## Today's Schedule (in EST!)

11:00-11:20 Introduction to CartograPlant - Dr. Jill Wegrzyn

11:20-11:40 Introduction to Data Submission with TPPS/TPPSc - Emily Grau

11:40-12:00 Introduction to Data Collection/Mobile Phenotyping with TreeSnap - Dr.

Margaret Staton

12:00-12:15 Break

12:15-12:35 Behind the Scenes of CartograPlant - Environmental Layers and Data - Risharde

Ramnath

12:35-12:55 Analytics with CartograPlant (GWAS and GEA). Part 1 - Gabriel Barrett

12:55-1:15 Analytics with CartograPlant (GWAS and GEA). Part 2 – Dr. Irene Cobo-Simon

1:15-1:30 Q&A



# Data Analysis with CartograPlant: Background

AG2PI WORKSHOP | JULY 12, 2023

1.6k Plants

Me...

5.8k Plants

4k Plants

3.3k Plants

983 Plants

1k Plants

Gabriel Barrett, MSc.

Plant Dataset Sources

Y Filters

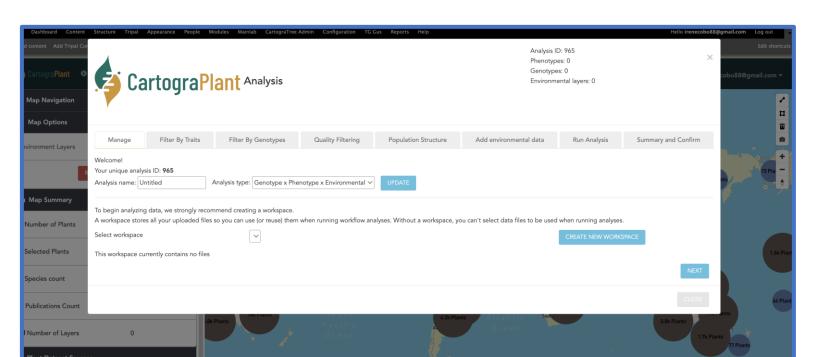
MSc. degree in biology student

University of Rhode Island



## AVAILABLE STEPS FOR DATA ANALYSIS IN CARTOGRAPLANT

- Data quality filtering
- Genotype Imputation
- Genome Wide Association Analysis (GWAS: GxP, GxE)
- Population Structure



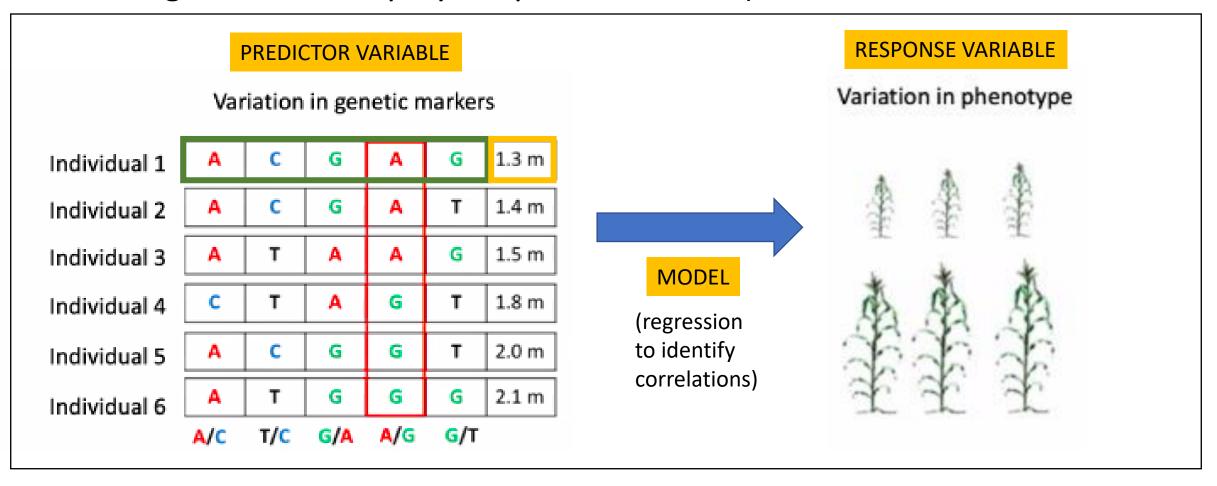
## HOW DOES CARTOGRAPLANT DO ANALYSIS?

- To perform data analysis, CartograPlant makes use of Galaxy (<a href="https://galaxyproject.org/">https://galaxyproject.org/</a>)
- Galaxy is a user-friendly platform that allows the bioinformatic analysis of high throughput data without the need of coding skills.



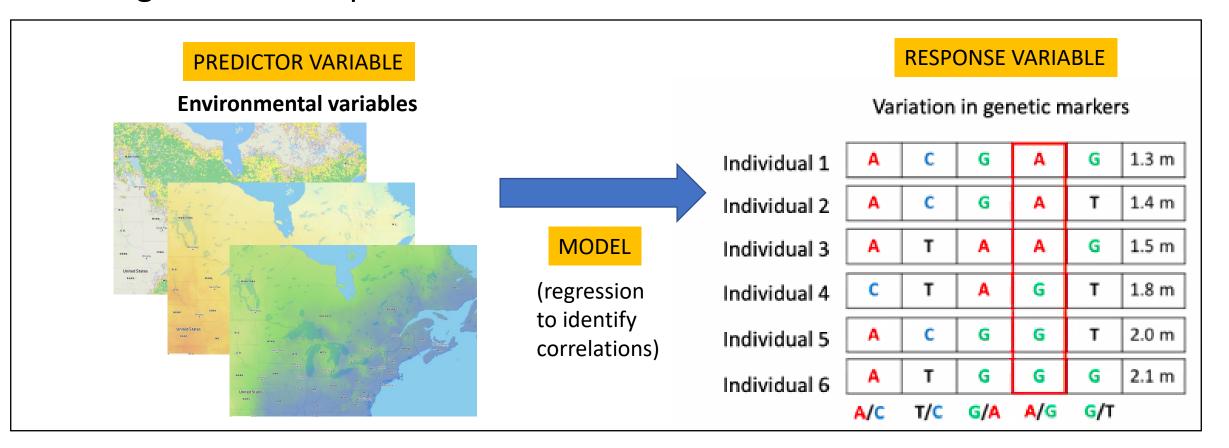
### WHAT IS GWAS?

• A genome-wide association study (GWAS) is an approach used in genetics research to associate specific genetic variations (usually single nucleotide polymorphisms or SNPs) with traits of interest.

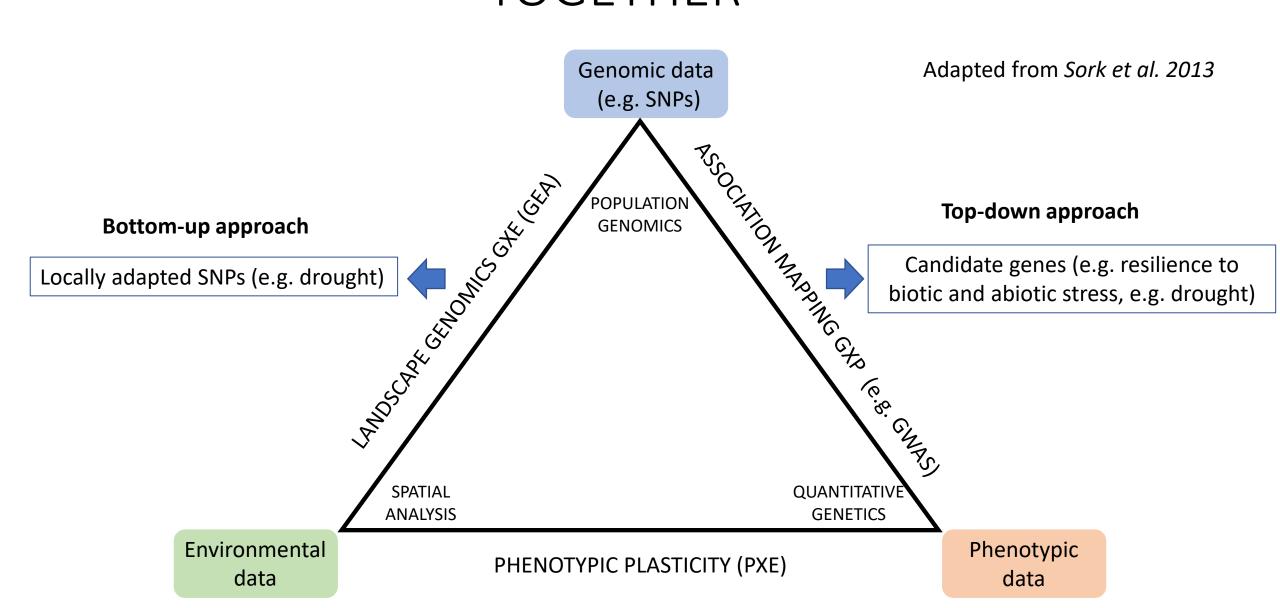


## WHAT IS LANDSCAPE GENOMICS?

• Landscape genomics (GxE) a.k.a. GEA is an approach used in genetics research to identify relationships between environmental factors and genetic variations (usually SNPs) to investigate the genetic adaptation of organisms in response to these environmental factors of interest.



## USEFULNESS OF PERFORMING GXP AND GXE TOGETHER



## USEFULNESS OF PERFORMING GXP AND GXE TOGETHER

Genomic data (e.g. SNPs)

Adapted from Sork et al. 2013

## Identify markers putatively under selection within that

environment or causing the expression of that phenotype

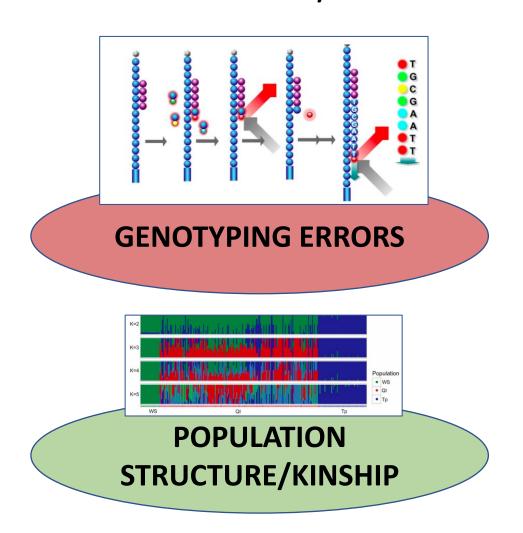
SPATIAL ANALYSI QUANTITATIVE GENETICS

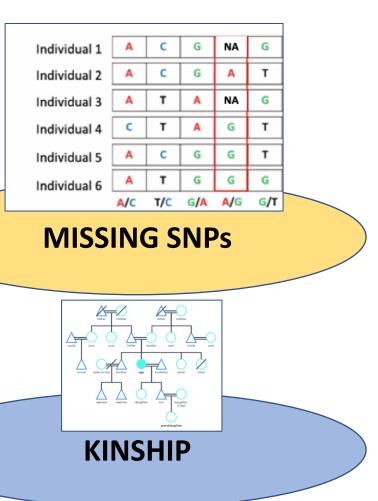
Environmental data

PHENOTYPIC PLASTICITY (PXE)

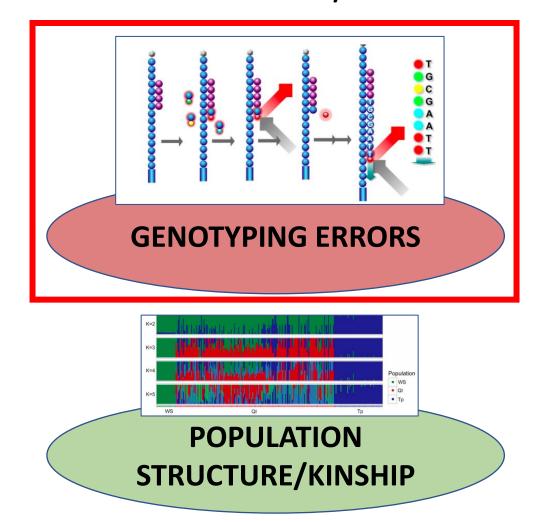
Phenotypic data

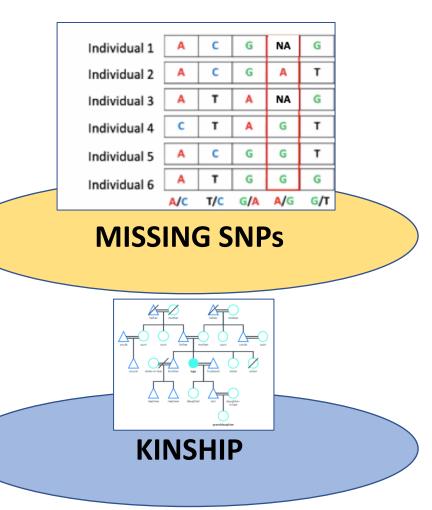
## FACTORS THAT CAN AFFECT THE ACCURACY OF THE GWAS/GEA RESULTS





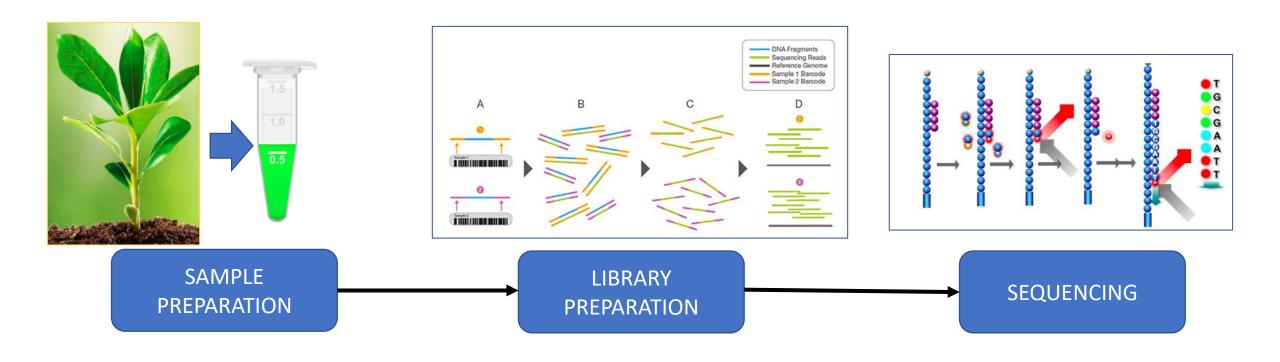
## FACTORS THAT CAN AFFECT THE ACCURACY OF THE GWAS/GEA RESULTS





## GENOTYPING ERRORS

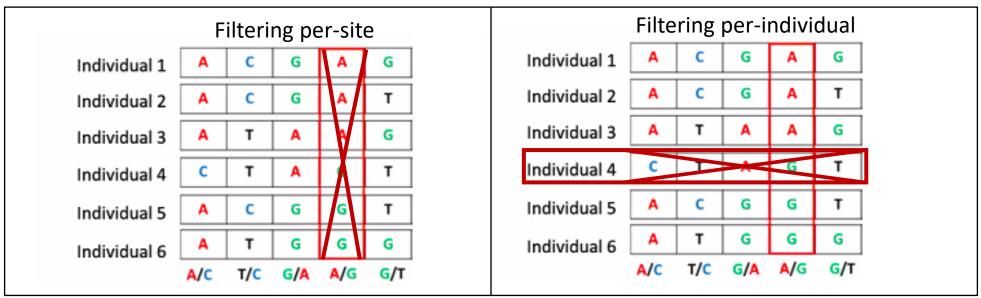
• A **genotype error** describes when the **observed genotype** does not correspond with the **true genotype** 



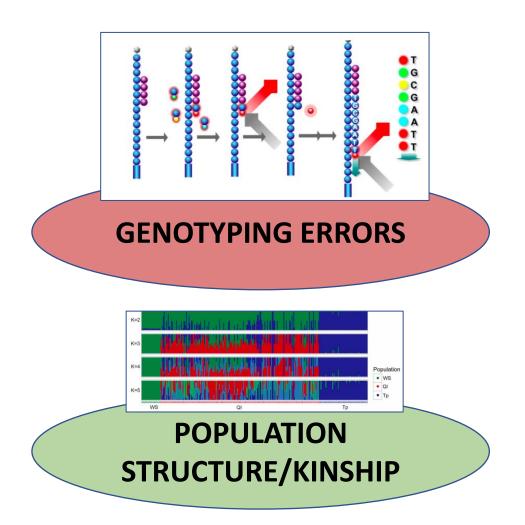
### GENOTYPING ERRORS

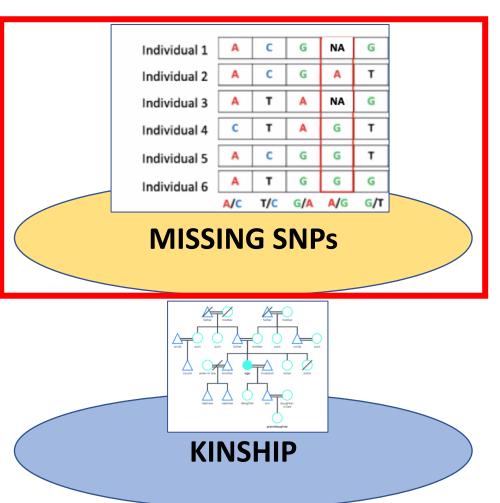
#### **SOLUTION:**

- Filtering Data Based on Quality Metrics (prior to analysis)
- Remove markers and individuals:
  - Low quality scores
  - High levels of missing genotypes
  - Minimum genotype counts (minor allele count)



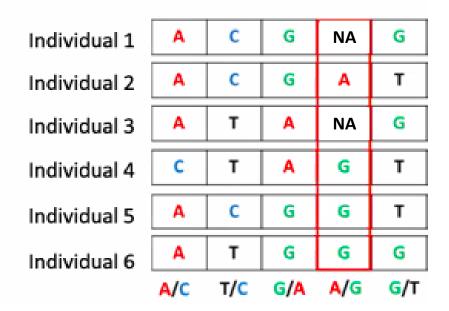
## FACTORS THAT CAN AFFECT THE ACCURACY OF THE GWAS/GEA RESULTS





## MISSING SNPs

- Even after performing data quality filtering, missing SNPs may remain in our dataset.
- Missing SNPs can reduce the statistical power of our GWAS/GEA, especially in the case of low density SNP panels.

