VARIATION IN CARBON SEQUESTRATION

IN RESPONSE TO WATER LIMITATION

IN A DIVERSE PANEL OF SWITCHGRASS GENOTYPES

by

ANITA GIABARDO

(Under the Direction of Ali Missaoui)

ABSTRACT

Switchgrass (*Panicum virgatum* L.) has been identified as a "model" herbaceous species for bioenergy production by the U. S. Department of Energy. This work focuses on the evaluation of soil C storage of 150 switchgrass genotypes in response to water-stressed conditions by measuring yield and soil Permanganate Oxidizable Carbon (POXC) content. The overall objectives are to evaluate the variability in C sequestration over time, also in response to water limitation conditions, to investigate the relationship between C sequestration and yield, and to evaluate drought tolerance. POXC was significantly lower at deeper soil layers and under the drought treatment. In addition, we found a slightly positive, significant correlation ($p<.001$) between yield and POXC under the drought treatment. In order to evaluate drought tolerance among the 150 genotypes involved, we calculated a drought adaptation index for POXC and yield in both 2021 and 2022. We were able to identify four best performers.

INDEX WORDS: switchgrass, carbon sequestration, drought tolerance

# VARIATION IN CARBON (C) SEQUESTRATION

# IN RESPONSE TO WATER LIMITATION

# IN A DIVERSE PANEL OF SWITCHGRASS GENOTYPES

by

ANITA GIABARDO

BS, Università degli Studi di Padova, Italy, 2020

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2023

VARIATION IN (C) CARBON SEQUESTRATION

IN RESPONSE TO WATER LIMITATION

IN A DIVERSE PANEL OF SWITCHGRASS GENOTYPES

by

ANITA GIABARDO

Major Professor: Ali Missaoui
Committee: Miguel L. Cabrera
Francesco Morari

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
August 2023

DEDICATION

To the person that has accompanied me since the beginning of my sentient life – from my favorite sandbox playmate to my best lifetime friend and partner in laughter – who I admire and love beyond my ability to express: *mio fratello*.

ACKNOWLEDGEMENTS

This manuscript is the result of two years of research work carried out at the University of Georgia, where I arrived as a dual degree student from the University of Padua, in 2021. I am truly grateful for the support that my major professor, Dr. Missaoui, and the other committee members, Dr. Cabrera and Dr. Morari, have granted me throughout this journey.

All the necessary fieldwork and data collection wouldn’t have been possible without the support of all the Missaoui lab members: Jonathan, Shiva, Jazib, Angel, Holly, Razi, Chloe, Reneliza, Lydia, and Kendall. Thank you all so much. I also would like to thank Dr. Kishan Mahmud, who started this project and patiently guided me through its completion.

Finally, I would like to thank my family and my friends, who believed in me and made my stay in Athens as sweet as it could be. Thank you for being by my side.

TABLE OF CONTENTS

# LIST OF TABLES

Page

LIST OF FIGURES

# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

## The Importance of Switchgrass as a Bioenergy Crop

Among the challenges the current generation of scientists and researchers is concerned with is how to ensure sufficient food and energy for a world population that is expected to reach 11 billion by 2100 (United Nations, 2019). Achieving such a goal is made particularly arduous by the current context of ever-scarcer natural resources and increasingly concentrated atmospheric $CO_2$, one of the primary Greenhouse gasses (GHGs) responsible for climate change (Lacis et al., 2010).

In 2016, the primary source of GHG emissions was energy use (World Resources Institute, 2021), with fossil fuel combustion being the main contributor. One of the possible strategies which could reduce the dependence on fossil fuels – a finite, non-renewable resource – while reducing the amount of $CO_2$ released into the atmosphere is to decrease the share of energy derived from fossil fuels in favor of bioenergy (Lemus and Lal, 2005). According to the Intergovernmental Panel on Climate Change (IPCC) *Special Report on Renewable Energy Sources and Climate Adaptation* (Chum et al., 2011), "bioenergy has significant GHG mitigation potential, provided that the resources are developed sustainably and that efficient bioenergy systems are used." In fact, even though biofuels production and combustion processes generate some $CO_2$, the net contribution to atmospheric $CO_2$ enrichment is much lower than of fossil fuels', as the C released during combustion is offset by the C sequestrated earlier, during plant biomass growth (Turhollow and Perlack, 1991).

Lignocellulosic biomass is the primary raw material necessary for bioethanol production (McMillan, 1997). Such biomass may be derived from bioenergy crops, forestry residues, agricultural or industrial residues and is generally comprised of cellulose, hemicellulose, and lignin (Agarwal et al., 2017). Plant species generally considered to be bioenergy crops include cellulosic plants, perennial grasses, non-edible oil crops, and oil plants. Yadav *et al*. (2019) classified bioenergy crops – intended as species purposely cropped for bioenergy production – into five groups: first-generation, second-generation, and third-generation bioenergy crops, dedicated bioenergy crops, and halophytes. First-generation bioenergy crops include species that are also a source of food for human consumption, such as corn (*Zea mays*), sugarcane (*Saccharum officinarum* L.), and sweet sorghum (*Sorghum bicolour* L.). Second-generation bioenergy crops include perennial forages such as Switchgrass (*Panicum virgatum* L.), species from the *Miscanthus* genus, alfalfa (*Medicago sativa* L.), and reed canary grass (*Phalaris arundinacea* L.). Third-generation bioenergy crops include boreal plants (*e. g.*, perennials such as *Phleum pratense* L. and *Phalaris arundinacea* L.), Crassulacean Acid Metabolism plants, *Eucalyptus* sp., *Agave* sp., and Microalgae; halophytes constitute a group of species which can grow in coastal areas, where they can take advantage of high salt concentrations.

As of 2021, in the US, renewable energy accounted for 7.9% of the total energy supply, 60% of which was sourced from biomass (IEA, 2021). Since the year 2000, the overall share of bioenergy has been growing, with liquid biofuels being the main growth driver, particularly bioethanol. Biodiesels account for a lower share compared to bioethanol, followed by biogases (IEA, 2021). The primary crops involved in the production of liquid transportation biofuels in the US are corn grain for bioethanol and soybean for biodiesel. As of 2010, about 38% of corn grain produced was used in ethanol production, for a total of 50 billion liters of production (U. S. Department of Energy, 2011).

Increasing biofuel production would require expanding the amount of land dedicated to biofuel crops' biomass production. Food and energy security are strictly interconnected challenges (Karp and Richter, 2011, Buchanan and Orbach, 2014, Perrone and Hornberger, 2014), and first-generation bioenergy crops, whose land use is in competition with food production, are generally seen as in conflict with food security (Mohr and Raman, 2013). In this regard, second-generation bioenergy crops such as Switchgrass, poplar, red canary grass, etc., could play an important role in ensuring energy and food security while contributing to tackling climate change. The present work will focus on Switchgrass, one of the most promising second-generation biofuel crops. The next paragraphs will provide an overview of Switchgrass as a biofuel crop in the United States.

Switchgrass (*Panicum virgatum* L.) is a monocot $C_4$ perennial grass native to the prairies of North America. Member of the *Poaceae* family, subfamily *Panicoideae*, it is found in most of the United States, with the exception of a few far west states such as California, Oregon, and Washington state (U. S. Department of Agriculture, 2011). Although it started to be an intentionally cropped species only in the last decades, it is nowadays recognized as a "multipurpose crop species" (Parrish and Fike, 2005). In fact, Switchgrass is recognized as a provider of several ecosystem services, among which biodiversity harboring and enhancement (Werling et al., 2014), soil erosion control (Wang et al., 2020), runoff vegetative filtering, reclamation and revegetation of disturbed land (Munshower, 1994). In addition, Switchgrass may be cropped on marginal lands (Khanna et al., 2021), which is an important land resource for biofuel feedstock production (Feng et al., 2017, Fan et al., 2020).

Switchgrass breeding started at the University of Nebraska in the 50s with the aim of improving forage quality (Eberhart and Newell, 1959). The focus on Switchgrass as a promising biofuel herbaceous crop has shifted the emphasis on biomass yield gain as one of the main breeding objectives (Sanderson et al., 2006), together with flowering time and biomass composition. Crop improvement efforts started with germplasm collection and evaluation, proceeded with conventional breeding and selection approaches, and are now supported by molecular approaches (Sanderson et al., 2006).

In recent years, Switchgrass has been identified as a “model” herbaceous species for bioenergy production by the U. S. Department of Energy’s Herbaceous Energy Crops Program (HECP), which was initiated in 1978 at the Oakridge National Laboratory (ORNL) (Mclaughlin and Kszos, 2005). The U. S. Department of Agriculture (USDA) began breeding and selecting native perennial grasses in the 1930s, increasingly focusing on Switchgrass (U. S. Department of Energy, 2011). High biomass production under low-input management, adaptation to a wide range of U. S. soils and climates, and potential profitability for farmers are among the main reasons behind this choice (Parrish and Fike, 2005, Wright and Turhollow, 2010, Mitchell et al., 2012). Even when grown in lower-yielding marginal cropland, Switchgrass tends to average between 5.2 and 11.1 Mg $ha^{-1}$ biomass yield (Schmer et al., 2008).

Over time, the interest of the scientific community, as highlighted by the amount of published scientific publications on Switchgrass as a biofuel crop, has been constantly increasing (Parrish et al., 2012). Nevertheless, there are still some constraints to switchgrass use in bioenergy production, including satisfying establishment, fertilization and nutrient management, and efficient conversion technologies (Sanderson et al., 2006).

Besides the gains in reduced GHG emissions from switchgrass-sourced bioethanol – which are estimated to be > 90% lower than the ones from gasoline (Schmer et al., 2008) – an aspect which is of particular importance in the case of Switchgrass grown for bioenergy production is soil C sequestration: given the potential role of biofuel crops as relevant players in the global effort towards a reduction of the energy industry's carbon footprint, it appears pivotal not only to clearly define their net contribution to atmospheric $CO_2$ but also to explore the possibility of plant breeding for improved carbon sequestration capacity. In particular, to provide a reliable Life Cycle Assessment of switchgrass cultivation and use as a bioenergy crop, it is fundamental to gain knowledge on belowground C storage dynamics, which thus far have only been marginally investigated, especially in relation to breeding programs.

