottom, fungal isolate2)

Description	Total Score	Query Cover	E Value	% Identity	Accession
Melanoporia nigra (ITS1, 5.8S rRNA gene, ITS2)	1138	94%	0.0	99.52%	KC543172.1
Melanoporia nigra (ITS1m 5,8S rRNA gene, ITS2)	1131	94%	0.0	99.52%	KC585356.1

NOTES: Basidiomycota, family Fomitopsidaceae, most species in this family are parasitic on woody plants and tend to cause brown rots.

RC0007_ST_F1 Reedy Creek JRPS, soil core top, fungal isolate 1)

Description	Total Score	Query Cover	E Value	% Identity	Accession
Cladosporium cladosporioides isolate UASWS2026 (ITS 1, partial sequence; 5.8S rRNA gene and ITS 2)	966	94%	0.0	100%	MN833925.1
Dothideomycetes sp. isolate FT1O-2 (ITS 1, partial sequence; 5.8S rRNA gene and ITS 2)	966	94%	0.0	100%	KX641948.1
Cladosporium sp. isolate FS9-1 (ITS1, partial sequence; 5.8S rRNA gene and ITS 2)	966	94%	0.0	100%	KX641947.1

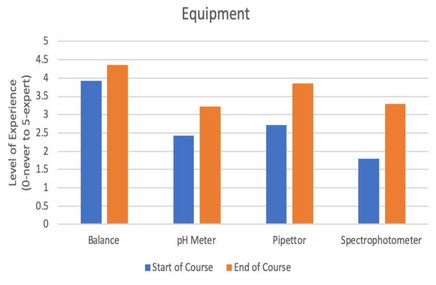
NOTES: Ascomycota, found worldwide, indoor and outdoor and is an agent of plant disease.

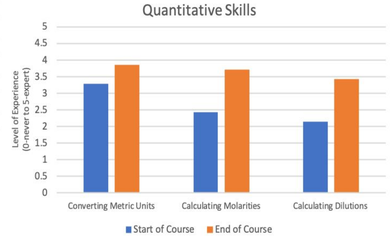
RC0002_F2 (PMK2) (Reedy Creek JRPS, soil core, fungal isolate 1)

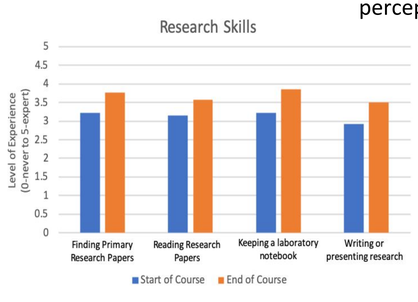
Description	Total Score	Query Cover	E Value	% Identity	Accession
Montagnula opulenta (ITS1, 5.8S rRNA gene, ITS2)	908	92%	0.0	99.23%	LT796834.1
Camarosporium sp. (ITS1m 5,8S rRNA fene, ITS2)	894	93%	0.0	97.92%	KT180771.1

NOTES: Both of these genera are part of the order *Pleosporales*. The majority of species in this order are saprobes of decaying plant material, however there are several species that have been identified to have roles as parasites, epiphytes or endophytes on living plants.

Impact of Course Experience on students perceptions of level of experience in three areas; Equipment, Quantitative Skills and Research Skills (n=16)







Methods

Soil cores (1.8cm dia X 20cm depth) were taken from 10 unique sites within the JRPS. 1g of each sample was suspended in 10 mls H_2O, diluted 1:10 5X, and 1ml of the final dilution placed on YM film media. After 1 week incubation at 25°C, individual fungal isolates were transferred to PD plates and allowed to grow for 7-14 days. DNA was extracted from a subset of the isolates and DNA barcoding using primers for ITS sequences (Forward:ITS1 5' –TCCGTAGGTGAACCTGCGG-3' Reverse ITS-4 5'-TCCTCCGCTTATTGATATGC-3')was used to identify a subset of the isolated fungi.

Students were asked to evaluate their equipment, quantitative and research skills on a brief survey at the beginning and end of the course.

Summary

Fungal DNA barcoding was used in a research module in a first-year transfer laboratory course. The method was simple, provided opportunities to learn and apply different laboratory and quantitative skills, and allowed students to apply existing knowledge to formulate hypotheses and interpret data. Students identified Ascomycota and Basidiomycota soil fungi that have different roles in the James River Park soil microbiome. Different primers or primer combinations will be used in the future to get a broader picture of the fungal diversity. In addition, plant primers will be used to identify seedlings and Bryophytes isolated from soil samples.

Overall students reported a gain in quantitative and research skills and also expressed increased confidence to use the equipment used in the laboratory. Modifications to course activities and additional survey questions will provide more experience with scientific equipment and research techniques as well as feedback on student perceptions of skills.

References

W. H.van der Putten, Science 355, 134 (2017)

R. D.Bardgett, W. H.van der Putten, Nature 515, 505 (2014)

O'Brien et al. Appl. Environ. Microbiol. 71, 2005