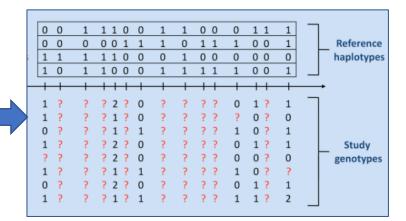


## MISSING SNPs

#### **SOLUTION**

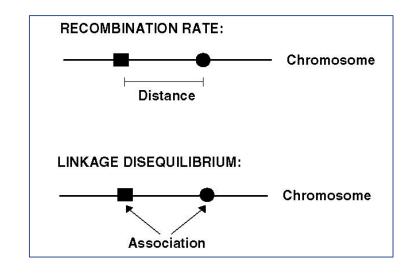
#### Imputation of lacking SNPs

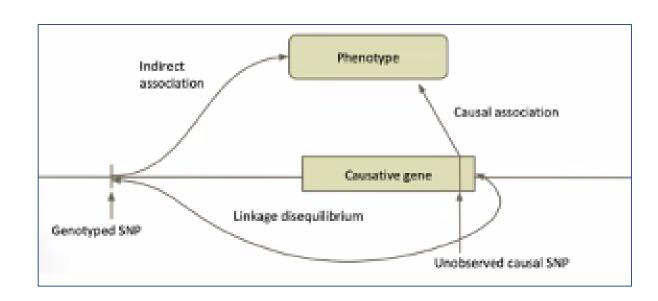
- This imputation or inference of the lacking SNPs can be performed by different methods:
  - Using a Reference panel of SNPs (same species, similar population): Non-suitable for non-model species
  - Based on Linkage disequilibrium: Looking for SNPs in linkage disequilibrium with the lacking SNPs: suitable for non-model species



## WHAT IS LINKAGE DISEQUILIBRIUM (LD)?

- Non-random association between two or more loci in the population (two or more loci that tend to be inherited together)
- The closer two markers are, the higher LD (due to recombination)
- It is also the basis of the identification of causative/locally adapted genes in GWAS/GEA





### MISSING SNPs

#### **SOLUTION**

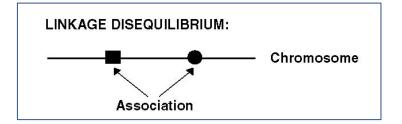
#### LinkImputeR

- **Tests** for different combinations of SNP data **quality filtering thresholds** (aka "Cases").
- <u>Objective</u>: Maximize the quantity and quality of the resulting SNPs, while maintaining accuracy.
- Imputation for non-model organisms
  - No requires ordered markers or a reference panel
  - LD-kNN Imputation, based on Linkage Disequilibrium (LD)

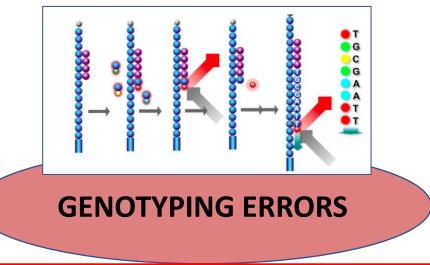
#### **CASES**

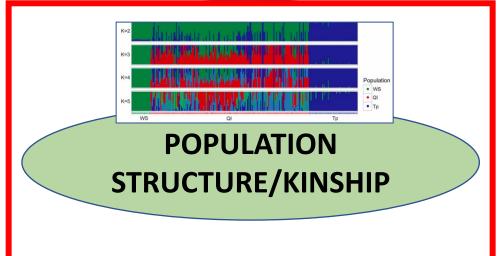
						1
Name	Samples	Positio	ns	Accurac	y Correlation Filters Additional	
Case 1	100	343652	0.9684	0.9428	PositionMiss(0.7),SampleMiss(0.7),MAF(0.01)	Depth(2)
Case 2	100	422531	0.9669	0.9425	PositionMiss(0.8),SampleMiss(0.8),MAF(0.01)	Depth(2)
Case 3	100	930660	0.9558	0.9189	PositionMiss(0.9),SampleMiss(0.9),MAF(0.01)	Depth(2)
Case 4	100	325977	0.9639	0.9405	PositionMiss(0.7),SampleMiss(0.7),MAF(0.02)	Depth(2)
Case 5	100	404026	0.9618	0.9360	PositionMiss(0.8), SampleMiss(0.8), MAF(0.02)	Depth(2)
Case 6	100	907122	0.9494	0.9095	PositionMiss(0.9),SampleMiss(0.9),MAF(0.02)	Depth(2)
Case 7	100	252294	0.9788	0.9578	PositionMiss(0.7),SampleMiss(0.7),MAF(0.01)	Depth(4)
Case 8	100	316696	0.9750	0.9464	PositionMiss(0.8),SampleMiss(0.8),MAF(0.01)	Depth(4)
Case 9	100	808533	0.9616	0.9244	PositionMiss(0.9),SampleMiss(0.9),MAF(0.01)	Depth(4)
Case 10	100	216859	0.9740	0.9527	PositionMiss(0.7),SampleMiss(0.7),MAF(0.02)	Depth(4)
Case 11	100	277145	0.9736	0.9484	PositionMiss(0.8),SampleMiss(0.8),MAF(0.02)	Depth(4)
Case 12	100	761913	0.9571	0.9218	PositionMiss(0.9),SampleMiss(0.9),MAF(0.02)	Depth(4)
Case 12	100	761913	0.9571	0.9218	PositionMiss(0.9), SampleMiss(0.9), MAF(0.02)	Depth(4)

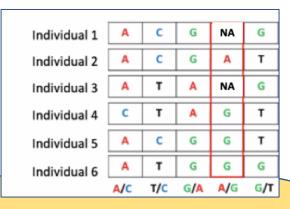


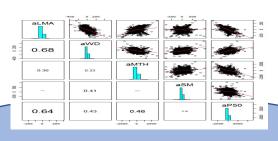


## FACTORS THAT CAN AFFECT THE ACCURACY OF THE GWAS/GEA RESULTS









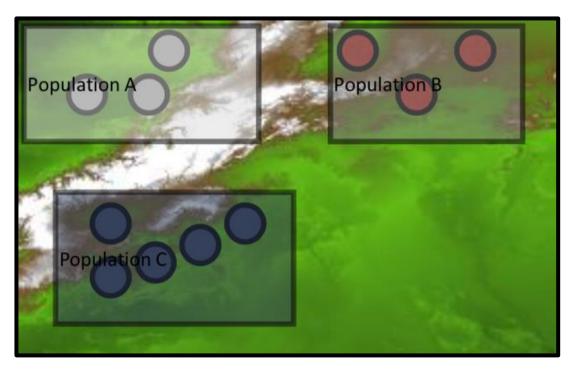
MISSING SNPs

**MULTICOLLINEARITY** 

## POPULATION STRUCTURE/KINSHIP CORRECTION

#### **SOLUTION:**

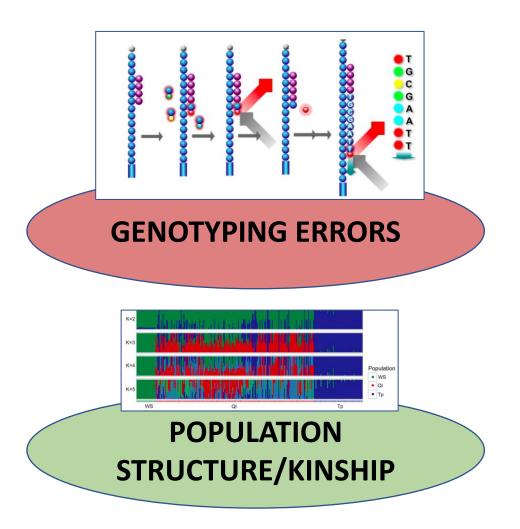
- Account for confounding variables in the model
- Objective of GWAS/GEA: To identify alleles associated with the traits/environmental variables of interest.
- Reduce spurious associations or false positives in GWAS/GEA
  - **Population structure:** patterns caused by by isolation by distance, genetic drift, kinship etc.
- These hidden sample structures can act as confounding variables, leading to false positives or spurious associations



#### **Example of confounding variable**

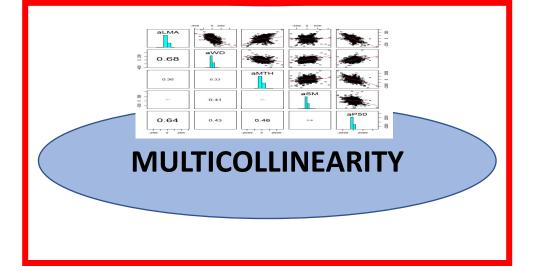
Sometimes the population structure **mimic** the environmental variable of interest

## FACTORS THAT CAN AFFECT THE ACCURACY OF THE GWAS/GEA RESULTS





MISSING SNPs

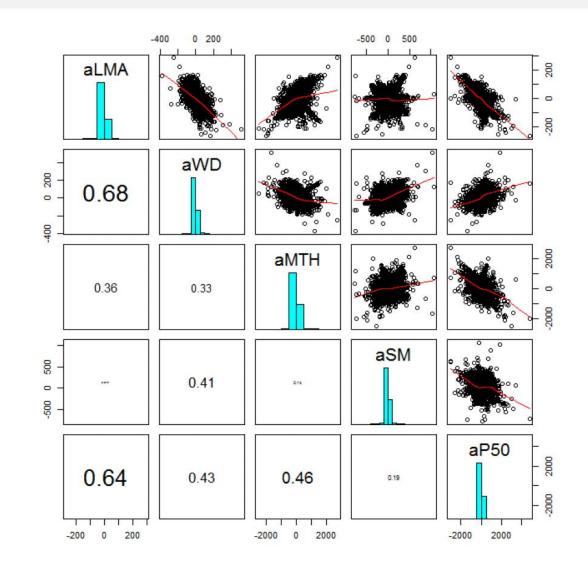


### OTHER GXE ISSUES: MULTICOLLINEARITY

• **Collinearity** refers to a linear relationship between two explanatory variables (environmental variables in our case).

#### **SOLUTION**

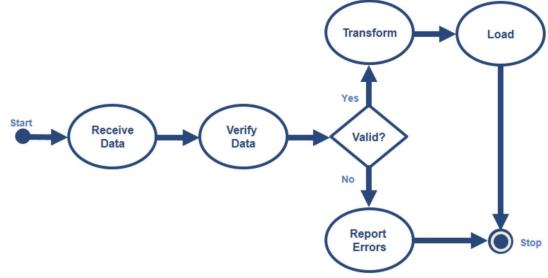
- Variable selection is a critical preliminary step in ecological modelling, including landscape genomics, to avoid overfitting and collinearity
- To help with this variable reduction decisions, CartograPlant offers Pearson correlation analyses of the environmental variables of interest, plotted in pairwise scatterplot matrices



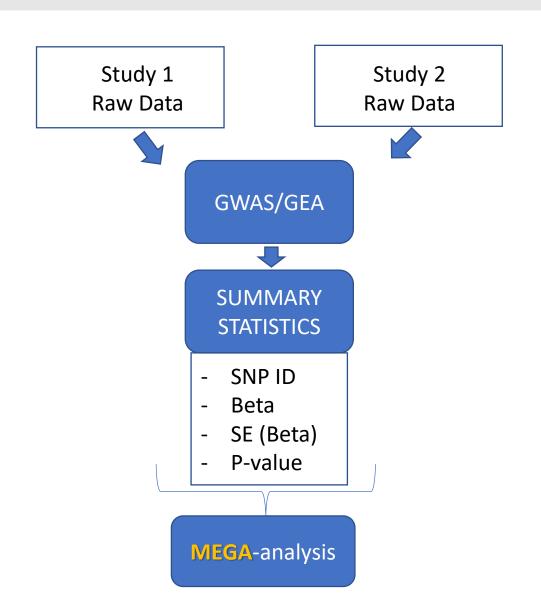
### CARTOGRAPLANT ANALYTIC WORKFLOWS OBJECTIVES

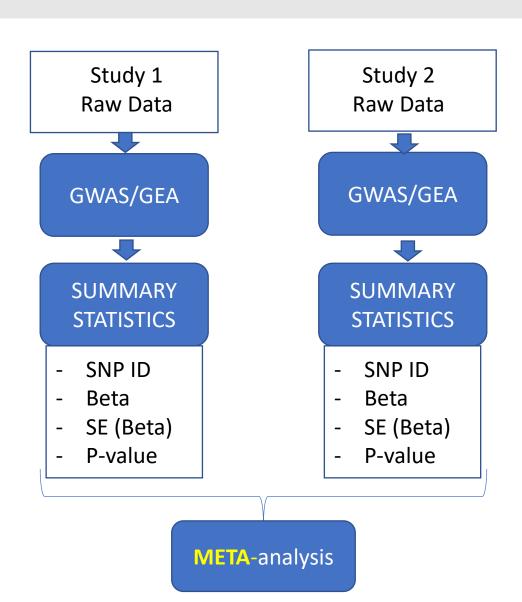
- Genome Wide Association Analysis (GWAS, G X P)
- Landscape genomics (G X E)
- Mega/meta-analysis



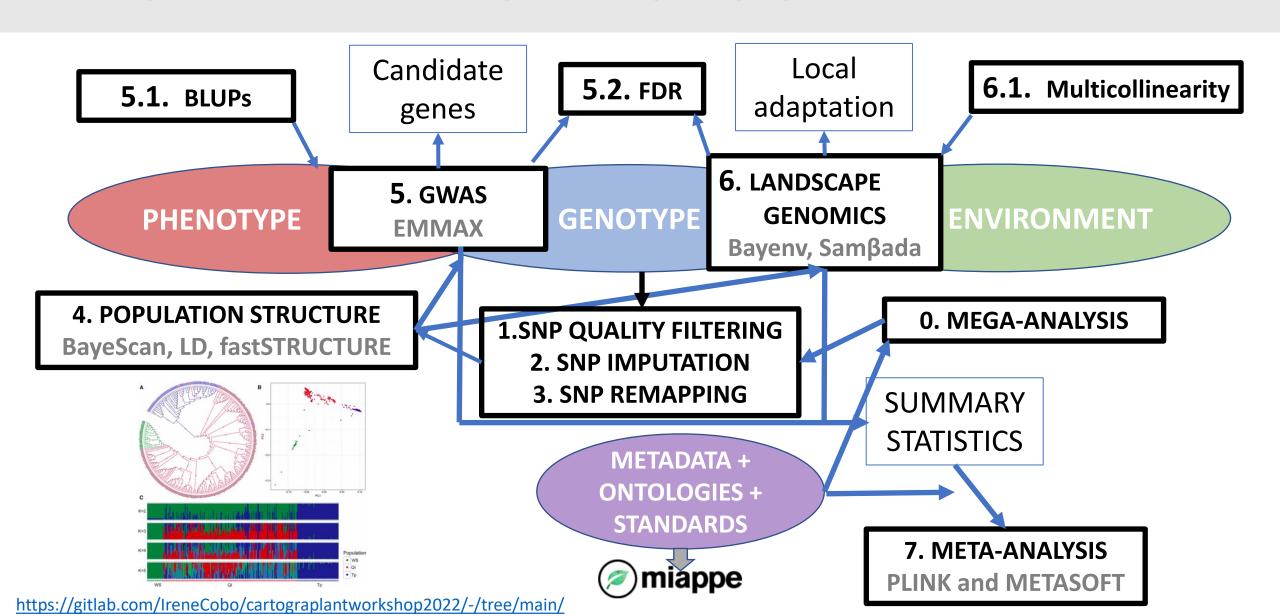


## WHAT ARE META-ANALYSIS AND MEGA-ANALYSIS?

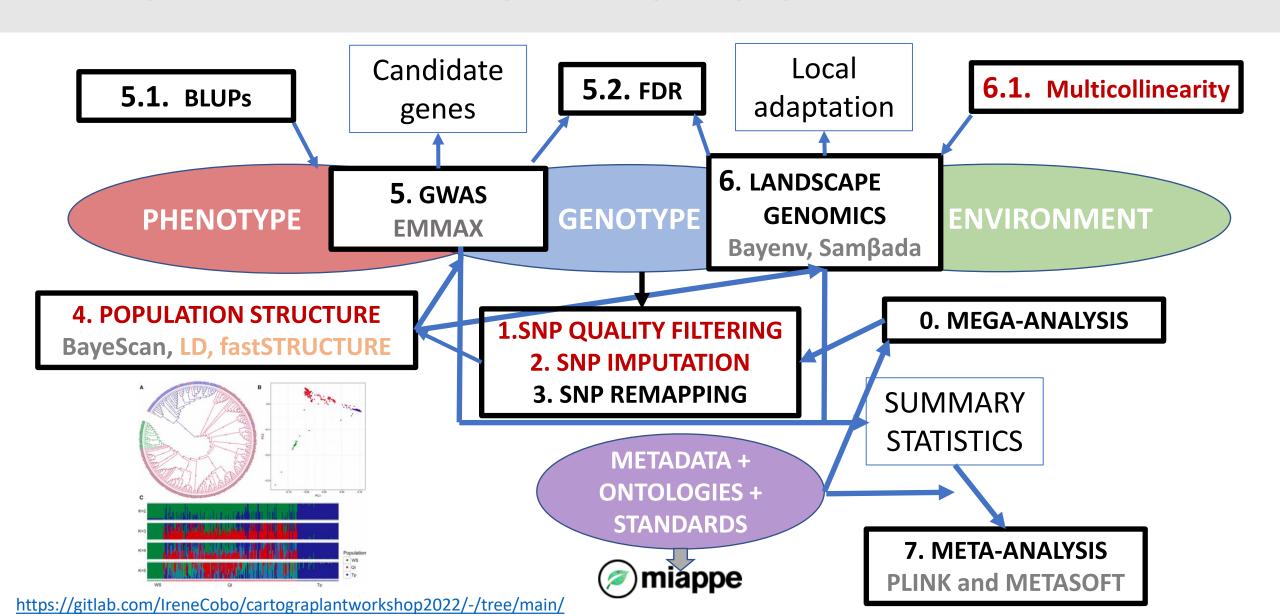




## CARTOGRAPLANT WORKFLOWS OVERVIEW

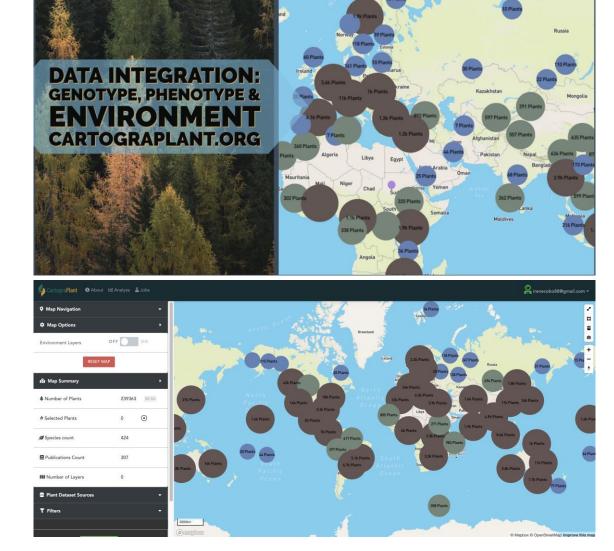


## CARTOGRAPLANT WORKFLOWS OVERVIEW



## CONCLUSIONS

- Cartograplant provides flexible analytic workflows
- Native support for mega and metaanalysis
- Is currently being maintained by a team of software developers
  - Constantly adding new features and updates to stay up to date with current research standards
- A centralized platform to integrate, visualize and analyze high-throughput biological data for plants



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1:15-1:30 Q&A

## Analytics with CartograPlant (GWAS and GEA): Demo



Irene Cobo Simón, PhD.