This work explores the ability to increase the underground soil C of different switchgrass genotypes under drought conditions. The following section will present an overview of selected published literature on switchgrass genetics and genetic improvement efforts, with a particular focus on drought tolerance and on switchgrass' carbon sequestration capabilities.

**The Origin of Ecotypes**

Switchgrass is a highly polymorphic species showing multiple ploidy levels, high heterozygosity, and a strong preference for cross-pollination as a reproductive strategy, with nearly 100% gametophytic self-incompatibility (Martínez-Reyna and Vogel, 2002). The main taxonomic division recognizes two phenotypically different ecotypes: lowland and upland. The phenotypic divergence between the two ecotypes is probably ascribable to differences in habitat and latitudinal adaptation. In fact, upland ecotypes are generally found north of the 34° N latitude (temperate climates), while lowland ecotypes are adapted to southern latitudes (subtropical climates), spanning from up to 42° N in the western to 45° N in the eastern North American grasslands, where the ocean mitigates the climate (Casler, 2012).

While the basic number of chromosomes in Switchgrass is x = 9, ploidy variation appears to be ecotype-specific, as many studies have suggested. Lowland ecotypes are generally tetraploid (2n = 4x = 36 chromosomes), while upland ecotypes can be found at both tetraploid and octoploid (2n = 8x = 72) levels (Casler, 2012). A phylogenetic analysis by Lu et al. (2013) performed using Single Nucleotide Polymorphism markers suggested that upland tetraploids evolved from upland octoploids.

Besides the divergence in the ploidy structure, the two ecotypes also show evident morphological differences: lowland plants are generally taller, have fewer, thicker tillers, longer and wider leaf blades, thicker stems, and flower later compared to the upland plants (Casler, 2012). Recently, Lovell et al. (2021) introduced a third coastal ecotype occupying the same geographic area as the lowland ecotype but showing upland leaf characteristics and lowland plant architecture.

A study by Casler et al. (2004) revealed that considerable variation in biomass yield and survival among different genotypes across latitudes ranging from 36° to 46° N are attributable to adaptive differences among ecotypes. More recently, Lovell et al. (2021) investigated the genomic basis of climatic adaptation of Switchgrass and showed how higher biomass accumulation is manifested whenever locally adapted cultivars are cropped. The authors studied 700 genotypes grown within ten common gardens spanning over 1,862 km of latitude and a variety of climatic conditions. They found that biomass yield for each genotype was maximized when planted in gardens more climatically similar to their original collection location. Through winter mortality analysis, the authors observed strong evidence that climatic adaptive evolution played an important role in ecotype divergence. Thus, climatic adaptation appears to be one of the main drivers for biomass yield.

**Switchgrass Breeding**

Given its strong outcrossing nature and severe inbreeding depression, traditional switchgrass breeding has been generally carried out through recurrent selection and population improvement. Though attempts towards hybrid breeding have been made, the preferred breeding strategy remains synthetic cultivar development. As to 2018, there are no commercially available switchgrass cultivars in the US (Clifton-Brown et al., 2018). Switchgrass phenotyping is particularly labor-intensive, and yield evaluation must be carried out throughout multiple seasons as to ensure sufficient yield stability, a trait that is of particular importance in the biofuel industry. Traditional switchgrass breeding strategies could benefit greatly from the latest developments and applications of molecular breeding tools, such as Marker Assisted Selection (MAS). MAS is a technology that allows for the early selection of the most promising individuals in a population at a very early stage based on genetic markers. MAS has the potential to speed-up the breeding cycle therefore increasing the genetic gain. For this purpose, in recent years, there has been active research on the identification and validation of Quantitative Trait Loci (QTL) associated with traits of interest, such as biomass yield (Serba et al., 2014, Chang et al., 2016, Chang et al., 2022, Nayak et al., 2022, Razar et al., 2022), flowering time (Tornqvist et al., 2018), and abiotic stress adaptation (Poudel et al., 2019).

**Drought Tolerance**

Response to water limitation is a feature of interest for two main reasons: firstly, biofuel crops such as Switchgrass are expected to be grown on marginal lands under low-input management. Therefore, they need to be able to thrive in unfavorable or harsh abiotic conditions.

Secondly, climate change could exacerbate these conditions (Strzepek et al., 2010), increasing the frequency and intensity of dry spells, hence undermining the performance of this crop, and calling for an even more urgent need to develop drought-resistant cultivars (Oliver et al., 2009). Plants possessing the $C_4$ photosynthetic pathway, such as Switchgrass, tend to show higher water use efficiency (Pearcy and Ehleringer, 1984) compared to $C_3$ plants. Nevertheless, the $C_4$ pathway is not a sufficient condition to guarantee higher drought tolerance. Both $C_3$ and $C_4$ plants exhibit a wide range of variation in terms of performance under water stress conditions, even within the same species (Pearcy and Ehleringer, 1984). In fact, although severe drought has been proved to significantly reduce switchgrass growth and biomass in simulation experiments as well as in field experiments (Lovell et al., 2016, Hui et al., 2018), water use efficiency varies among genotypes, making improvements possible (Stroup et al., 2003, Barney et al., 2009).

How water limitation will affect soil carbon sequestration by Switchgrass is of concern as the sustainability of Switchgrass as a biofuel crop is strictly related to its ability to offset C emissions, also through belowground C storage. Moreover, soil carbon content changes are of interest in terms of soil quality repercussions and sustainable soil management. In addition, a higher ability to sequestrate carbon into the soil is likely to guarantee higher drought-withstanding capacity in the long term since soil organic carbon content is one of the main drivers of soil structure stability, water retention capacity, and overall soil quality (Bünemann et al., 2018).

## References

AGARWAL, A. K., AGARWAL, R. A., GUPTA, T. & GURJAR, B. R. 2017. Biofuels Technology, Challenges and Prospects. In: AGARWAL, A. K., AGARWAL, R. A., GUPTA, T. & GURJAR, B. R. (eds.) *Green Energy and Technologies.* Springer Nature Singapore Pte. Ltd.

BLAIR, G. J., LEFROY, R. D. B. & LISLE, L. 1995. Soil Carbon Fractions Based on their Degree of Oxidation, and the Development of a Carbon Management Index for Agricultural Systems. *Australian Journal of Agricultural Research,* 46**,** 1459-1466.

BUCHANAN, G. A. & ORBACH, R. L. 2014. Convergence of Food Security, Energy Security and Sustainable Agriculture. *In:* DAVID D. SONGSTAD, J. L. H., DWIGHT T. TOMES (ed.) *Convergence of Food Security, Energy Security and Sustainable Agriculture.* Springer Heidelberg New York Dordrecht London.

BÜNEMANN, E. K., BONGIORNO, G., BAI, Z., CREAMER, R. E., DE DEYN, G., DE GOEDE, R., FLESKENS, L., GEISSEN, V., KUYPER, T. W., MÄDER, P., PULLEMAN, M., SUKKEL, W., VAN GROENIGEN, J. W. & BRUSSAARD, L. 2018. Soil Quality – A Critical Review. *Soil Biology and Biochemistry,* 120**,** 105-125.

CASLER, M. D. 2012. Switchgrass Breeding, Genetics, and Genomics. *In:* MONTI, A. (ed.) *Switchgrass: A Valuable Biomass Crop for Energy.* Springer.

CASLER, M. D., VOGEL, K. P., TALIAFERRO, C. M. & WYNIA, R. L. 2004. Latitudinal Adaptation of Switchgrass Populations. *Crop Sciences,* 44**,** 293-403.

CHANG, D., DONG, H., BAI, S. & WU, Y. 2022. Mapping QTLs for Spring Green-up, Plant Vigor, and Plant Biomass in Two Lowland Switchgrass Populations. *Molecular Breeding,* 42.

CHANG, D., WU, Y., LIU, L., LU-THAMES, S., DONG, H., GOAD, C., BAI, S., MAKAJU, S. & FANG, T. 2016. Quantitative Trait Loci Mapping for Tillering-Related Traits in Two Switchgrass Populations. *Plant Genome,* 9.

CHUM, H., FAAIJ, A., MOREIRA, J., BERNDES, G., DHAMIJA, P., DONG, H., GABRIELLE, B., GOSS ENG, A., LUCHT, W., MAPAKO, M., MASERA CERUTTI, O., MCINTYRE, T., MINOWA, T., PINGOUD, K. 2011. Bioenergy. *In:* IPCC Special Report on Renewable Energy Sources and Climate Change Adaptation [EDENHOFER, O., PICHS-MADRUGA, R., SOKONA, Y., SEYBOTH, K., MATSCHOSS, P., KADNER, S., ZWICKEL, T., EICKEMEIER, P., HANSEN, G., SCHLÖMER, S., VON STECHOW, C. (eds.)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

CLIFTON-BROWN, J., HARFOUCHE, A., CARSLER, M. D., JONES, H. D., MACALPINE, W. J., MURPHY-BOKERN, D., SMART, L. B., ADLER, A., ASHMAN, C., AWTY-CARROLL, D., BASTIEN, C., BOPPER, S., BOTNARI, V., BRANCOURT-HULMEL, M., CHEN, Z., CLARK, L. V., COSENTINO, S., DALTON, S., DAVEY, C., DOLSTRA, O., DONNISON, I., FLAVELL, R., GREEF, J., HANLEY, S., HASTING, A., HERTZBERG, M., HSU, T.-W., HUANG, L. S., IURATO, A., JENSEN, E., JIN, X., JØRGENSEN, U., KIESEL, A., KIM, D.-S., LIU, J., MCCALMONT, J. P., MCMAHON, B. G., MOS, M., ROBSON, P., SACKS, E. J., SANDU, A., SCALICI, G., SCHWARZ, K., SCORDIA, D., SHAFIEI, R., SHIELD, I., GANCHO, S., STANTON, B. J., SWAMINATHAN, K., GAIL, T., TORRES, A., F., TRINDADE, L. M., TSCHAPLINSKI, T., TUSKAN, G. A., YAMADA, T., YU, C. Y., ZALESNY JR, R. S., ZONG, J. & LEWANDOWSKI, I. 2018. Breeding Progress and Preparedness for Massscale Deployment of Perennial Lignocellulosic Biomass Crops Switchgrass, Miscanthus, Willow and Poplar. *GCB Bioenergy,* 11**,** 118-151.

EBERHART, S. A. & NEWELL, L. C. 1959. Variation in Domestic Collections of Switchgrass, Panicum virgatum L. *Agronomy Journal*.

FAN, P., HAO, M., DING, F., JIANG, D. & DONG, D. 2020. Quantifying Global Potential Marginal Land Resources for Switchgrass. *Energies,* 13.

FENG, Q., CHAUBEY, I., ENGEL, B., CIBIN, R., SUDHEER, K. P. & VOLENEC, J. 2017. Marginal land suitability for Switchgrass, Miscanthus and hybrid poplar in the Upper Mississippi River Basin (UMRB). *Environmental Modelling & Software,* 93**,** 356-365.

HUI, D., YU, C.-L., DENG, Q., DZANTOR, E. K., ZHOU, S., DENNIS, S., SAUVE, R., JOHNSON, T. L., FAY, P. A., SHEN, W. & LUO, Y. 2018. Effects of Precipitation Changes on Switchgrass Photosynthesis, Growth, and Biomass: a Mesocosm Experiment. *PLos ONE***,** 13.

INTERNATIONAL ENERGY AGENCY 2021. Implementation of Bioenergy in the United States - 2021 update. *In:* PELKMANS, L. (ed.). IEA Bioenergy [online]. Available: https://www.ieabioenergy.com [accessed May 2023].

KARP, A. & RICHTER, G. M. 2011. Meeting the Challenge of Food and Energy Security. *J Exp Bot,* 62**,** 3263-71.

KHANNA, M., CHEN, L., BASSO, B., CAI, X., FIELD, J. L., GUAN, K., JIANG, C., LARK, T. J., RICHARD, T. L., SPAWN-LEE, S. A., YANG, P., ZIPP, K. Y. 2021. Redefining Marginal Land for Bioenergy Crop Production. GBC Bioenergy, 13**,** 1590-1609.

LACIS, A. A., SCHMIDT, G. A., RIND, D. & RUEDY, R. A. 2010. Atmospheric CO2: Principal Control Knob Governing Earth’s Temperature. *Science,* 330**,** 356-359.

LEMUS, R. & LAL, R. 2005. Bioenergy Crops and Carbon Sequestration. *Critical Reviews in Plant Sciences,* 24**,** 1-21.

LOVELL, J. T., MACQUEEN, A. H., MAMIDI, S., BONNETTE, J., JENKINS, J., NAPIER, J. D., SREEDASYAM, A., HEALEY, A., SESSION, A., SHU, S., BARRY, K., BONOS, S., BOSTON, L., DAUM, C., DESHPANDE, S., EWING, A., GRABOWSKI, P. P., HAQUE, T., HARRISON, M., JIANG, J., KUDRNA, D., LIPZEN, A., IV, T. H. P., PLOTT, C., QI, P., SASKI, C. A., SHAKIROV, E. V., SIMS, D., SHARMA, M., SHARMA, R., STEWART, A., SINGAN, V. R., TANG, Y., THIBIVILLIER, S., WEBBER, J., WENG, X., WILLIAMS, M., WU, G. A., YOSHINAGA, Y., ZANE, M., ZHANG, L., ZHANG, J., BEHRMAN, K. D., BOE, A. R., FAY, P. A., FRITSCHI, F. B., JASTROW, J. D., LLOYD-REILLEY, J., MARTÍNEZ-REYNA, J. M., MATAMALA, R., MITCHELL, R. B., JR, F. M. R., RONALD, P., SAHA, M., TOBIAS, C. M., UDVARDI, M., WING, R. A., WU, Y., BARTLEY, L. E., CASLER, M., DEVOS, K. M., LOWRY, D. B., ROKHSAR, D. S., GRIMWOOD, J., JUENGER, T. E. & SCHMUTZ, J. 2021. Genomic Mechanisms of Climate Adaptation in Polyploid Bioenergy Switchgrass. *Nature,* 590**,** 438-444.

LOVELL, J. T., SHAKIROV, E. V., SCHWARTZ, S., LOWRY, D. B., ASPINWALL, M. J., TAYLOR, S. H., BONNETTE, J., PALACIO-MEJIA, J. D., HAWKES, C. V., FAY, P. A. & JUENGER, T. E. 2016. Promises and Challenges of Eco-Physiological Genomics in the Field: Tests of Drought Responses in Switchgrass. *Plant Physiology,* 172**,** 734-748.

LU, F., LIPKA, A. E., GLAUBITZ, J., ELSHIRE, R., CHERNEY, J. H., CASLER, M. D., BUCKLER, E. S. & COSTICH, D. E. 2013. Switchgrass Genomic Diversity, Ploidy and

Evolution: Novel Insight from a Network-Based SNP Discovery Protocol. *PLOS Genetics,* 9**,** 1-14.

MARTÍNEZ-REYNA, J. M. & VOGEL, K. P. 2002. Incompatibility Systems in Switchgrass. *Crop Sciences,* 42**,** 1800-1805.

MCLAUGHLIN, S. B. & KSZOS, L. A. 2005. Development of Switchgrass (Panicum virgatum) as a Bioenergy Feedstock in the United States. *Biomass and Bioenergy,* 28**,** 515-535.

MCMILLAN, J. D. 1997. Bioethanol Production: Status and Prospects. *Renewable Energy,* 10**,** 295-302.

MITCHELL, R. B., VOGEL, K. P. & UDEN, D. R. 2012. The feasibility of Switchgrass for biofuel production. *Biofuels*, 3**,** 47-59.

MOHR, A. & RAMAN, S. 2013. Lessons From First Generation Biofuels and Implications for the Sustainability Appraisal of Second Generation Biofuels. *Energy Policy,* 63**,** 114-122.

MUNSHOWER, F. F. 1994. Practical Handbook of Disturbed Land Revegetation. CRC Press, Boca Raton, FL, USA.

NAYAK, S., BHANDARI, H., SAHA, M. C., ALI, S., SAMS, C. & PANTALONE, V. 2022. Identification of QTL Associated with Regrowth Vigor Using the Nested Association Mapping Population in Switchgrass. *Plants (Basel),* 11.

OLIVER, R. J., FINCH, J. W. & TAYLOR, G. 2009. Second Generation Bioenergy Crops and Climate Change: a Review of the Effects of Elevated Atmospheric $CO_2$ and Drought on Water Use and the Implications for Yield. *GCB Bioenergy,* 1**,** 97-114.

PARRISH, D. J., CASLER, M. D. & MONTI, A. 2012. The Evolution of Switchgrass as an Energy Crop. *In:* MONTI, A. (ed.) *Green Energy and Technology.* Springer-Verlag London.

PARRISH, D. J. & FIKE, J. H. 2005. The Biology and Agronomy of Switchgrass for Biofuels. *Critical Reviews in Plant Sciences,* 24**,** 423-459.

PEARCY, R. W. & EHLERINGER, J. 1984. Comparative Ecophysiology of $C_3$ and $C_4$ plants. *Plant, Cell and Environment,* 7**,** 1-13.

PERRONE, D. & HORNBERGER, G. M. 2014. Water, Food and Energy Security: Scrambling for Resources or Solutions? *WIREs water,* 1**,** 49-68.

POUDEL, H. P., SANCIANGCO, M. D., KAEPPLER, S. M., BUELL, C. R. & CASLER, M. D. 2019. Quantitative Trait Loci for Freezing Tolerance in a Lowland x Upland Switchgrass Population. *Front Plant Sci,* 10**,** 372.

RAZAR, R. M., QI, P., DEVOS, K. M. & MISSAOUI, A. M. 2022. Genotyping-by-Sequencing and QTL Mapping of Biomass Yield in Two Switchgrass F(1) Populations (Lowland x Coastal and Coastal x Upland). *Front Plant Sci,* 13**,** 739133.

SANDERSON, M. A., ANDLER, P. R., BOATENG, A. A., CASLER, M. D. & SARATH, G. 2006. Switchgrass as a Biofuels Feedstock in the USA *Canadian Journal of Plant Science,* 86**,** 1315-1325.

SCHMER, M. R., VOGEL, K. P., MITCHELL, R. B. & PERRIN, R. K. 2008. Net Energy of Cellulosic Ethanol from Switchgrass. *PNAS,* 105**,** 464-469.

SERBA, D. D., DAVERDIN, G., BOUTON, J. H., DEVOS, K. M., BRUMMER, E. C. & SAHA, M. C. 2014. Quantitative Trait Loci (QTL) Underlying Biomass Yield and Plant Height in Switchgrass. *BioEnergy Research,* 8**,** 307-324.

STROUP, J. A., SANDERSON, M. A., MUIR, J. P., MCFARLAND, M. J. & REED, R. L. 2003. Comparison of Growth and Performance in Upland and Lowland Switchgrass Types to Water and Nitrogen Stress. *Bioresource Technology,* 86.

STRZEPEK, K., YOHE, G., NEUMANN, J. & BOEHLERT, B. 2010. Characterizing Changes in Drought Risk for the United States from Climate Change. *Environmental Research Letters,* 5.

TORNQVIST, C. E., TAYLOR, M., JIANG, Y., EVANS, J., BUELL, C. R., KAEPPLER, S. M. & CASLER, M. D. 2018. Quantitative Trait Locus Mapping for Flowering Time in a Lowland x Upland Switchgrass Pseudo-F2 Population. *Plant Genome,* 11.

TURHOLLOW, A. F. & PERLACK, R. D. 1991. Emissions of CO2 from Energy Crop Production. *Biomass and Bioenergy,* 1**,** 129-135.

UNITED NATIONS, DEPARTMENT OF ECONOMIC AND SOCIAL AFFAIRS. 2019. *World Population Prospects 2019* [Online]. Available: https://population.un.org/wpp/Graphs/DemographicProfiles/Line/900 [Accessed May 2023].

U. S. DEPARTMENT OF AGRICULTURE. 2011. Plant fact sheet for Switchgrass (*Panicum virgatum* L.). USDA - Natural Resources Conservation Service [Online]. Available: https://plants.sc.egov.usda.gov [Accessed May 2023].

U. S. DEPARTMENT OF ENERGY. 2011. U. S. Billion-ton update - Biomass Supply for a Bioenergy and Bioproducts Industry. PERLACK, R. D. & STOKES, B. J. (Leads), ORNL/TM/-2011/224. Oak Ridge National Laboratory, Oak Ridge, TN.