Postdoctoral Research Associate/Postdoctoral Fellow

CSIC CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS





University of Connecticut/Institute of Forest Science (ICIFOR-INIA, CSIC), Spain

#### **CARTOGRAPLANT WEBSITE**



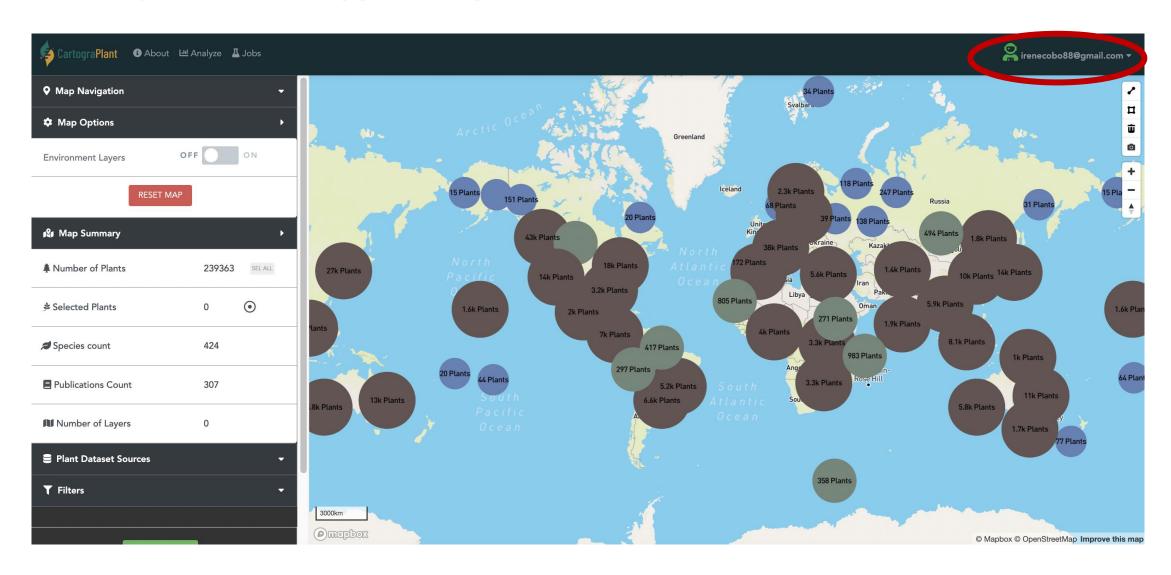
Long-lived and sessile plant species serve as ideal models to assess population structure and adaptation to the environment. Despite the availability of comprehensive data, the researchers who study them are challenged to integrate data describing genotype, phenotype, and the environment. Towards this goal, the web application CartograTree (now known as CartograPlant), was designed and implemented as an open repository and open-source analytic web-based framework for all three.







Detailed documentation, tutorials and resources



https://cartograplant.org/

#### **LEFT PANEL**

The action panel from which users can interact with the map and the plants. It is located at the left of the screen



#### **LEFT PANEL**

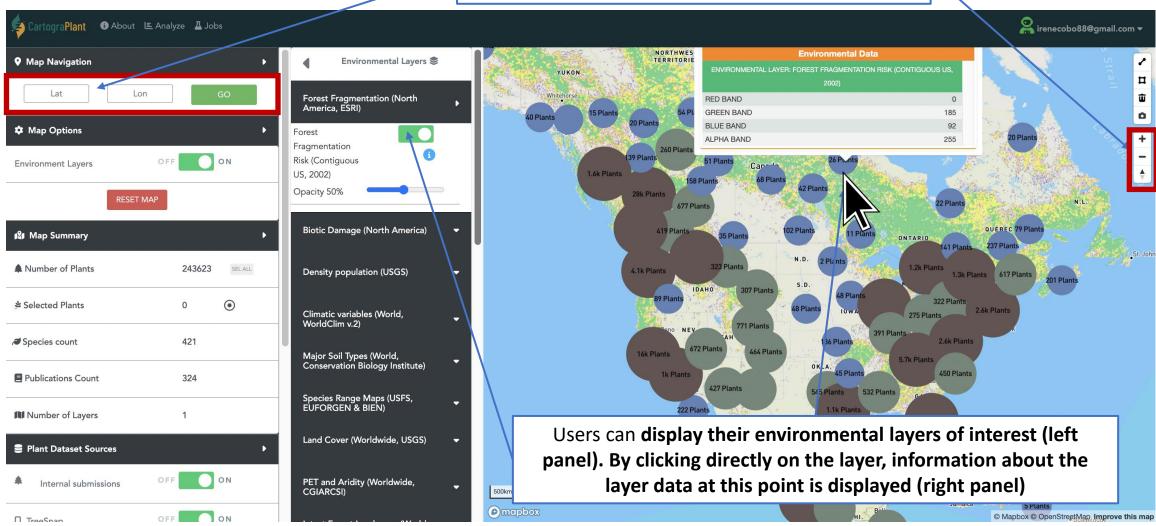
The action panel from which users can interact with the map and the plants. It is located at the left of the screen



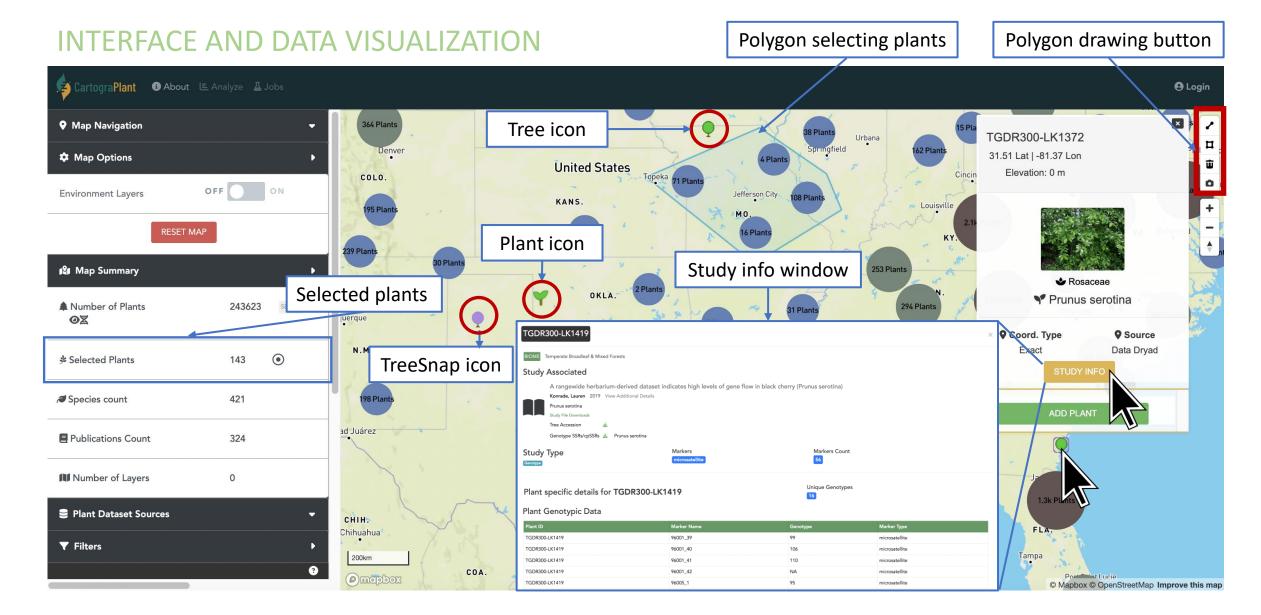
#### **RIGHT PANEL**

An interactive map, showing the plants and the environmental layers that were selected on the left panel

Users can **navigate** on the map by using the **zoom buttons** or by **typing the coordinates of interest** 



https://cartograplant.org/

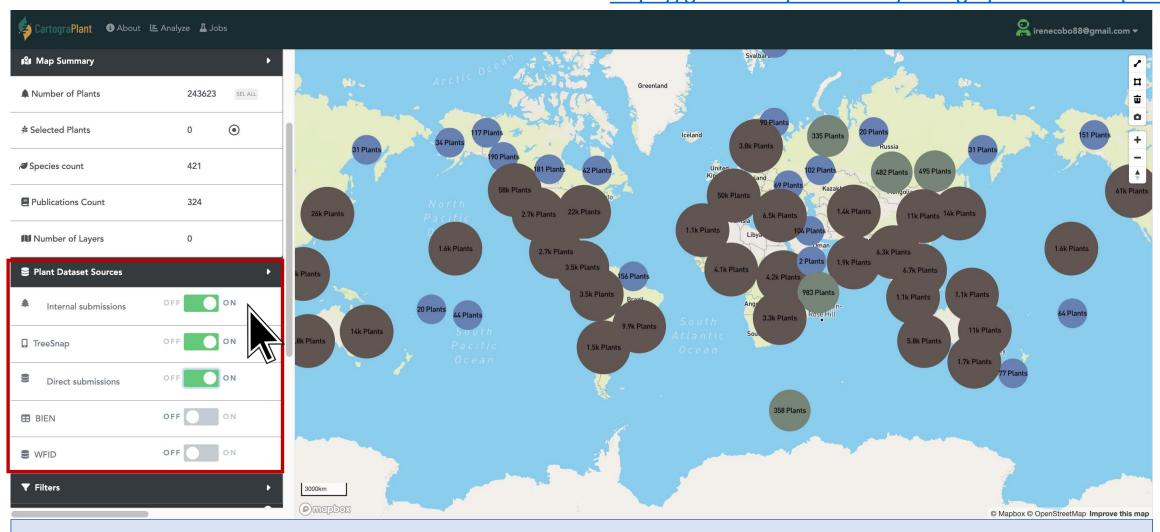


https://cartograplant.org/

https://gitlab.com/IreneCobo/cartograplantworkshop2022/

### https://cartograplant.org/

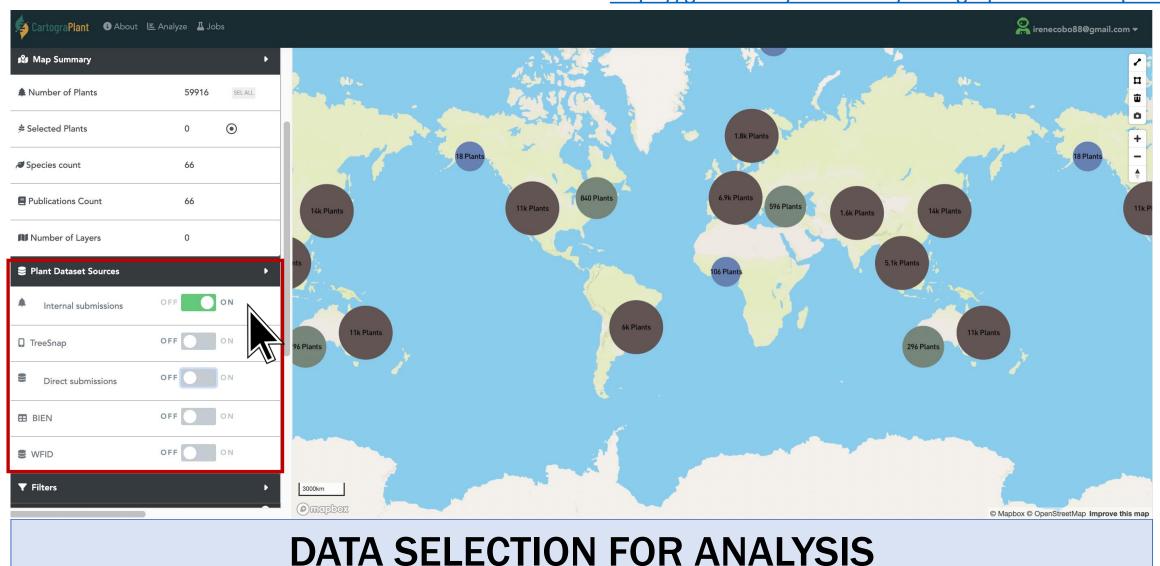
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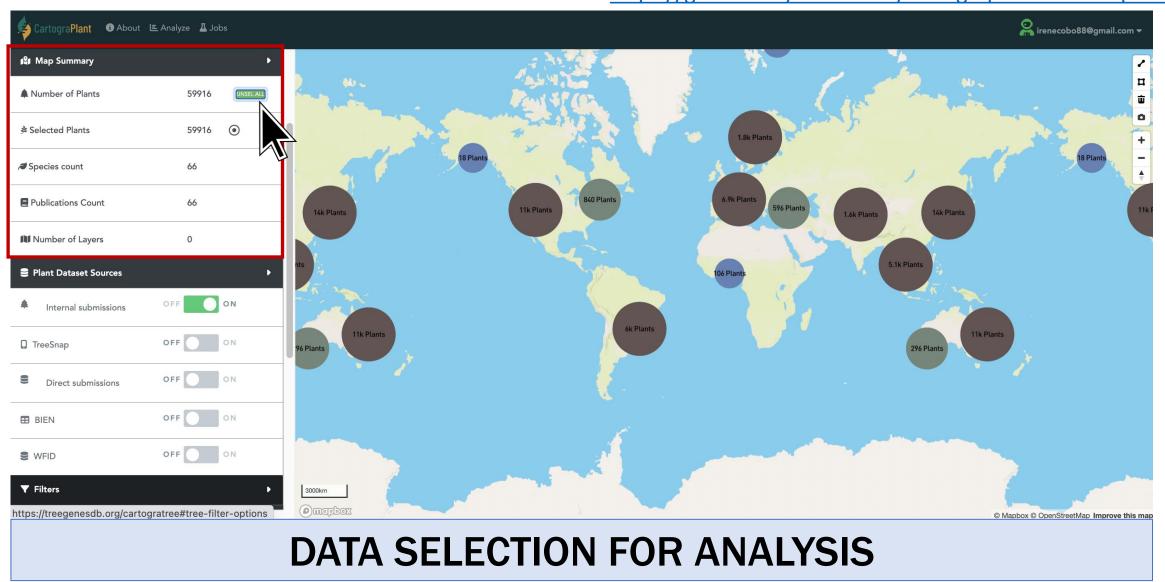
#### DATA SELECTION FOR ANALYSIS