WANG, E., CRUSE, R. M., SHARMA-ACHARYA, B., HERZMANN, D. E., GELDER, B. K., JAMES, D. E., FLANAGAN, D. C., BLANCO-CANQUI, H., MITCHELL, R. B. & LAIRD, D. A. 2020. Strategic Switchgrass (*Panicum virgatum*) Production within Row Cropping Systems: Regional-scale Assessment of Soil Erosion Loss and Water Runoff Impacts. *GCB Bioenergy,* 12**,** 955-967.

WERLING, B. P., DICKSON, T. L., ISAACS, R., GAINES, H., GRATTON, C., GROSS, K. L., LIERE, H., MALMSTROM, C. M., MEEHAN, T. D., RUAN, L., ROBERTSON, B. A., ROBERTSON, P. G., SCHMIDT, T. M., SCHROTENBOER, A. C., TEAL, T. K., WILSON, J. K. & LANDIS, D. A. 2014. Perennial Grasslands Enhance Biodiversity and Multiple Ecosystem Services in Bioenergy Landscapes. *PNAS,* 111**,** 1652-7.

WORLD RESOURCES INSTITUTE. 2021. *World Greenhouse Gas Emissions: 2016* [Online]. Available: https://www.wri.org/data/world-greenhouse-gas-emissions-2016 [Accessed May 2023].

WRIGHT, L. & TURHOLLOW, A. 2010. Switchgrass selection as a "model" bioenergy crop: A history of the process. Biomass and Bioenergy, 34:6, 851-868.

YADAV, P., PRIYANKA, P., KUMAR, D., YADAV, A. & YADAV, K. 2019. Biofuel and Biorefinery Technologies. *In:* RASTEGARI, A. A., YADAV, A. N. & GUPTA, A. (eds.) *Prospects of Renewable Bioprocessing in Future Energy Systems.* Springer Nature Switzerland AG.

# CHAPTER 2

# VARIATION IN CARBON (C) SEQUESTRATION IN RESPONSE TO WATER LIMITATION IN A DIVERSE PANEL OF SWITCHGRASS GENOTYPES

[1]Anita Giabardo and Ali Missaoui. To be submitted to *Crop Science*

**Abstract**

Switchgrass (*Panicum virgatum* L.) is a monocot $C_4$ perennial grass native to North America. Although it has been used for decades as pasture species, it is now categorized as a “multipurpose crop species”. Switchgrass provides several ecosystem services, among which are biodiversity harboring, soil erosion control, runoff vegetative filtering, and reclamation of disturbed land. In recent years, Switchgrass has been identified as a “model” herbaceous species for bioenergy production by the U. S. Department of Energy. Besides the gains in reduced GHG emissions from switchgrass-derived biofuel, an aspect that is of particular importance is soil Carbon (C) sequestration. The objective of this study was to evaluate the variability in C sequestration, also in response to water limitation conditions, and to investigate the relationship between C sequestration and other traits of agronomic interest. For this purpose, dry biomass yield and Permanganate Oxidizable Carbon (POXC) content at 0- to 15, 15- to 30, and 30- to 60 cm depths are measured for 150 different switchgrass genotypes. We found that drought lowers the amount of POXC in the soil. In addition, POXC is lower with increasing soil depth. When looking at the correlation between POXC and yield, we found a positive correlation ($r = 0.27$, $p < 0.05$) within the drought-treated plot. This study provides insight on the impact of Switchgrass on soil POXC over time and at different depths and provides a framework for future evaluation of root-related traits in Switchgrass, also in relation to drought stress.

**Keywords**: Switchgrass, POXC, yield, drought

## Introduction

Switchgrass (*Panicum virgatum* L.) is a monocot $C_4$ perennial grass native to North America. Member of the *Poaceae* family, subfamily *Panicoideae*, it is found in most of the United States (U. S. Department of Agriculture, 2011). Switchgrass is recognized as a "multipurpose crop species" (Parrish and Fike, 2005). In fact, Switchgrass provides several ecosystem services, among which biodiversity harboring and enhancement (Werling et al., 2014), soil erosion control (Wang et al., 2020), runoff vegetative filtering, reclamation and revegetation of disturbed land (Munshower, 1994).

In recent years, Switchgrass has been identified as a "model" herbaceous species for bioenergy production by the U. S. Department of Energy's Herbaceous Energy Crops Program (HECP), which was initiated in 1978 at the Oakridge National Laboratory (ORNL) (Mclaughlin and Kszos, 2005). The U. S. Department of Agriculture (USDA) began breeding and selecting native perennial grasses in the 1930s, increasingly focusing on Switchgrass (U. S. Department of Energy, 2011). High biomass production under low-input management, adaptation to a wide range of U. S. soils and climates, and potential profitability for farmers are among the main reasons behind this choice (Parrish and Fike, 2005, Wright and Turhollow, 2010, Mitchell et al., 2012). In addition, Switchgrass may be cropped on marginal lands, which is an important land resource for biofuel feedstock production (Feng et al., 2017, Fan et al., 2020). Even when grown in lower-yielding marginal cropland, Switchgrass tends to average between 5.2 and 11.1 Mg $ha^{-1}$ biomass yield (Schmer et al., 2008).

### *Switchgrass as a Potential Carbon Sink*

As indicated by Lal and Follet (2009), soil carbon sequestration can be defined as the process of increasing the total C pool in the soil profile "through managerial interventions aimed at transferring atmospheric $CO_2$ to the soil C pool by moderating either organic and/or inorganic transformations".

Switchgrass is usually harvested yearly: although the optimum harvest time in the United States varies from state to state (Makaju et al., 2013), most of the leaves are still intact at harvest time. Therefore, any contribution to soil C sequestration is ascribable to the root-soil interface dynamics and below-ground C storage. Switchgrass has five times more below-ground biomass than corn (Zan et al., 1997). Such a developed rootstock allows Switchgrass to store soil organic carbon (SOC) not only at the soil surface but also at deeper depths, where C is generally less susceptible to mineralization and loss (Liebig et al., 2005). The wide aboveground polymorphism and the considerable rootstock that characterizes Switchgrass suggest that such phenotypic variability could be reflected belowground.

Bransby *et al.* (1998) reviewed the potential impacts of switchgrass cropping in terms of C and N balances. Their work points out how C sequestration benefits offered by Switchgrass will likely be superior compared to annual crops but not to grazed pastures. The authors also underline that the soil C sequestration one-time benefit is negligible compared to the impact on atmospheric $CO_2$ emissions and therefore suggest focusing future research on soil-related environmental benefits on N recovery rather than C sequestration. Furthermore, they highlight that since profits from row crops are higher than the ones from beef pastures, Switchgrass would likely replace the latter first, providing insignificant soil C sequestration gains.

Conversely, another review by Lemus and Lal (2005) stresses the importance of C sequestration in relation to a number of ecosystem services, such as degraded soil restoration, soil erosion control, and overall soil quality enhancement. Moreover, the authors estimate that bioenergy crops have the potential to sequester 20% of the total U. S. annual emissions, taking into account biomass yield, dedicated land, C sequestration potential, and conversion efficiency. Given the C trading market growth, they also suggest that the sequestered C could become a substantial new form of income for farmers.

In 2004, Frank *et al.* (2004) studied the C partitioning in two switchgrass cultivars through an in-field experiment in North Dakota. They found that SOC to 0.9 m depth increased at the rate of 1.01 kg C $m^{-2}$ $yr^{-1}$, suggesting that switchgrass fields could have the potential to store a substantial amount of C, although – as a study by Ma *et al.* (2000) suggests – it might take several years of switchgrass cropping before any increase in SOC becomes detectable. Nonetheless, the "active" SOC fraction has been proven to show a strong response to switchgrass root inputs (Ma et al., 2000).

The present work will focus on the "active" fraction of SOC, which is believed to be highly sensitive toward management. Although other traits of interest, such as plant height, tiller diameter, flag leaf length and width, crown perimeter, lodging tendency, greenness, and flowering time have been measured as part of the same experiment, in this study, the focus will be on the relationship between soil C and yield as the most important predictor for the agronomic success of Switchgrass in the sustainable biofuel industry. The next section will present a more detailed explanation of how the "active" fraction of SOC will be investigated.

*Permanganate Oxidizable Carbon (POXC) as a Measure of the "Active" Soil Carbon Pool*

Farmers, extension personnel, and researchers are increasingly interested in finding quick, easy, and inexpensive methods to monitor the amount of organic matter present in the soil, due to its pivotal role in determining and maintaining the fertility of agricultural fields, especially in response to changes in management. The soil carbon estimation method adopted in the present work has been introduced with this aim in the '80s and has been challenged and improved over time. The following paragraphs present a brief summary of the history of its development.

Permanganate Oxidizable Carbon (POXC) is a laboratory method first introduced by Loginow *et al.* (1987) with the aim of measuring the amount of labile carbon present in the soil, fractionating it according to its lability, and providing a technique to monitor small, short-term changes in Soil Organic Matter (SOM). The principle underlying the analysis is an oxidation reaction occurring in an aqueous solution in the presence of an oxidant agent, potassium permanganate ($KMnO_4$) (Tan, 2005). The method relied on using three different concentrations of $KMnO_4$ to oxidize increasing proportions of C over a fixed amount of time.

Later on – on the basis of a study by Lefroy *et al.* (1993) – Blair *et al.* (1995) presented a modified procedure that involved treating each soil sample with a single concentration (0.333 M) of $KMnO_4$ and introduced an index to monitor the rate of change in soil C dynamics of a system, named Carbon Management Index. Tirol-Padre and Ladha (2004) investigated the reliability of POXC as an index of SOC; The authors showed how POXC values obtained using the $KMnO_4$ concentration suggested by Blair *et al.* were strongly correlated with the total carbon content of soil rather than with water-soluble carbohydrates or Microbial Biomass Carbon (MBC) content. Based on this evidence, they suggested using POXC as an indicator of a stored, more recalcitrant carbon pool. Weil *et al.* (2003) proposed a lower working concentration for the $KMnO_4$ solution in order to improve the correlation of POXC with a smaller, more labile soil carbon pool, which they defined as "active C", and adapted the procedure to a protocol suitable for in-field analysis.

In 2012, Culman *et al.* further investigated the nature of the carbon fractions correlated with this method and proposed to adopt the name "Permanganate Oxidizable Carbon". The authors found POXC to be significantly related to particulate organic carbon (POC), microbial biomass carbon (MBC), and soil organic carbon (SOC) and therefore were able to suggest POXC as a method potentially suitable for soil quality monitoring and detection of differences due to management. Culman developed the protocol that has been adopted as a reference for this study.

The protocol can be found at: https://lter.kbs.msu.edu/protocols/133 (consulted on 14th September 2021). A brief description is presented in the "Materials and Methods" section of this manuscript.

**Objectives**

The objectives of the present work are:

1. Measuring active soil C under drought treatment withing a diverse panel of 150 switchgrass genotypes at three different soil depths (0- to 15 cm, 15- to 30 cm and 30- to 60 cm);
2. Investigating the relationship between POXC and yield, also in relation to drought conditions.

**Materials and Methods**

*Experimental Design*

The experimental plots under study are located within the University of Georgia's Gibbs Farm (Tifton, Tift County, GA; 31.4415007° N, 83.5799678° W, 116 m elevation). Soil is classified as fine-loamy, kaolinitic, thermic, Plinthic Kandudults. Four hundred five different genotypes are being tested under two treatments: (1) well-watered (or "uncovered", UC) and (2) water limitation (or "covered", CV). The average soil pH in 2018 was 6.30 in the UC plots and 6.70 in the CV plots. Drains were placed underneath the CV plot at about 1-m depth to prevent the water table from rising. Pipes located at the border of the CV plot allow for measuring the depth of the water table. Soil sensors measuring volumetric water content and soil temperature are present in the field. In addition, soil volumetric water content is measured throughout the year by means of a field scout digital moisture sensor (TDR 350 Soil Moisture Meter, Turf-Tec International). Climatic data of Tift County, including monthly minimum temperature, maximum temperature, and average rainfall, is provided in table 2.1 (UGA Weather Network, 2023).

The drought treatment is realized by means of a rain exclusion shelter, which prevents rainwater from reaching the plants. Fig. 2.1 shows a schematic representation of the experimental layout. In the UC plot, plants are exposed to rainwater (Fig. 2.2). For each of the two treatments, three replicates were set up, for a total of around 2400 individual switchgrass plants involved, arranged in a randomized complete block design (RCBD): within each experimental block, the accessions are randomly arranged to control statistical errors due to spatial variation. As a result, each replicate shows a different arrangement of genotypes, as it is typical of RCBDs.

This study involves a subset of one hundred fifty genotypes in two replicates. Of the one hundred fifty switchgrass genotypes involved, ninety-four are classified as lowland, forty as coastal, six as upland, and ten as unknown. Collection sites of the germplasm are located in over thirty US states spanning from 26.86972° to 46.38829° N latitude and 70.7594° to 103.609° W longitude. All the plants involved were planted in August 2018. Within each replicate, the plants are located 90 cm apart from each other and arranged into rows and ranges. The covered replicates consisted of seven rows with fifty-eight plants per row, while the uncovered ones included fourteen rows with twenty-nine plants per row.

*Management History*

With regards to previous management, the following crops had been cultivated on the land where the covered plots are located: cotton (*Gossypium hirsutum*) and peanut (*Arachis hypogaea*) in a rotation from 2000 to 2008 and from 2012 to 2017, and Switchgrass (2009-2011). On the land where the uncovered plots are located, a mixture of Bahiagrass (*Paspalum notatum*) and Bermudagrass (*Cynodon dactylon*) had been previously cultivated until 2018.

On a yearly basis, dead plants are replaced by switchgrass cultivar AP13 (Alamo) individuals in order to avoid allowing neighboring plants any competitive spatial advantage over the others. Biomass was harvested once a year, in December, after morphological data collection. Soil sampling was carried out after biomass harvest, starting in January.

<u>Soil Sampling</u>

In 2020, every plant located within the second replicate of each treatment has been subject to soil sampling at 0-15 cm depth, for a total of 800 soil samples collected. Samples were taken by means of a push auger of 2.5 cm diameter. Each sample represents a mixture of two sub-samples per plant, taken within 5 to 10 cm from the plant base. The soil samples were collected into paper bags which were left open for at least 24 hours to air-dry the soil and minimize reactivity without affecting organic carbon content (Tan, 2005). Prior to their analysis and after air-drying, the samples were ground and sieved through a 2-mm sieve.

Data from this first sampling will serve as a baseline reference for the following years. The soil sampling procedure adopted may be defined as systematic (samples are drawn systematically every time a switchgrass individual occurs; *ergo*, at 90 cm intervals), composited (every single sample used for analysis is a mixture of two sub-sampling units), disturbed (taken by means of an auger: as a consequence, a clear structure of soil aggregates is not observable), and homogenized (Tan, 2005). Samples grinding and sieving are an important mixing and homogenizing steps, which are functional to subsequent analysis and consistent with general, international (Tan, 2005) standards for the chemical analysis that the present work will discuss.

In January 2021, the second (first post-treatment) soil sampling round was performed. At this time, 150 genotypes selected on the basis of phenotypic diversity were subjected to soil sampling. The soil surrounding the corresponding 150 plants was sampled with a drill at three distinct depths (0- to 15, 15- to 30, and 30- to 60 cm), following the procedure described earlier, by means of a drill. The drill bid (2-cm diameter) was marked at the desired depths to make sure to keep the sampling depth constant. The sampling involved both the first and the second replicate for each of the two treatments, for a total of 1800 soil samples taken. The second post-treatment sampling round on the same 150 genotypes was completed in March 2022, according to the procedure followed in the previous sampling round.

Table 2.1: Average climatic data in Tifton, GA (31.446 N, 83.477 W) from 1923 to 2016. Elevation: 116 m. Data source: UGA Weather Network.

| **Time Period** | **Average Maximum Temperature (°C)** | **Average Minimum Temperature (°C)** | **Total Precipitation (mm)** | **Number of Rainy Days** |
|---|---|---|---|---|
| January | 16.0 | 3.6 | 108.458 | 9 |
| February | 17.6 | 4.8 | 106.68 | 9 |
| March | 21.2 | 8.2 | 122.174 | 9 |
| April | 25.4 | 12.1 | 98.806 | 7 |
| May | 29.2 | 16.5 | 82.296 | 8 |
| June | 32.0 | 20.2 | 117.094 | 11 |
| July | 32.7 | 21.5 | 137.922 | 14 |
| August | 32.7 | 21.3 | 123.952 | 13 |
| September | 30.7 | 19.0 | 96.774 | 9 |
| October | 26.3 | 13.0 | 57.658 | 6 |
| November | 21.2 | 7.7 | 63.5 | 7 |
| December | 17.0 | 4.5 | 92.456 | 9 |
| **Year** | **25.2** | **12.7** | **1,207.77** | **109** |

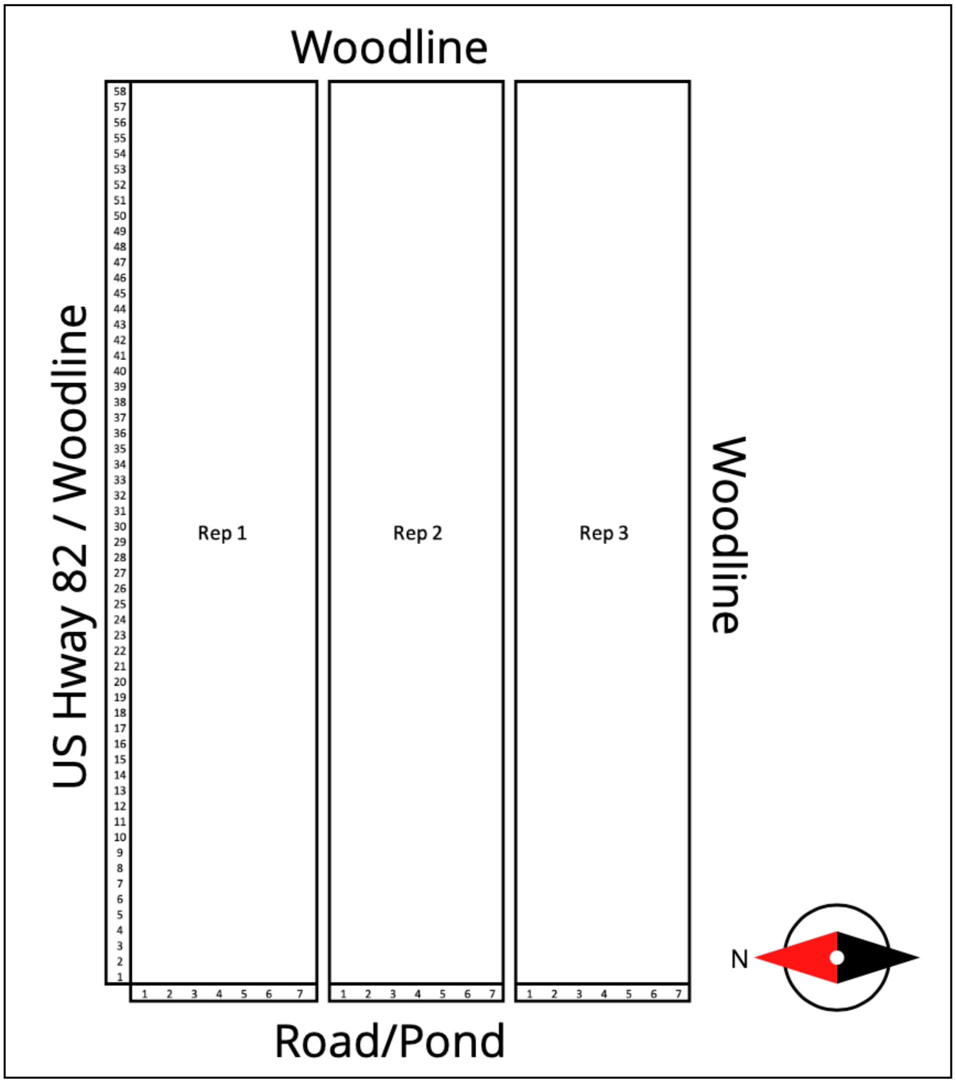


Figure 2.1: schematic representation of the CV (drought treatment) plot. The three replicates present 7 rows with 58 plants per row. Within each replicate, the accessions are randomly arranged to control for statistical errors due to spatial variation.