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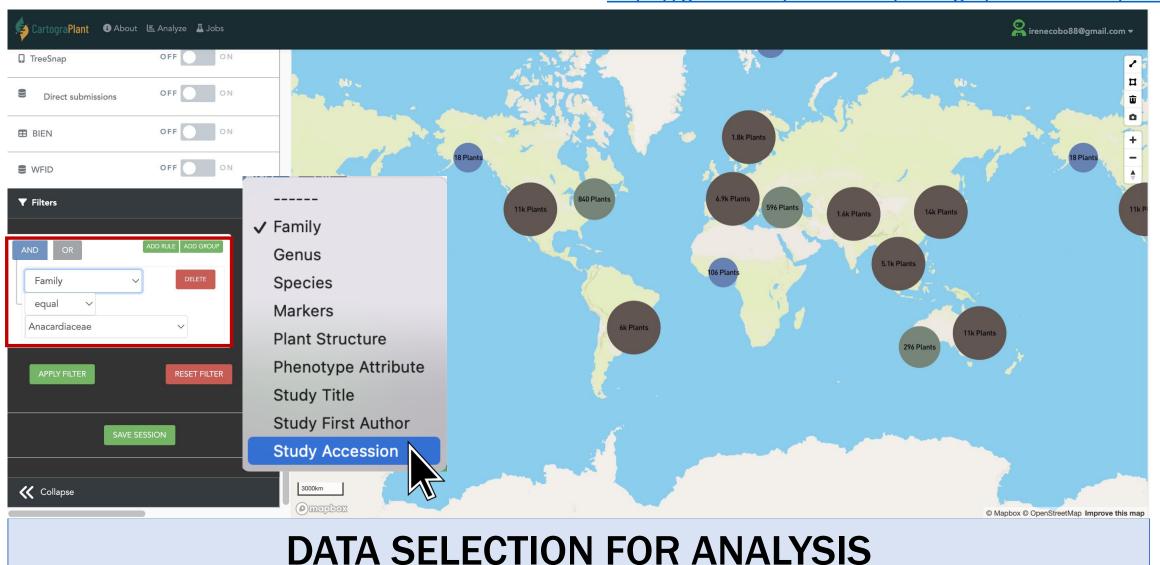
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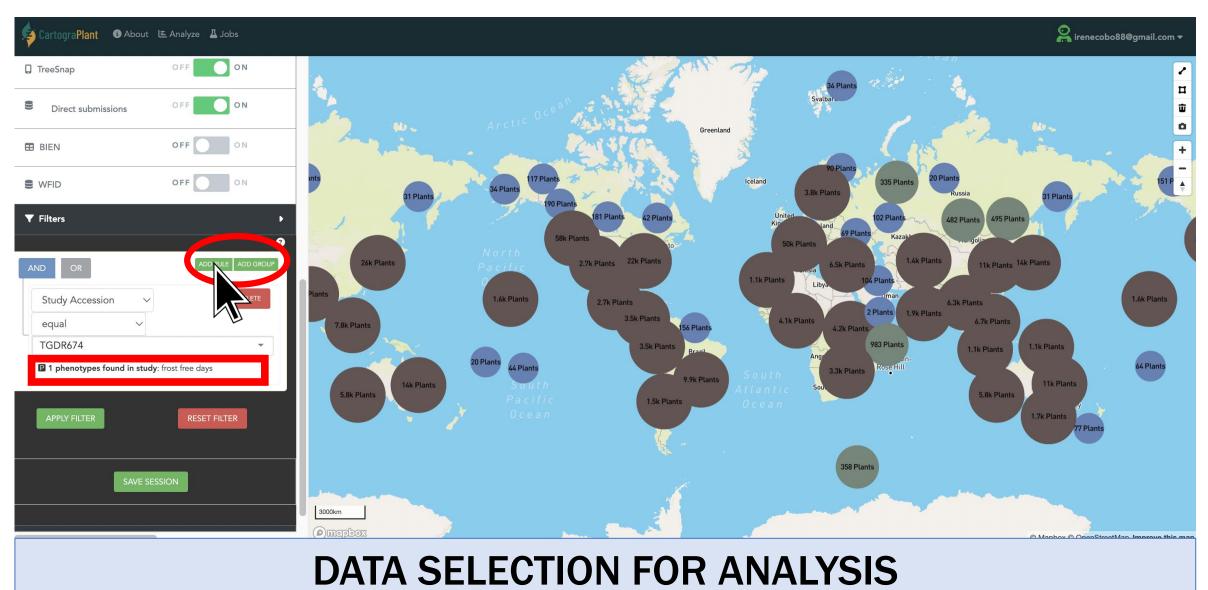


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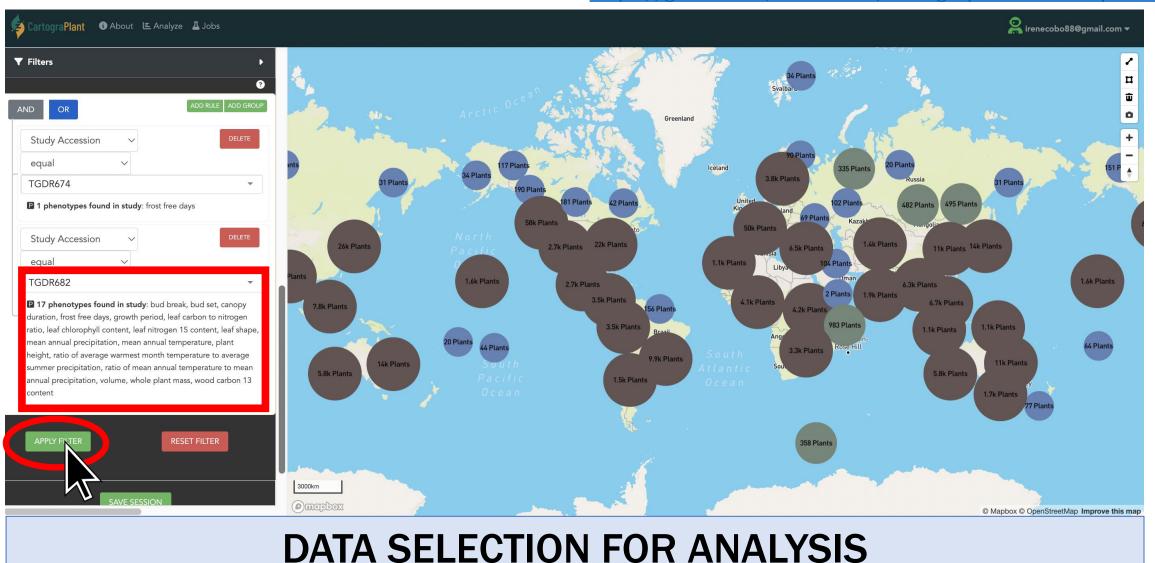


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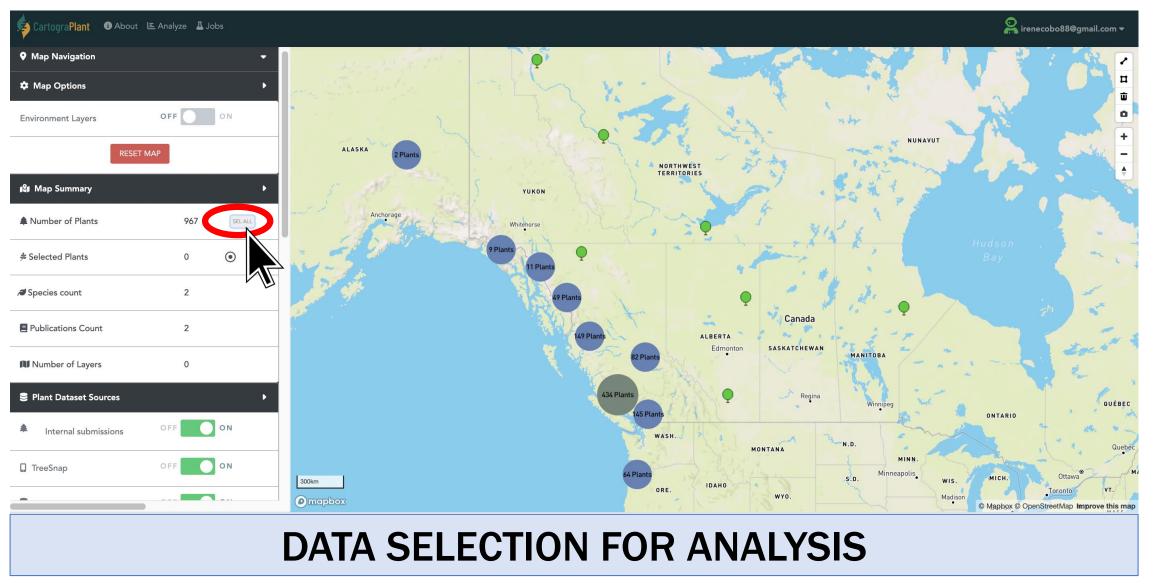
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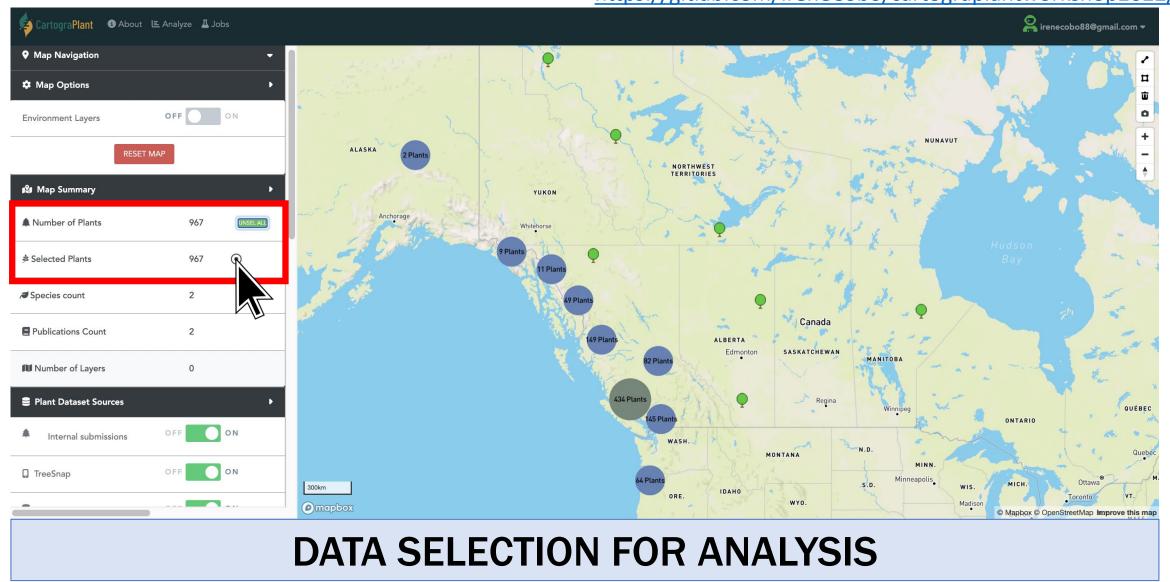
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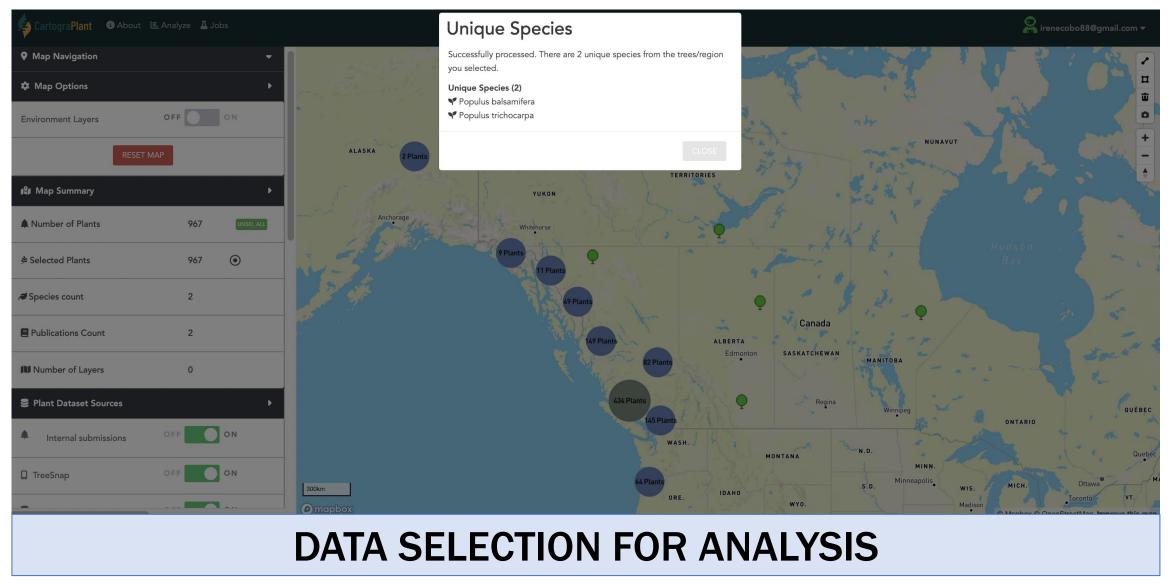
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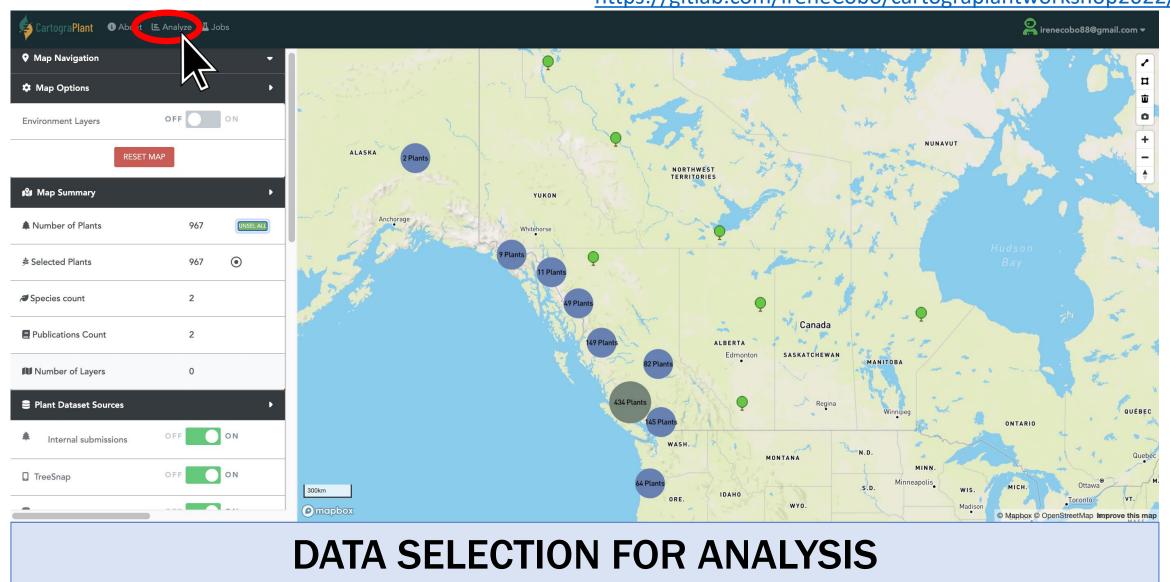
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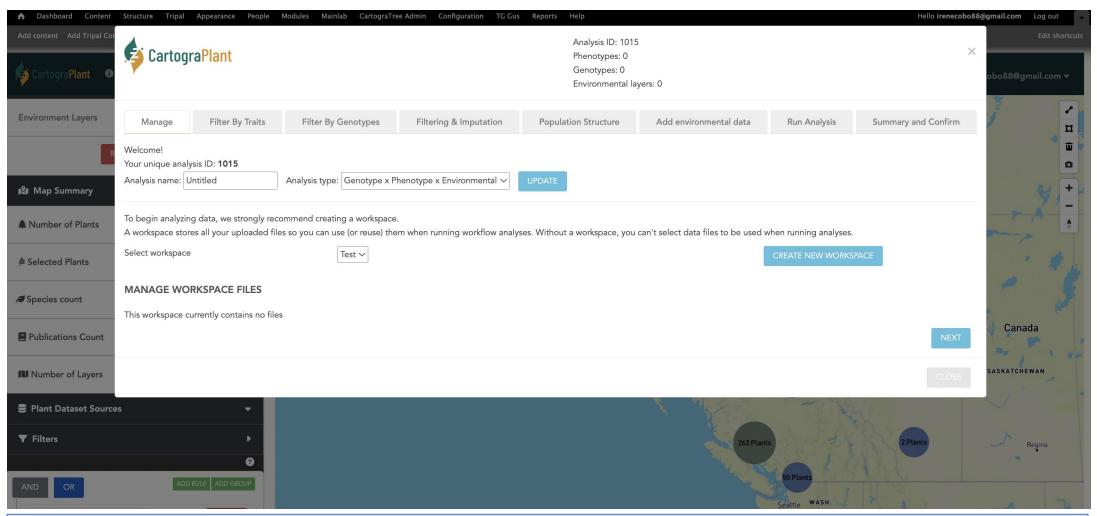


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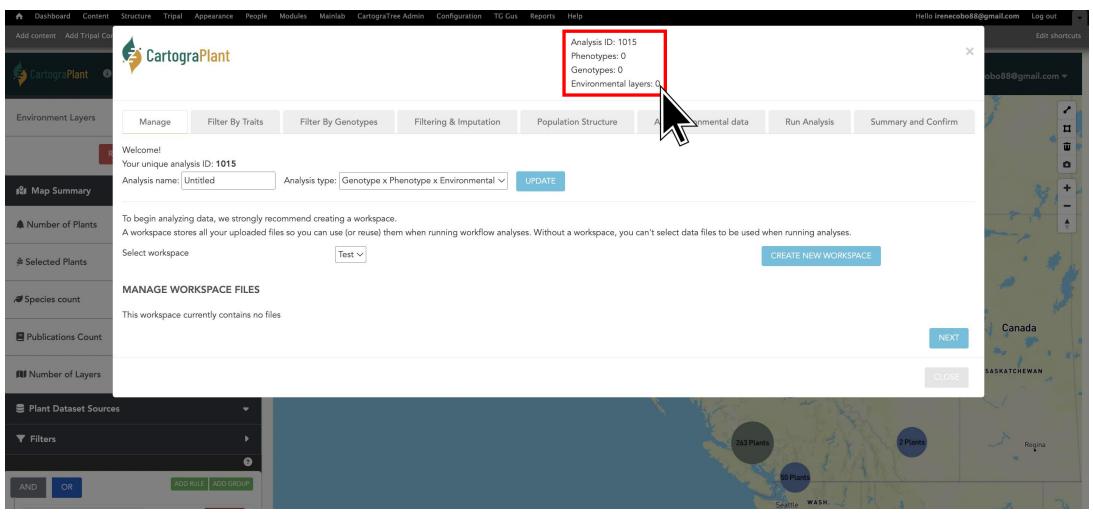
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DATA ANALYSIS: WORKSPACE CREATION