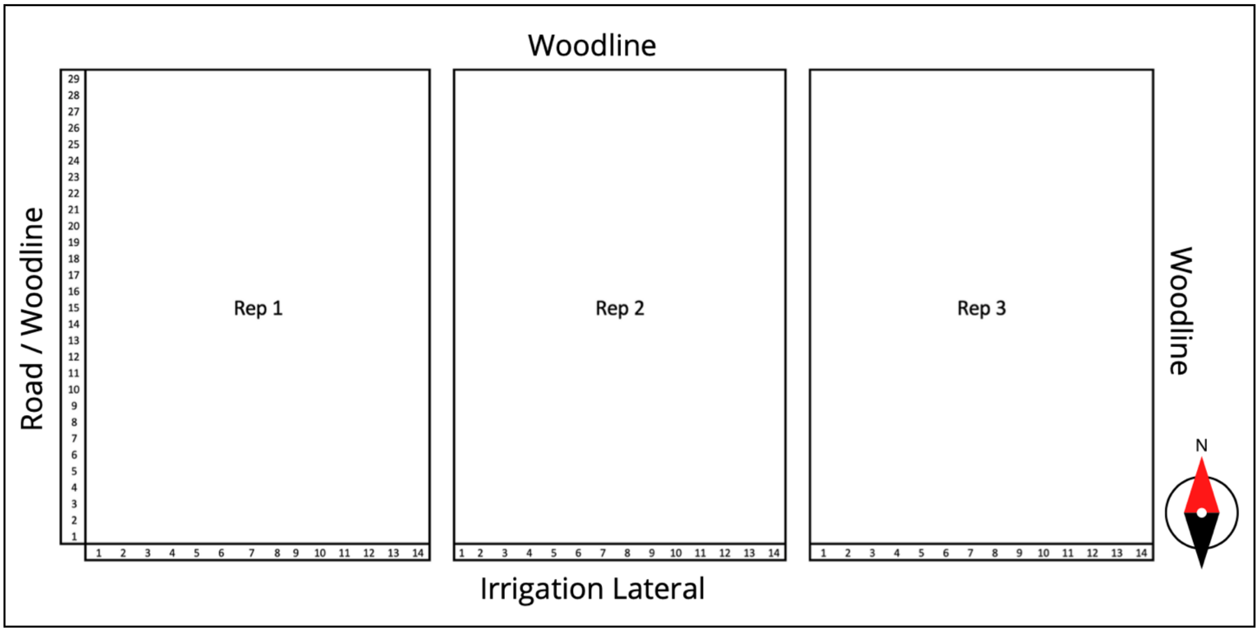


Figure 2.2: schematic representation of the UC ("uncovered", or control) plot. The three replicates present 14 rows with 29 plants per row. Within each replicate, the accessions are randomly arranged to control for statistical errors due to spatial variation.

Figure 2.3: picture of the CV (drought treatment) plot, with rain exclusion shelter.

Figure 2.4: picture of the UC (control) plot.

Figure 2.5: soil collection bucket with hole for drilling. The hole is close to the border of the bucket, to allow for sampling closer (about 5 cm) to the plant crown.

Soil sampling was performed in April 2023 on a subset of ten genotypes to check for differences in soil characteristics after three years of switchgrass cultivation and drought treatment. Sampling was performed at 0- to 15 and 15- to 30 cm depth. Among the analysis performed on these samples, there are pH measurements, % base saturation (%BS), cation exchange capacity (CEC), % organic matter (%OM), and % total organic carbon (%TOC). The protocols followed can be found at http://aesl.ces.uga.edu/soil.html.

*Biomass harvest*

Plants were individually harvested yearly. Fresh biomass weight was measured directly in the field. A sample of each plant was weighed, stored in a paper bag, and dried in order to later estimate the amount of dry biomass each plant produced. If the amount of fresh biomass of the individual plant was low enough for it to be stored entirely, then the entire harvested plant was weighed and stored in the paper bag. Plants are cut using a hand sickle or a chainsaw at a height of approximately 15 cm.

*Soil Analysis: POXC Protocol*

A 2.0 M potassium permanganate ($KMnO_4$) stock solution was prepared by dissolving 147 g of calcium chloride ($CaCl_2$) into 900 mL of deionized water and, once dissolution was complete, by adding 31.60 g of $KMnO_4$. The purpose of the $CaCl_2$ was to facilitate soil flocculation after the reaction is complete. Once the $KMnO_4$ was completely dissolved, pH was adjusted to 7.2 through sodium hydroxide (NaOH) addition. The shelf life of the stock solution is believed to be of at least three months, if stored in a dark bottle in a dark area. Starting from the $KMnO_4$ stock solution, four standard solutions were prepared. The standard solutions had five different $KMnO_4$ concentrations: 0.005, 0.01, 0.015, 0.02, and 0.025 M. The standard solutions were diluted daily to working standards by adding 0.5 mL of standard solution to 49.5 mL of deionized water.

For each sample, 2.50 g of soil was weighed and placed into a 50 mL centrifuge tube and 18.0 mL of water was added, together with 2 mL of $KMnO_4$ stock solution. The tubes were then rapidly placed on a shaker and shacked at 240 oscillations per minute. After 2 minutes, the tubes were placed in a dark area to settle for 10 minutes. Once settling time had passed, 0.5 mL of supernatant was quickly transferred into another 50 mL tube containing 49.5 mL of water. This constituted the final sample solution for the spectrophotometric reading at 550 nm. In order to perform the spectrophotometric reading, 250 µL of each sample and working standard were placed onto a 96-well plate.

The amount of POXC is a function of the amount of permanganate that is reduced. Hence, the higher the amount of POXC values, the lower the absorbance. A standard curve was constructed using the molarity and the absorbance of the standard solutions every time the spectrophotometric reading was carried out. In order to calculate POXC, the following equation was adopted (Weil et al., 2003).

$$\text{POXC (mg kg}^{-1}) = [0.02 \text{ mol/L} - (a + b \times \text{Abs})] \times (9000 \text{ mg C/mol}) \times (0.02 \text{ L solution/Wt})$$

Where:

0.02 mol/L = initial solution concentration;

a = intercept of the standard curve; b = slope of the standard curve;

Abs = absorbance of unknown sample;

9000 = milligrams of carbon oxidized by 1 mole of $MnO_4$ changing from $Mn^{7+}$ to $Mn^{2+}$;

0.02 L = volume of stock solution reacted;

Wt = weight of air-dried soil sample expressed in kg.

<u>*Statistical Analysis and Software*</u>

Data presented in this work was analyzed using linear models and linear mixed models in R software (R Core Team, 2018) which included the fixed effect of treatment, depth (when more than one depth was considered), post application year one and year two, genotype, and the random effect of block (or replication) when there were more than one. The model was computed using the *lme()* function from the lme4 R package (Bates *et al.*, 2015).

**Results**

<u>*POXC at Baseline*</u>

Data and results presented here are the results of the first (2020) – baseline – year of sampling, soil analyses, and data analyses. The model only included the fixed effect of treatment. The average POXC content was 315.3 (± 12.7) mg $kg^{-1}$ in the CV plot and 336.3 (± 14.5) mg $kg^{-1}$

in the UC plot. The ANOVA revealed that there was no statistically significant difference between the two plots at baseline ($F_{1, 274} = 1.9$, p-value = 0.16) (Fig. 2.6).

*POXC and Yield over Time, by Depth and Treatment*

In 2021 and 2022, samples were taken at three different depths (0-15, 15-30 and 30-60 cm depth). Table 2.2 summarizes the R output of the type II analysis of deviance. All of the fixed effects included in the model were significant, as well as their interactions ($p < 0.001$).

Post-hoc pairwise comparisons were carried out using the *emmeans()* function in the emmeans package (Lenth, 2023) (Table 2.3). Fig. 2.7 shows how average POXC changed over time by year, depth, and treatment. In 2021, under the CV treatment, the average POXC was 159.3 (± 5.5) mg $kg^{-1}$ at 0- to 15 cm depth and 163.2 (± 5.4) mg $kg^{-1}$ at 15- to 30 cm depth. Under the UC treatment, the average POXC was 255.5 (± 4.6) mg $kg^{-1}$ at 0- to 15 cm depth and 269.2 (± 6.3) mg $kg^{-1}$ at 15- to 30 cm depth. No significant difference between the 0- to 15 and 15- to 30 cm depth could be detected for both treatments. The deepest, 30- to 60cm depth showed a significantly lower POXC content compared to the previous two, under both treatments – average POXC was 132.4 (± 5.2) mg $kg^{-1}$ under the CV treatment, and 206.8 (± 4.4) mg $kg^{-1}$ under the UC treatment. In 2022, under the UC treatment, all three depths were significantly different from each other ($p < 0.05$). Average POXC was 260.6 (± 8.3) mg $kg^{-1}$ at 0- to 15 cm depth, 208. 8 (± 4.2) mg $kg^{-1}$ at 15- to 30 cm depth, and 159.1 (± 3.3) mg $kg^{-1}$ at 30- to 60 cm depth. Under the CV treatment, the average POXC content was 129.7 (± 4.0) at 0- to 15 cm depth and 128.9 (± 3.4) at 15- to 30 cm depth. The average POXC content at 30- to 60 cm depth was 110.8 (± 4.3) mg $kg^{-1}$ – significantly lower than the average POXC content at 0- to 15 cm depth ($p < 0.05$). Figures 2.8 to 2.10 show the variation in terms of POXC for each of the depths under study between 2020 and 2022. At 0- to 15 cm depth, the one for which three years of data are available, it is evident how POXC under the UC treatment remained stable through 2021 and 2022, whereas POXC under the CV treatment constantly decreased.

Fig. 2.11 shows how average yield changed over time by treatment. Similarly to what we observed for POXC, there was not much difference in terms of average yield between the two treatments in 2020. In the following years, the average yield in the control plot was greater than the average yield in the plot under drought treatment and than the average of 2020. In 2022, the average yield under both treatments was lower.

*Relationship between Yield and POXC*

When looking at the overall correlation between yield and POXC in 2021 and 2022, we found a positive and significant correlation, especially in 2022 and in deeper soil layers (15-30 and 30-60 cm) (Fig. 2.12). The positive correlation is due to the relationship realized between POXC and yield under the CV treatment (Fig. 2.14). In fact, under the UC treatment (control), there was no correlation between yield and POXC for any of the years or any of the depths under study (Fig. 2.13). The positive relationship between yield and POXC under the CV treatment was slight, nonetheless consistently present across both 2021 and 2022 and at all depths (Fig. 2.14). The strongest correlations are observed in 2022, at 15- to 30 cm depth ($r = 0.27$, $p < 0.0001$) and at 30- to 60 cm depth ($r = 0.27$, $p < 0.0001$). There is no correlation between both treatments in 2020 ($p > 0.05$).

Table 1.4 summarizes the Pearson correlation and Spearman rank-correlation coefficients for each combination of treatment, year, and depth of interest. The coefficients are color-coded in order to highlight the combinations of year, treatment, and depth that show a stronger correlation.

*Soil Characteristics*

Samples taken in April 2023 were analyzed for pH, % base saturation, cation exchange capacity, % organic matter, and % total organic carbon. Results provide information on soil characteristics after three years of drought treatment and switchgrass cultivation. Fig. 2.15 through 2.19 summarize these results. The UC plot has an overall higher pH (Fig. 2.18), % base saturation (Fig. 2.19), cation exchange capacity (Fig. 2.17), % organic matter (Fig. 2.16), and % total organic carbon (Fig. 2.15) at both sampling depths.

*Environmental data*

Soil temperature and volumetric water content were measured from 2020 through 2022. Volumetric water contend data is shown in Fig. 2.20. Volumetric water content was consistently higher under the UC treatment compared to the CV treatment. Fig. 2.21 shows soil temperature data. Soil temperature recorded in the UC plot was similar to the temperature recorded under the CV plot.

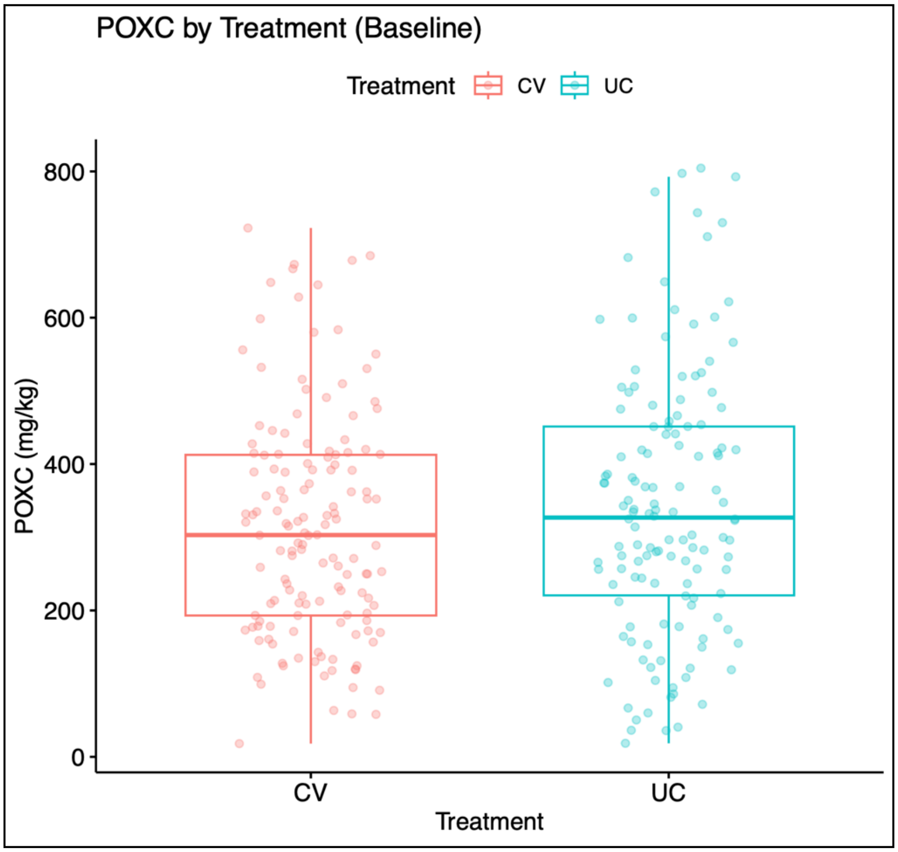


Figure 2.6: Boxplot showing soil POXC content (mg/kg) at baseline (2020), by treatment. Different colors indicate different treatments. Each data point represents one individual sample. All samples were taken from replication 2, at 0- to 15 cm depth. There is no statistically significant difference in the average POXC level of the two plots ($F_{1, 274} = 1.9$, p-value = 0.16).

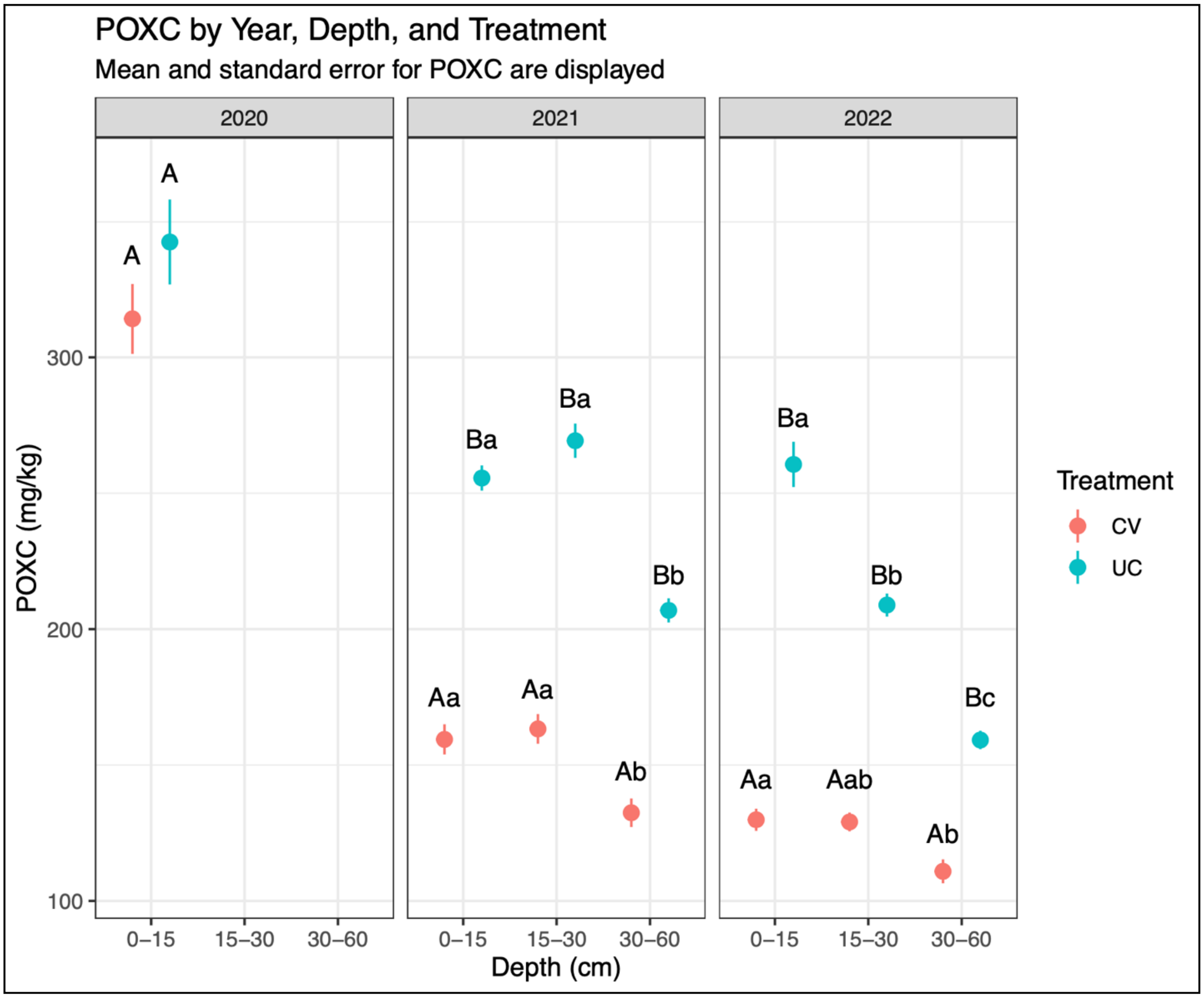


Figure 2.7: Average soil POXC content by depth, treatment, and year. Different colors indicate different treatments. The graph is faceted by year. Within each facet, average POXC (±se) for each treatment and depth is represented. Different upper-case letters indicate significant differences (p-value < 0.05) between treatments, within the same depth and year. Different lower-case letters indicate significant differences between depths, within the same treatment and year.