# https://cartograplant.org/

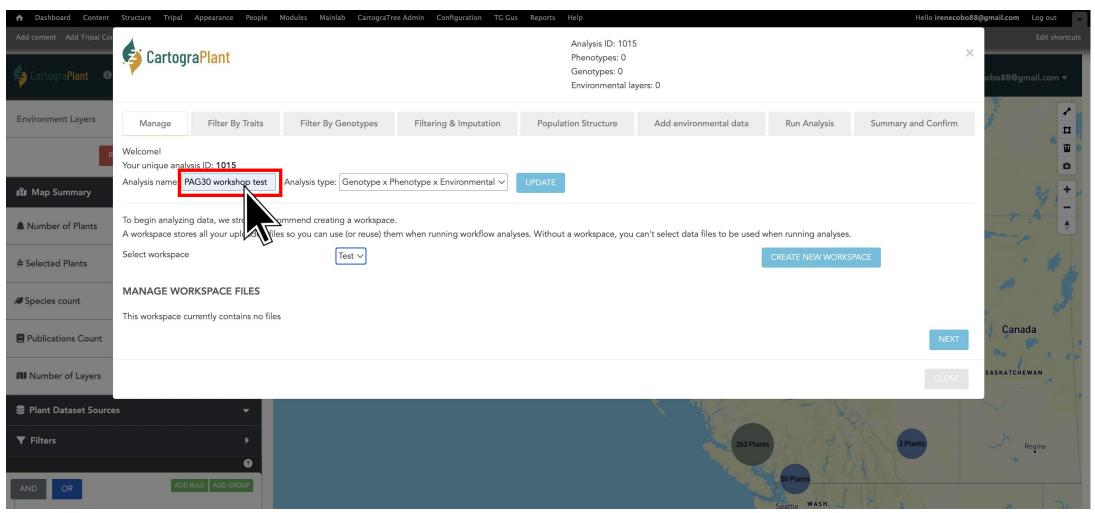
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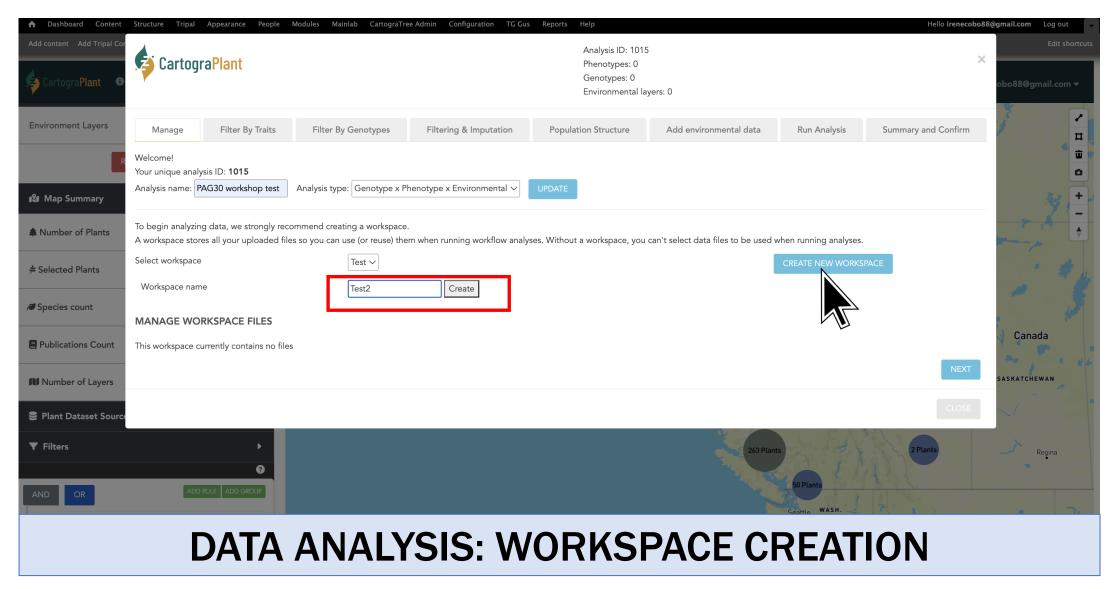
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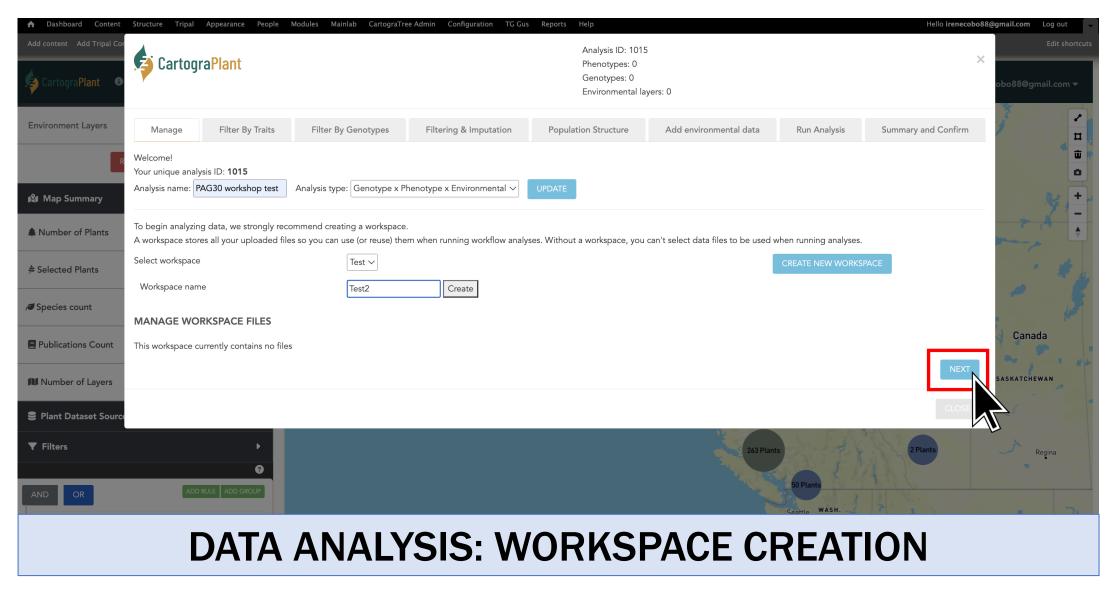


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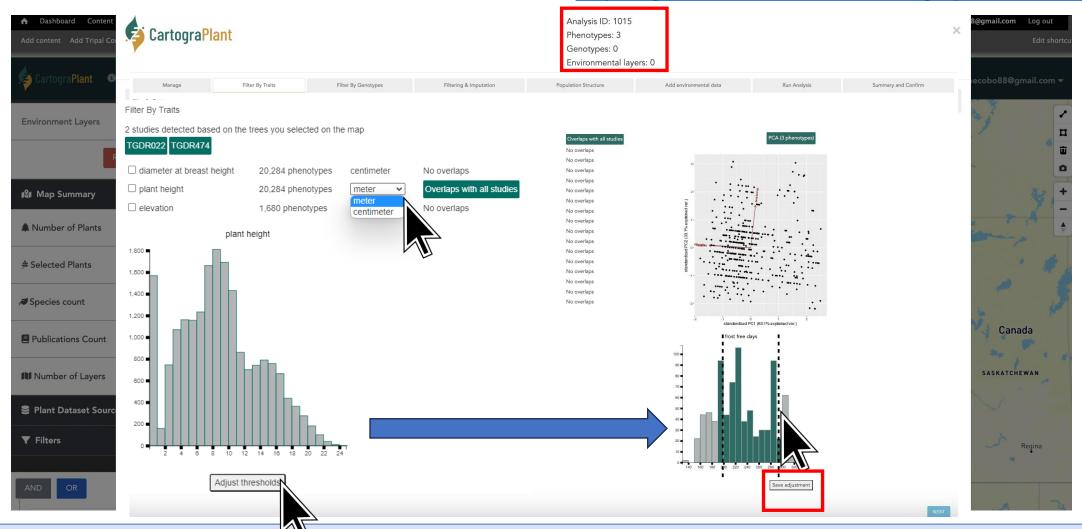


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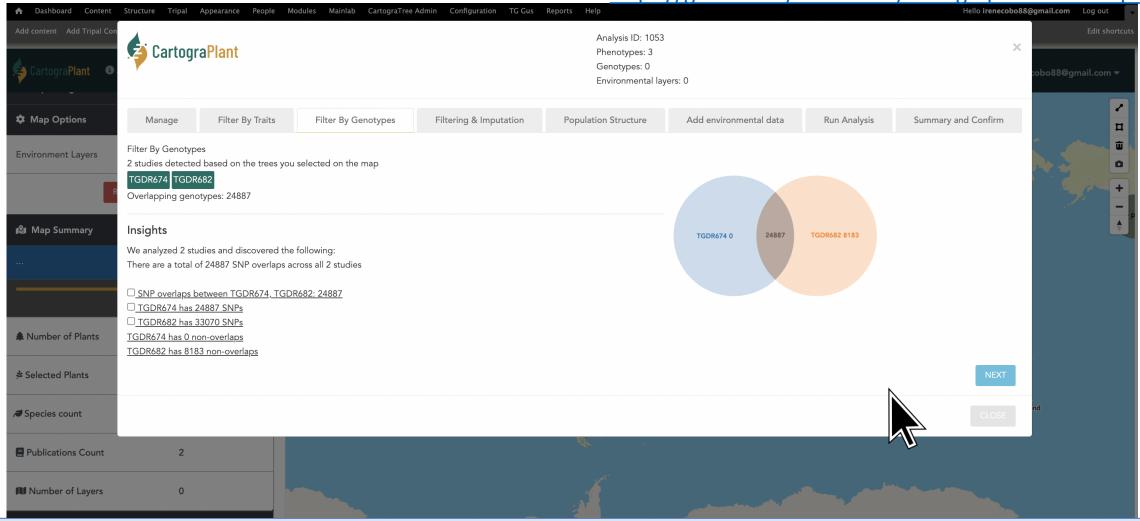
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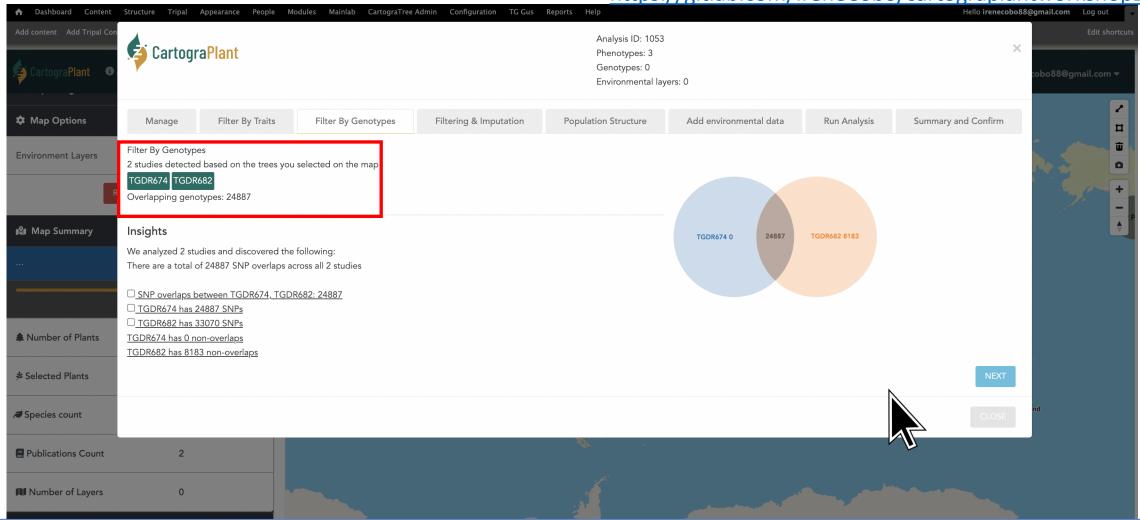
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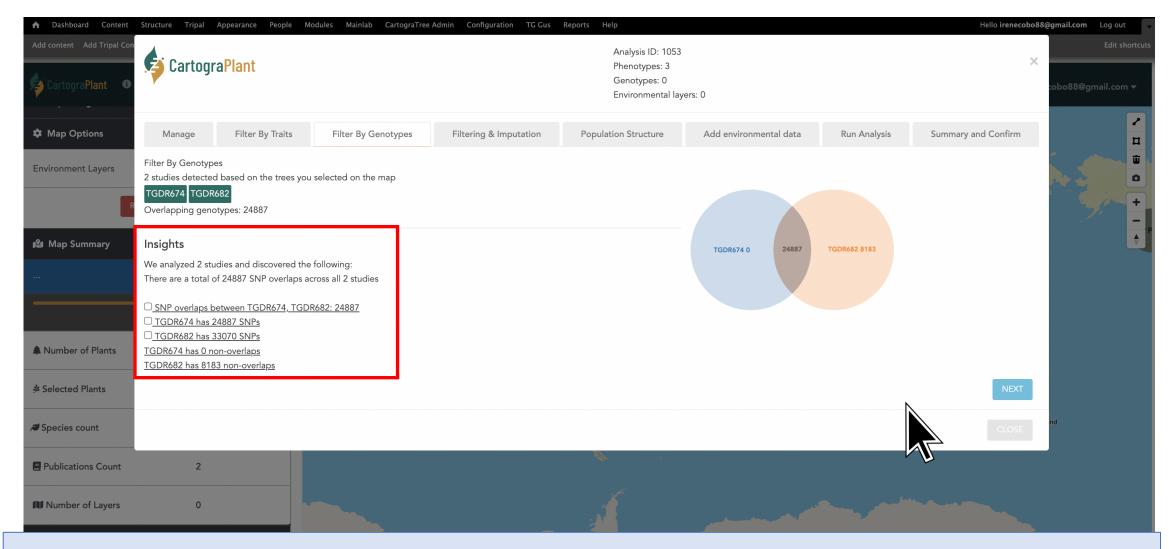


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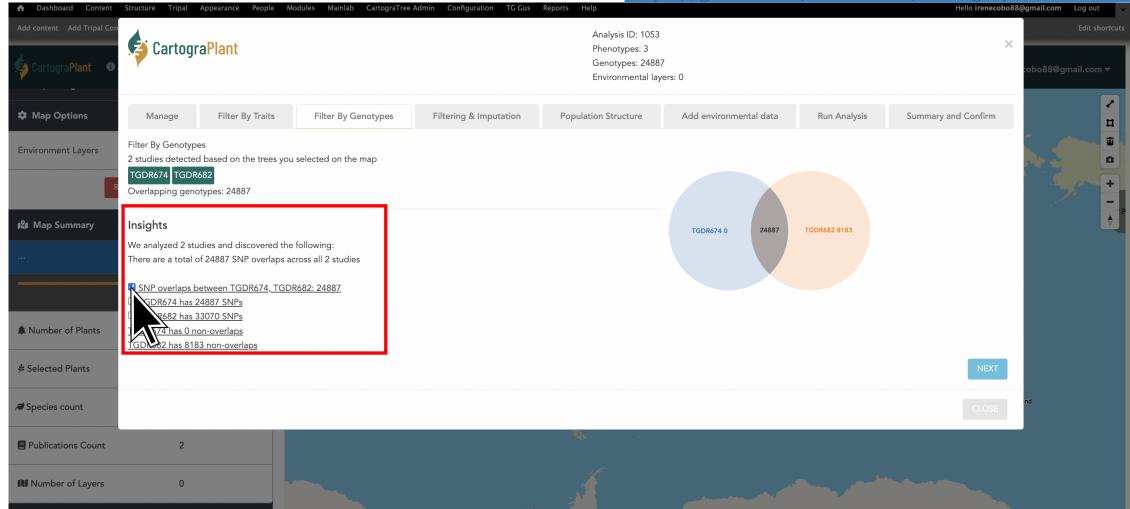


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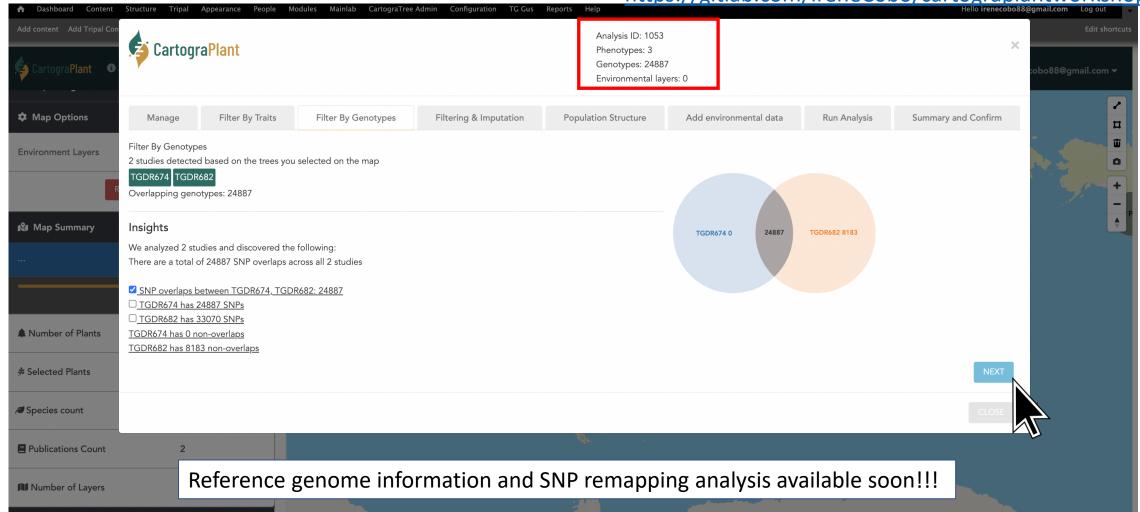
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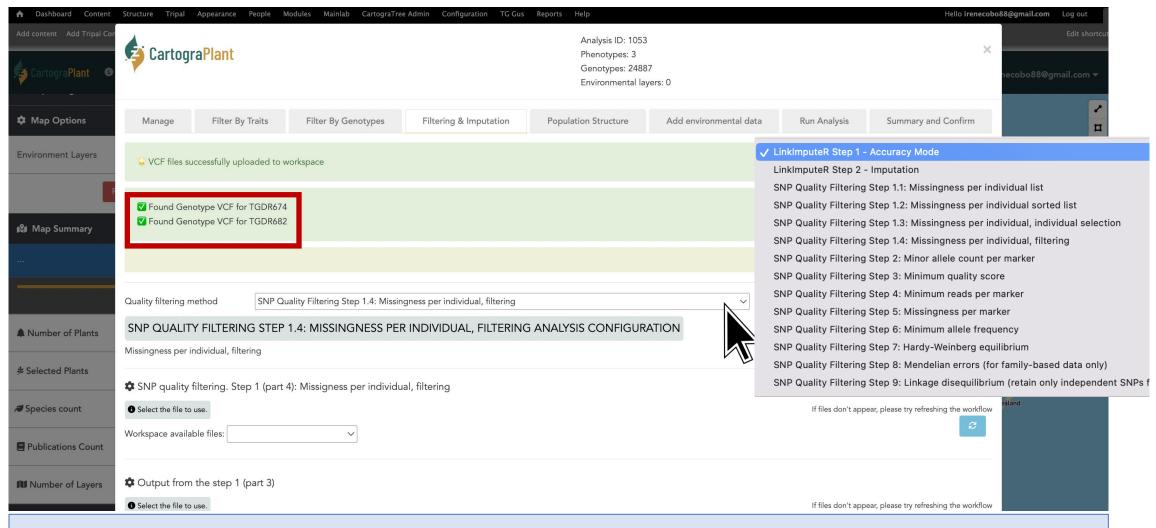
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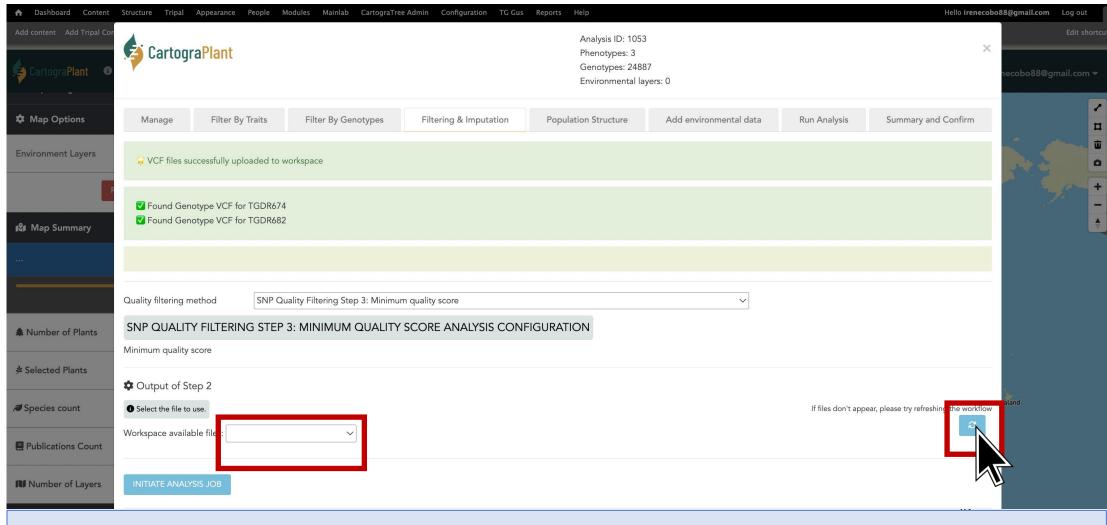
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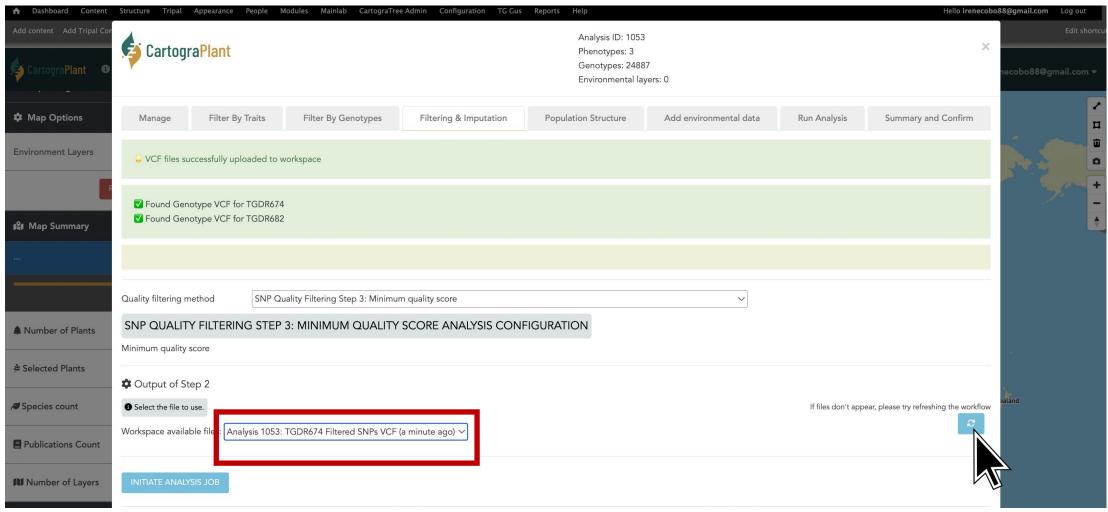
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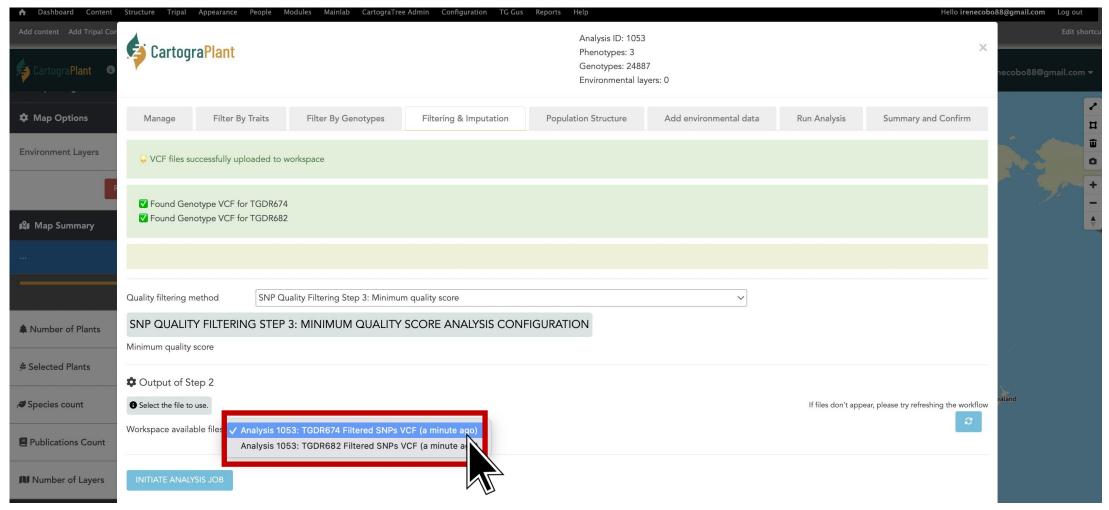
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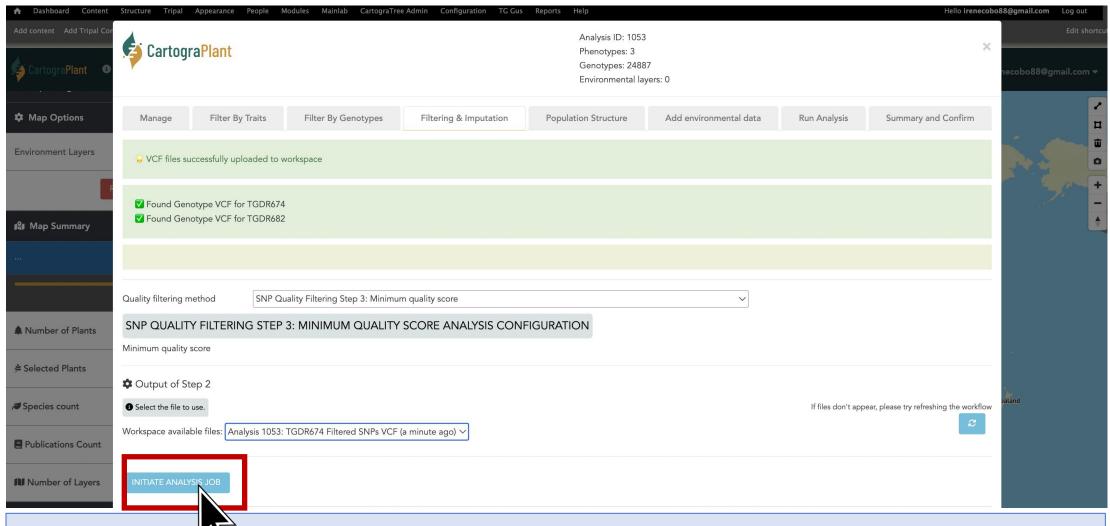
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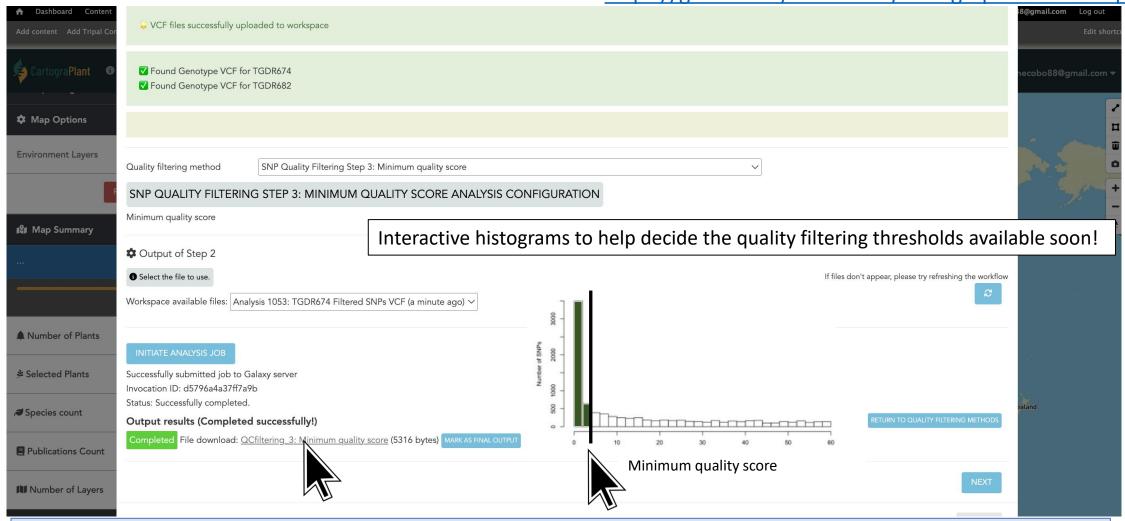
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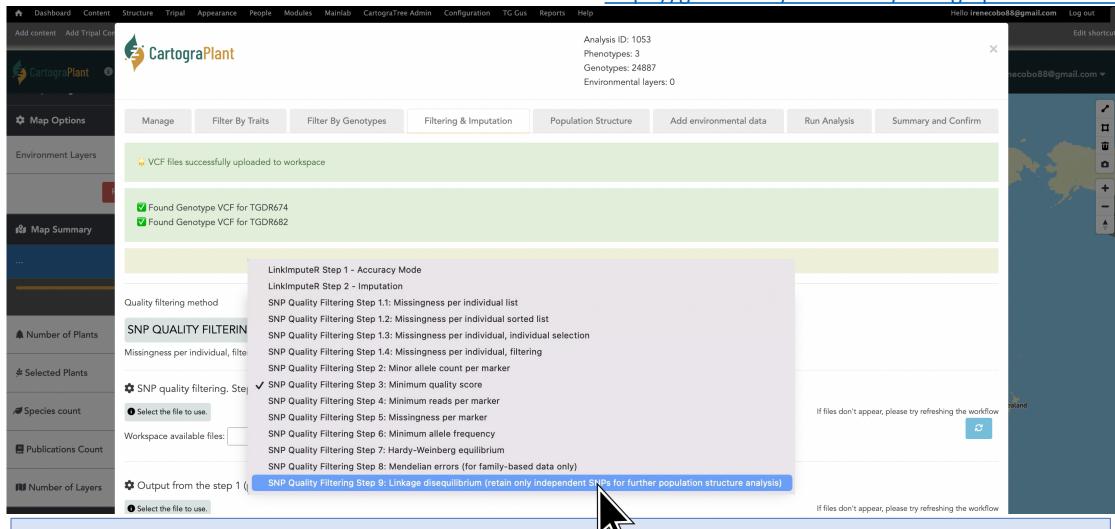
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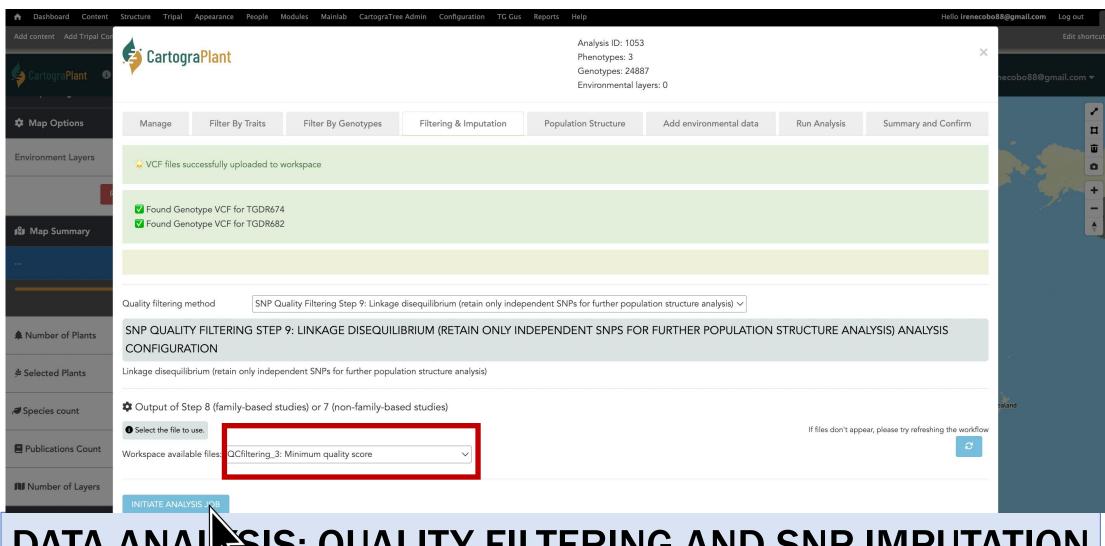
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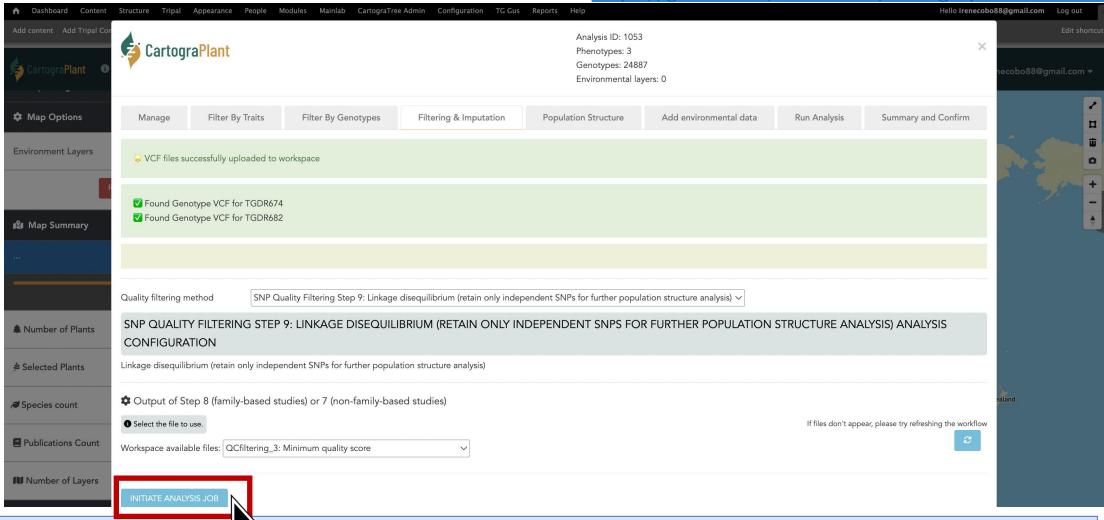
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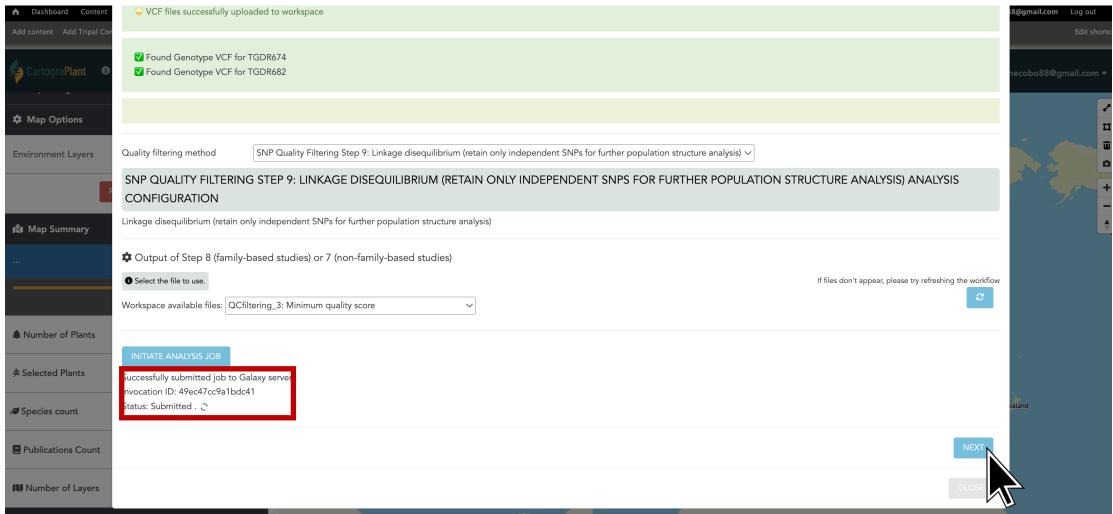
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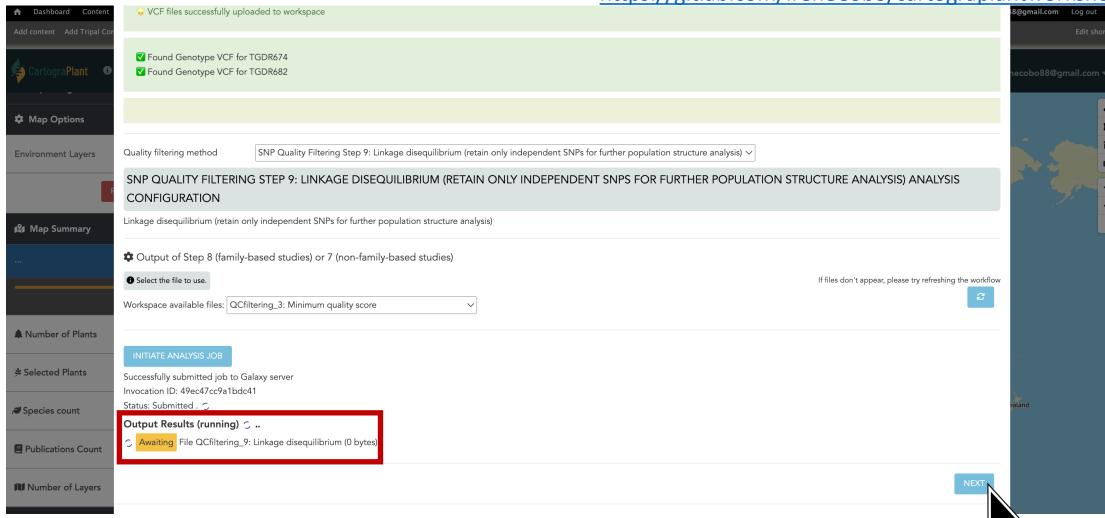
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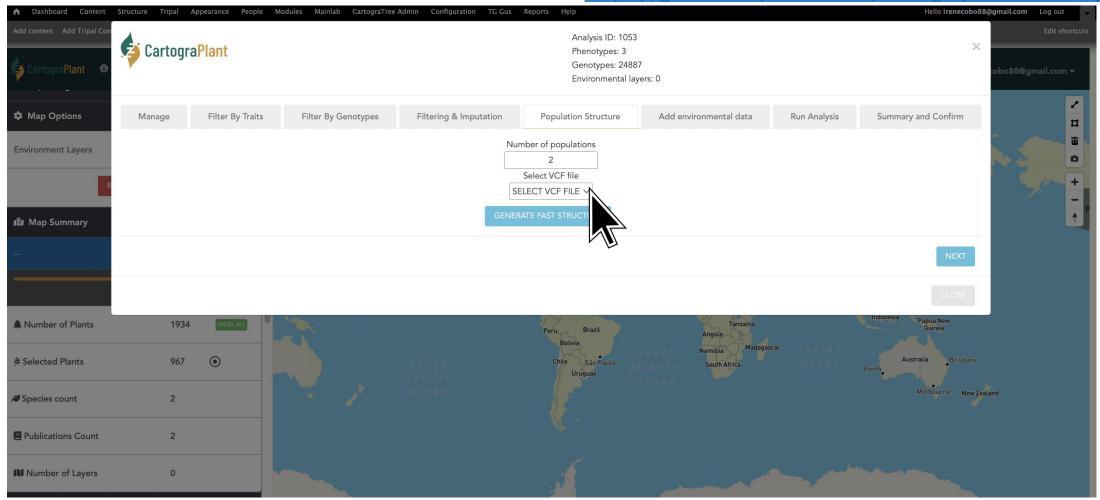
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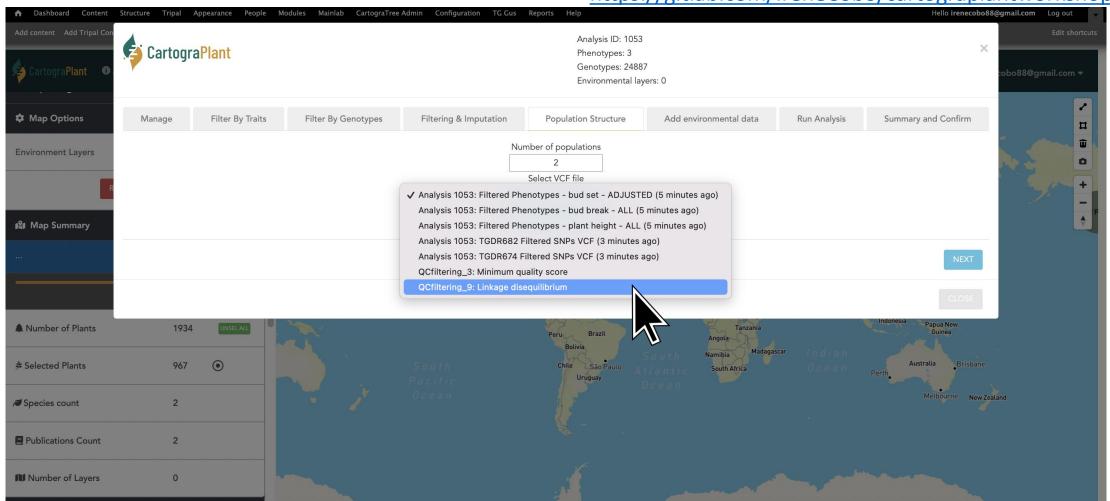
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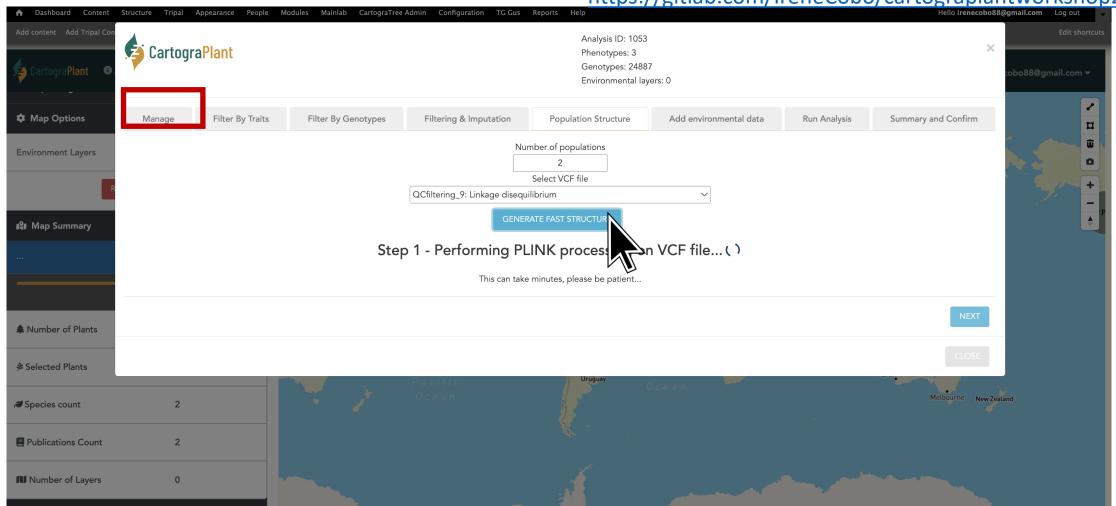
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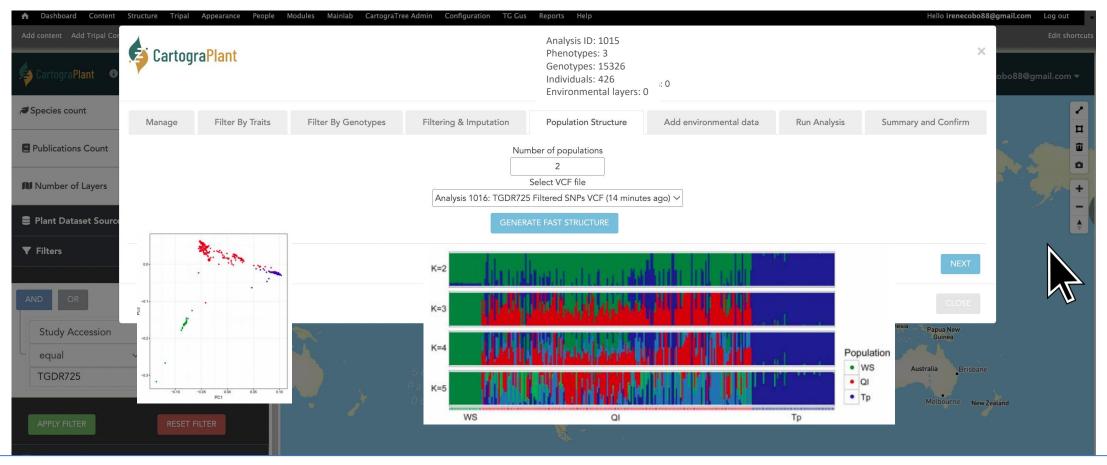
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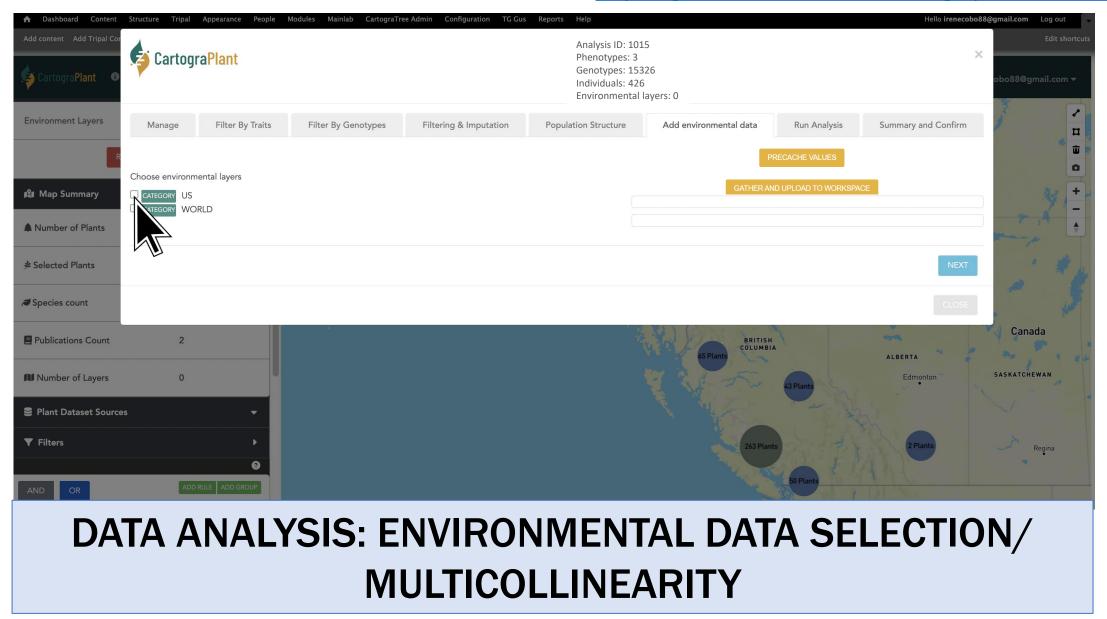
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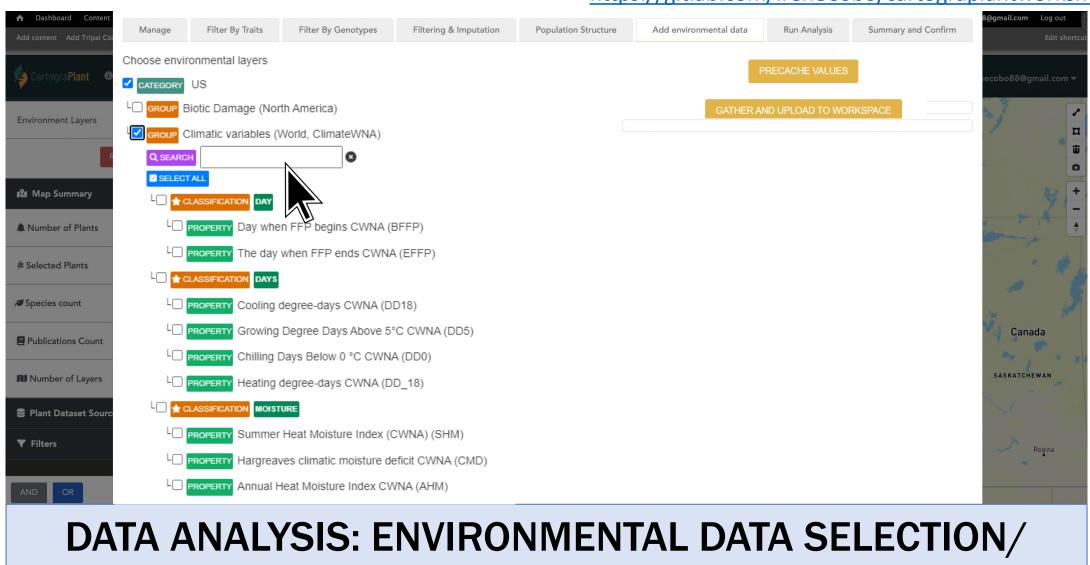
Population structure calculation (PCA and DAPC) and visualization (fastSTRUCTURE, PCA and DAPC) available soon!!!

# https://cartograplant.org/



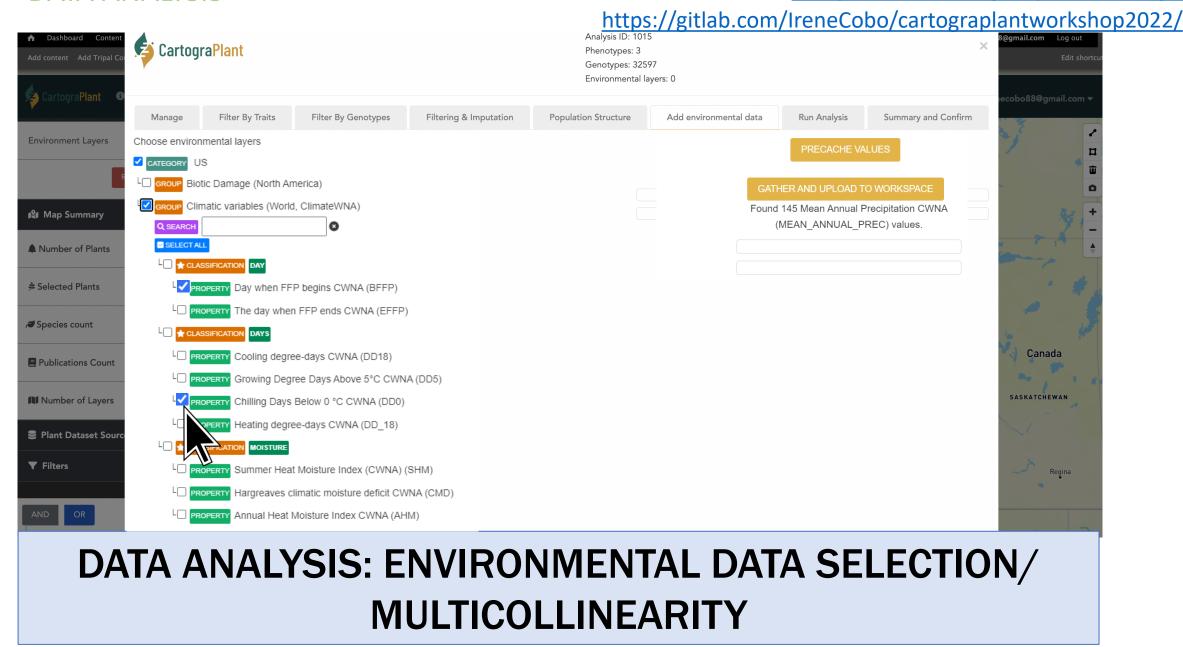
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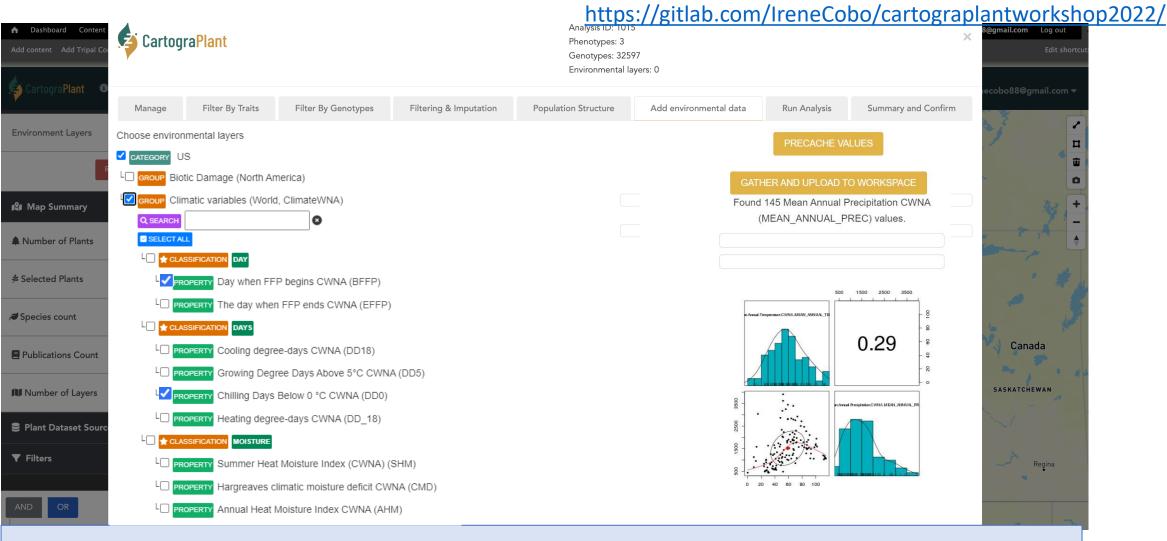


MULTICOLLINEARITY

https://cartograplant.org/



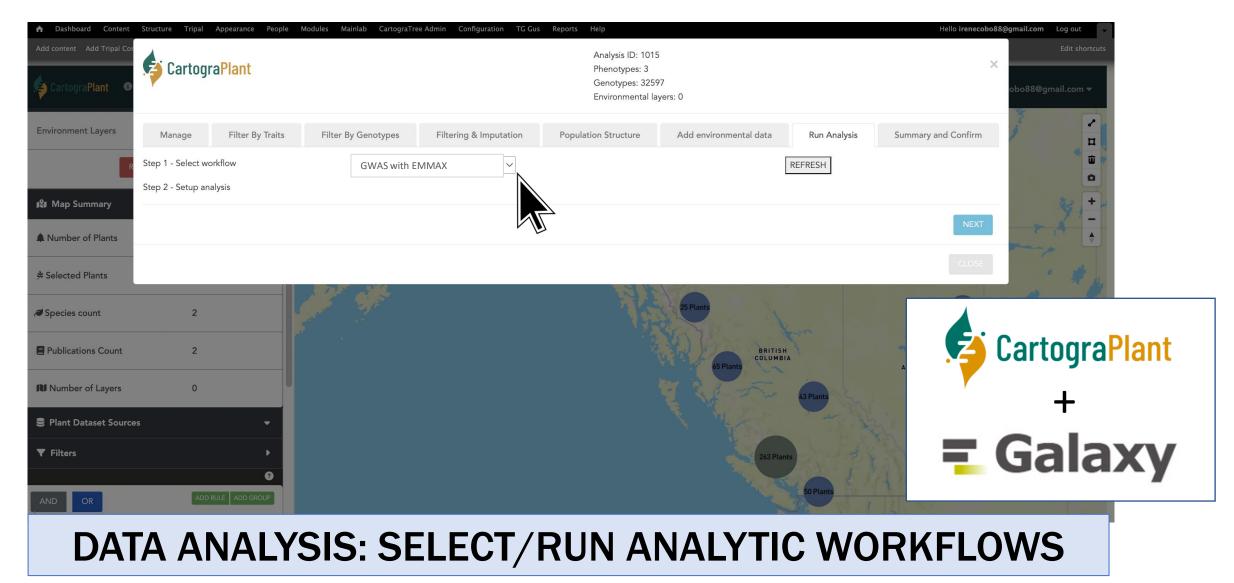
### https://cartograplant.org/



## DATA ANALYSIS: ENVIRONMENTAL DATA SELECTION/ MULTICOLLINEARITY

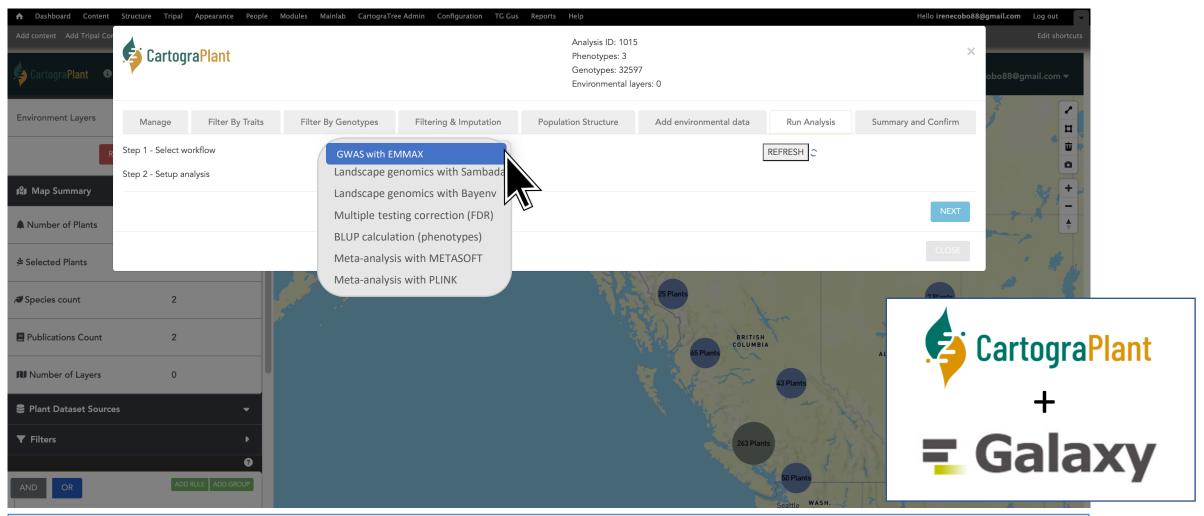
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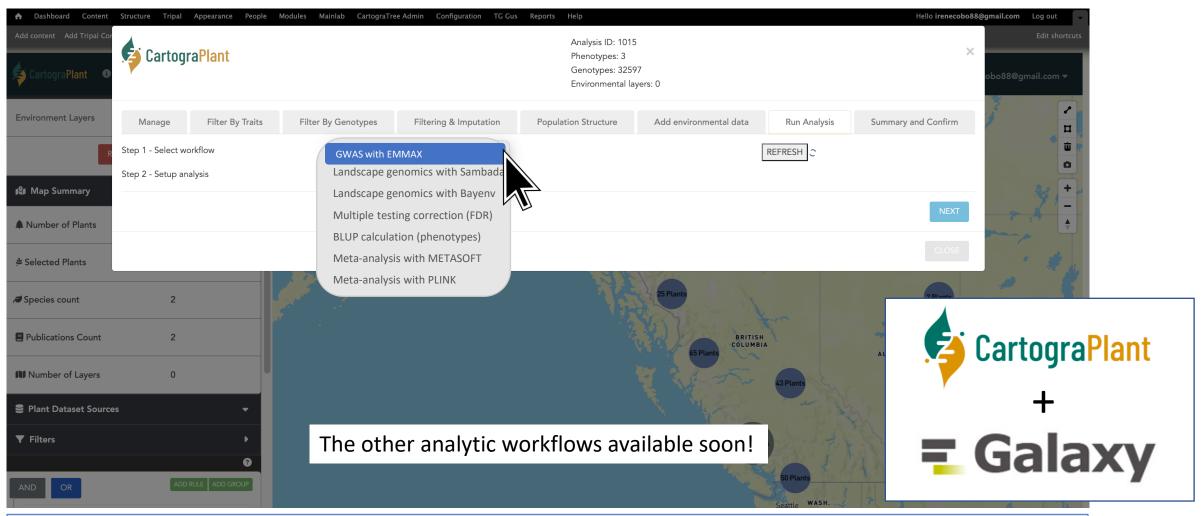
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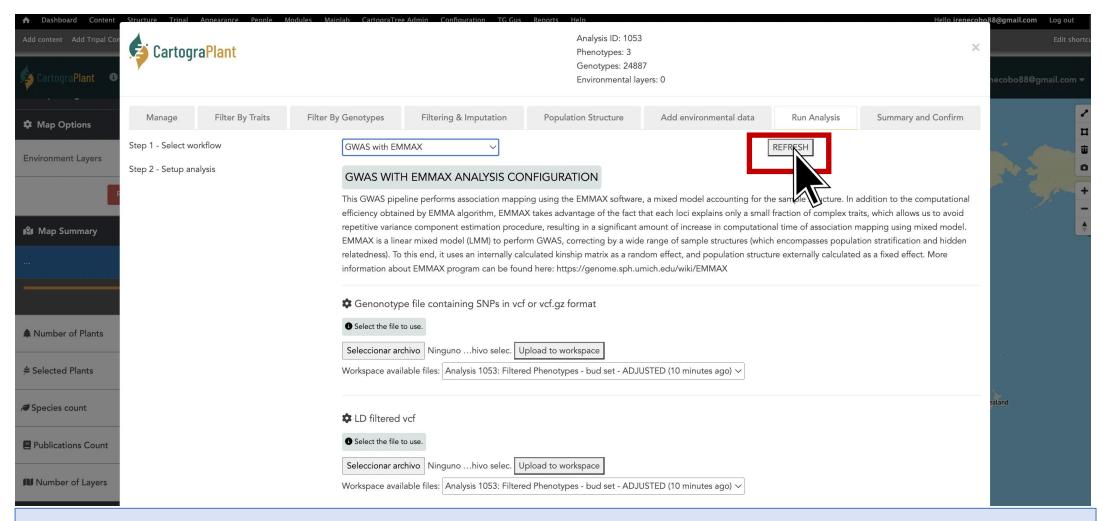
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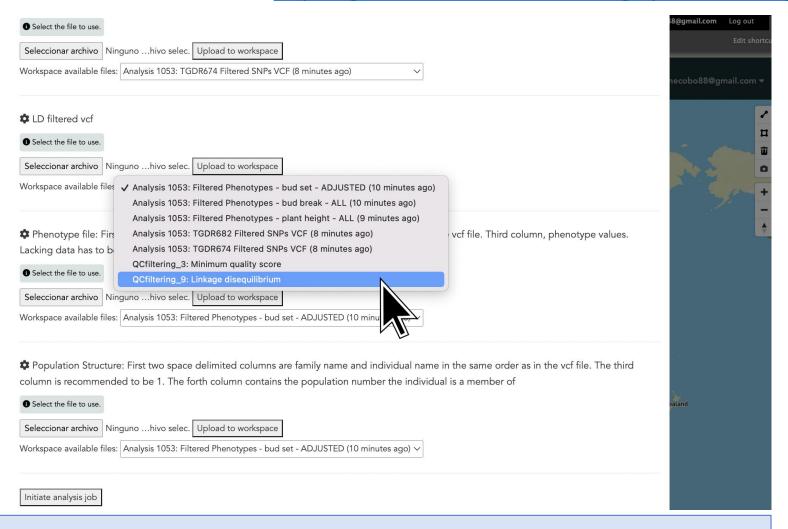
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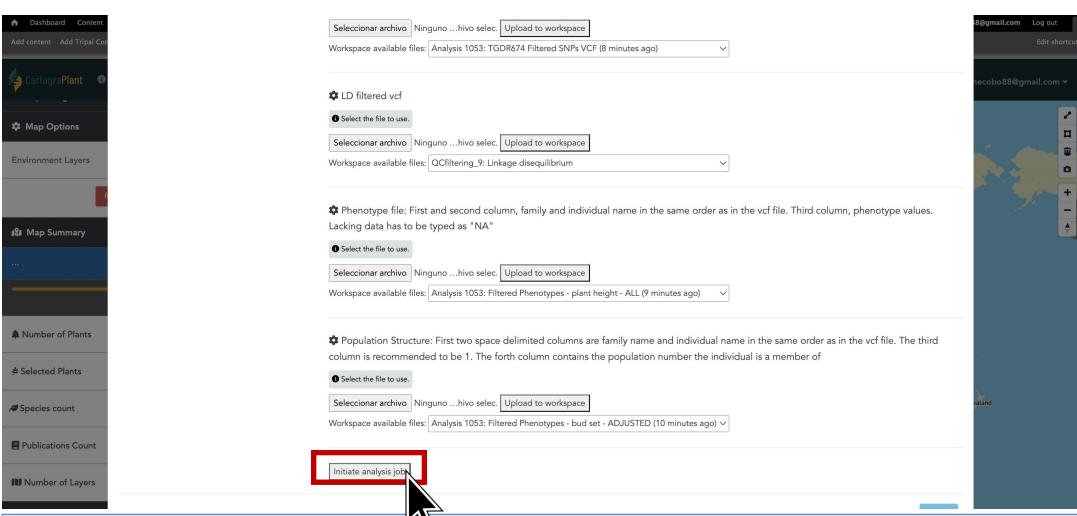
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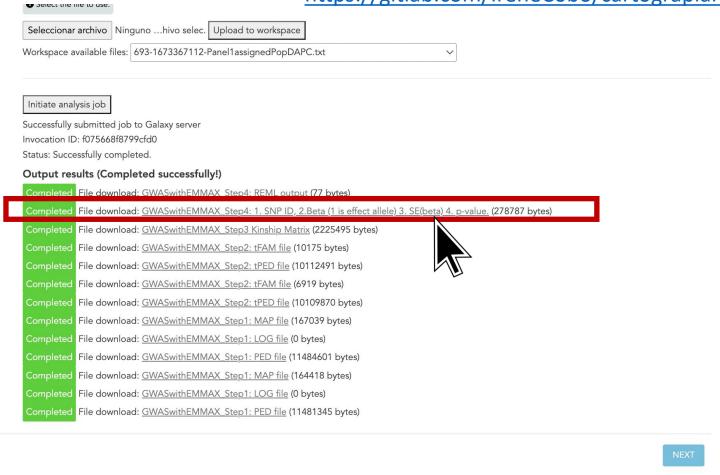
### https://cartograplant.org/

https://gitlab.com/IreneCobo/cartograplantworkshop2022/ Seleccionar archivo Ninguno ...hivo selec. Upload to workspace Workspace available files: 693-1673367112-Panel1assignedPopDAPC.txt Initiate analysis job Successfully submitted job to Galaxy server Invocation ID: f075668f8799cfd0 Status: Submitted ... Output Results (running) 🔿 . Awaiting File GWASwithEMMAX\_Step4: REML output (0 bytes) Awaiting | File GWASwithEMMAX\_Step4: 1. SNP ID, 2.Beta (1 is effect allele) 3. SE(beta) 4. p-value. (0 bytes) Awaiting File GWASwithEMMAX Step3 Kinship Matrix (2225495 bytes) Completed File download: GWASwithEMMAX\_Step2: tFAM file (10175 bytes) Completed File download: GWASwithEMMAX\_Step1: LOG file (0 bytes) Completed File download: GWASwithEMMAX\_Step1: PED file (11484601 bytes) Completed File download: <u>GWASwithEMMAX\_Step1: MAP file</u> (164418 bytes) Completed File download: GWASwithEMMAX\_Step1: LOG file (0 bytes) Completed File download: <u>GWASwithEMMAX\_Step1: PED file</u> (11481345 bytes)



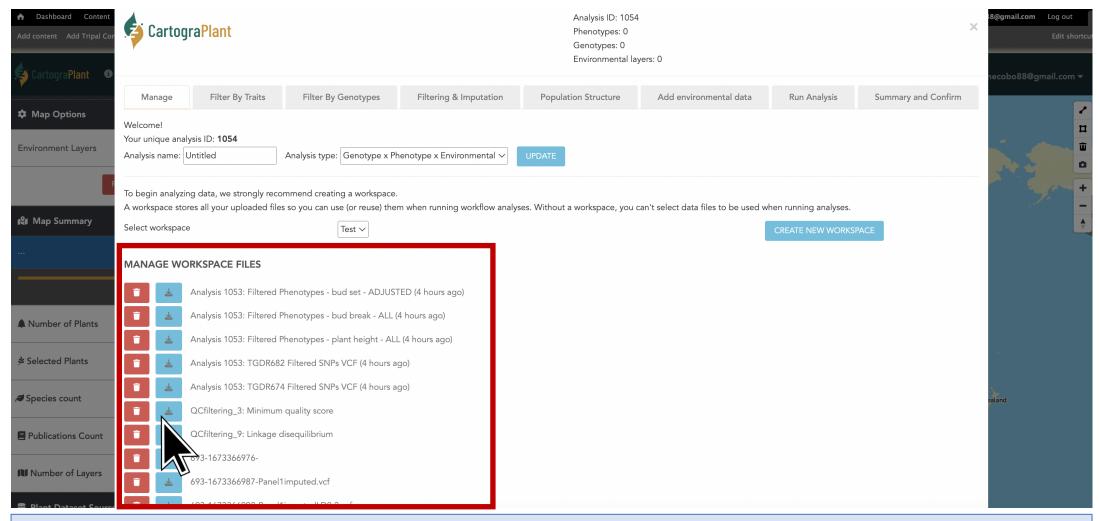
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# **TEAM MEMBERS! QUESTIONS?**

### Members of the project





- Stephen P. Ficklin

- Nic Herndon

This project has been funded by USDA-NIFA #2018-09223



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@TreeGenes



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- Risharde Ramnath
- Irene Cobo
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- Meghan Myles
- Emily Strickland
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- Margaret Staton
- Abdullah Almsaeed

