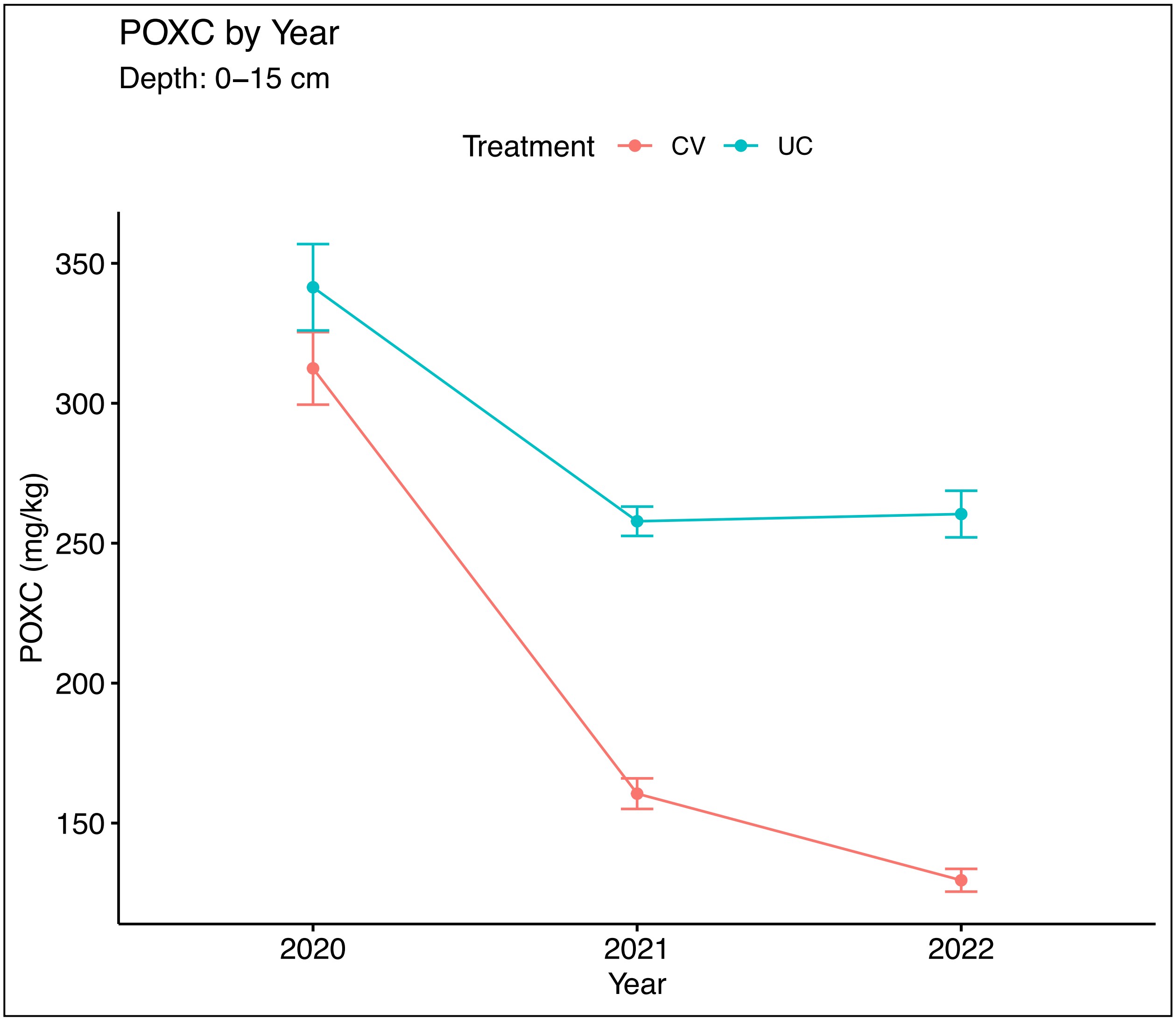


Figure 2.8: Average POXC (± se) at 0- to 15 cm depth, by treatment and year. Different colors indicate different treatments. It is possible to appreciate the divergence, in terms of POXC, at time passes, depending on the treatment. Under both treatments, average POXC tends to decrease in 2021. In 2022, POXC remains stable for the UC (control) treatment, whereas it decreases even more for the CV (National Drought Mitigation Center) treatment.

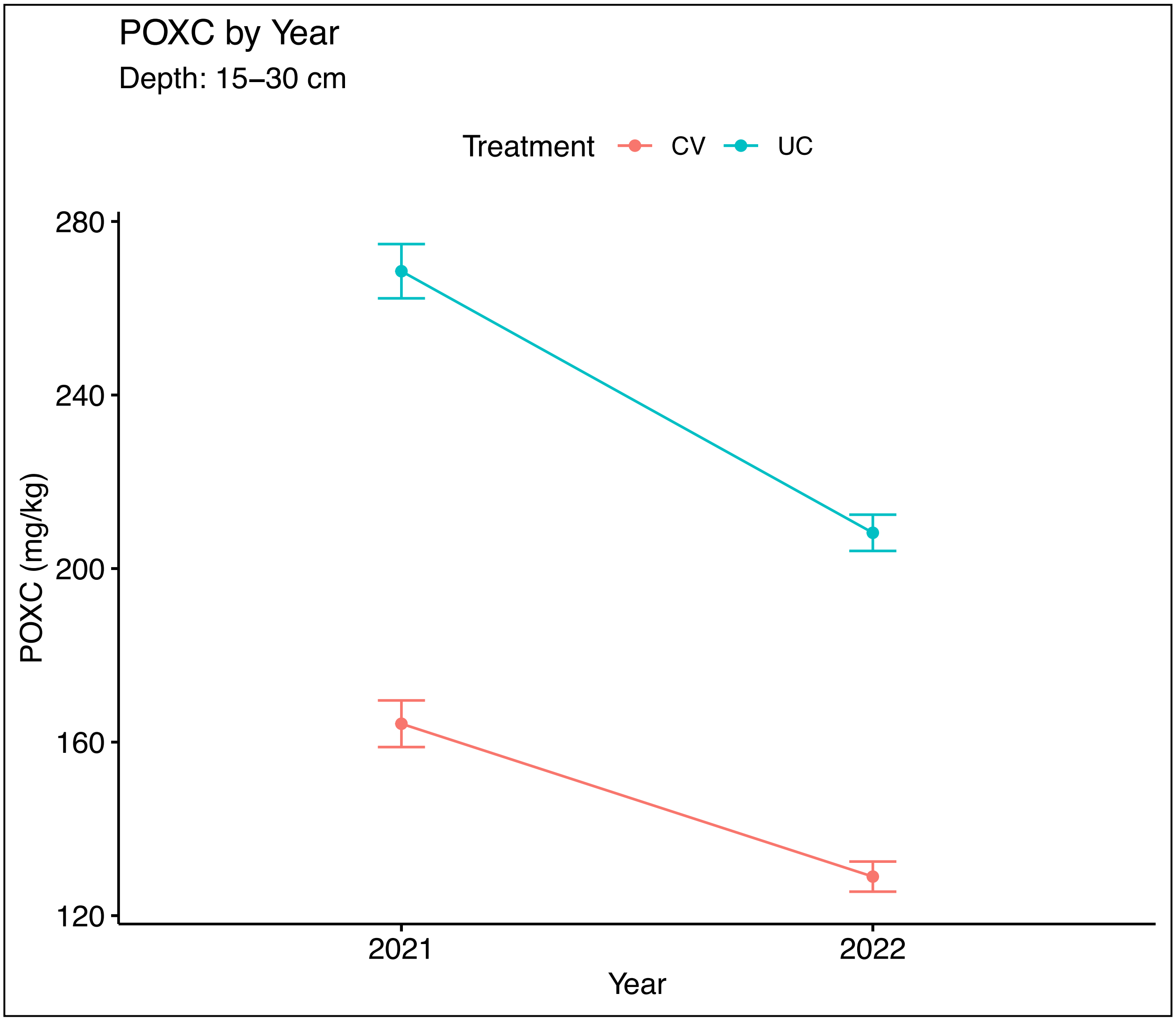


Figure 2.9: Average POXC (± se) at 15- to 30 cm depth, by treatment and year. Different colors indicate different treatments. Under both treatments, average POXC tends to decrease in 2022.

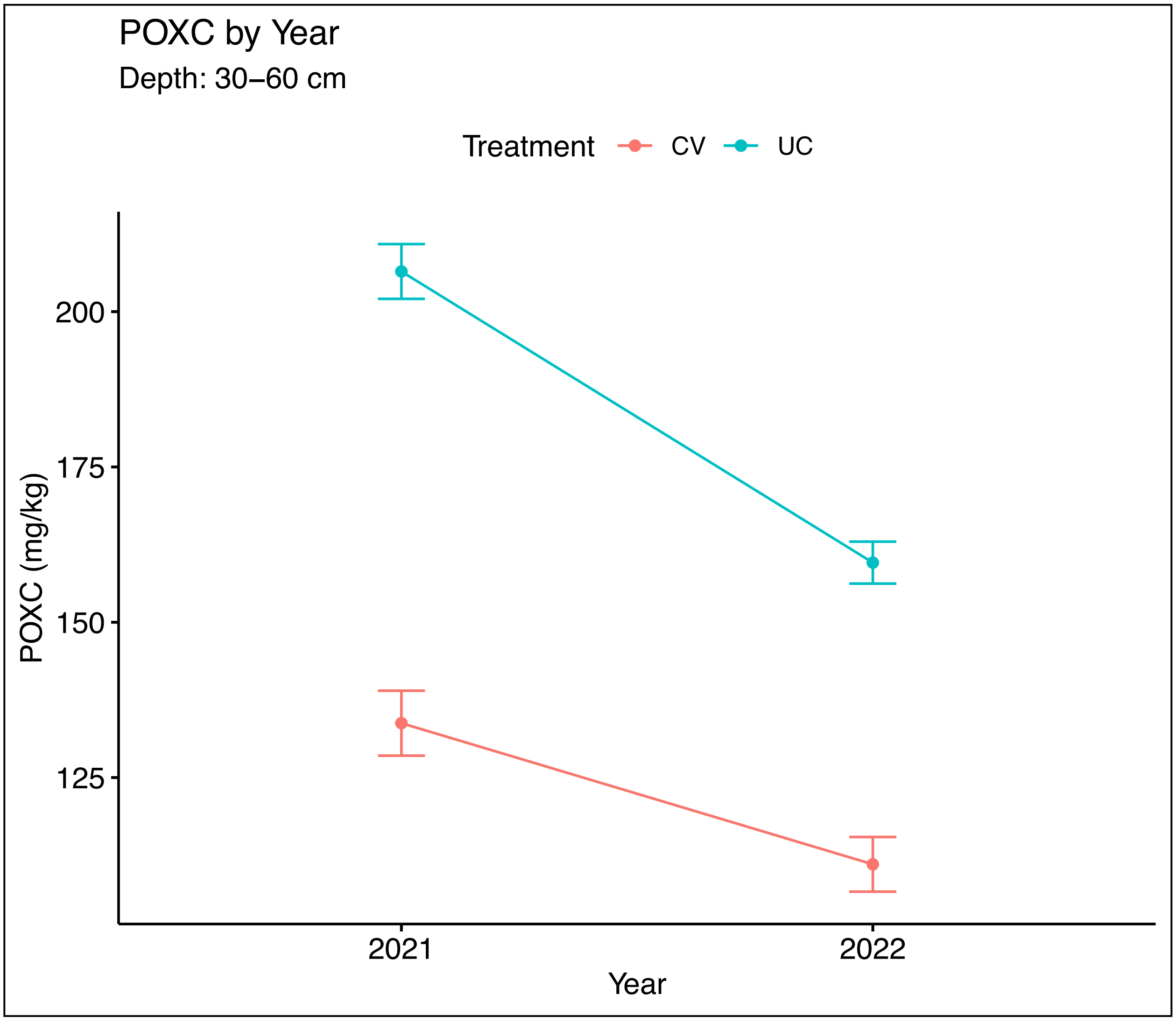

Figure 2.10: Average POXC (± se) at 30- to 60 cm depth, by treatment and year. Different colors indicate different treatments. Under both treatments, average POXC tends to decrease in 2022.

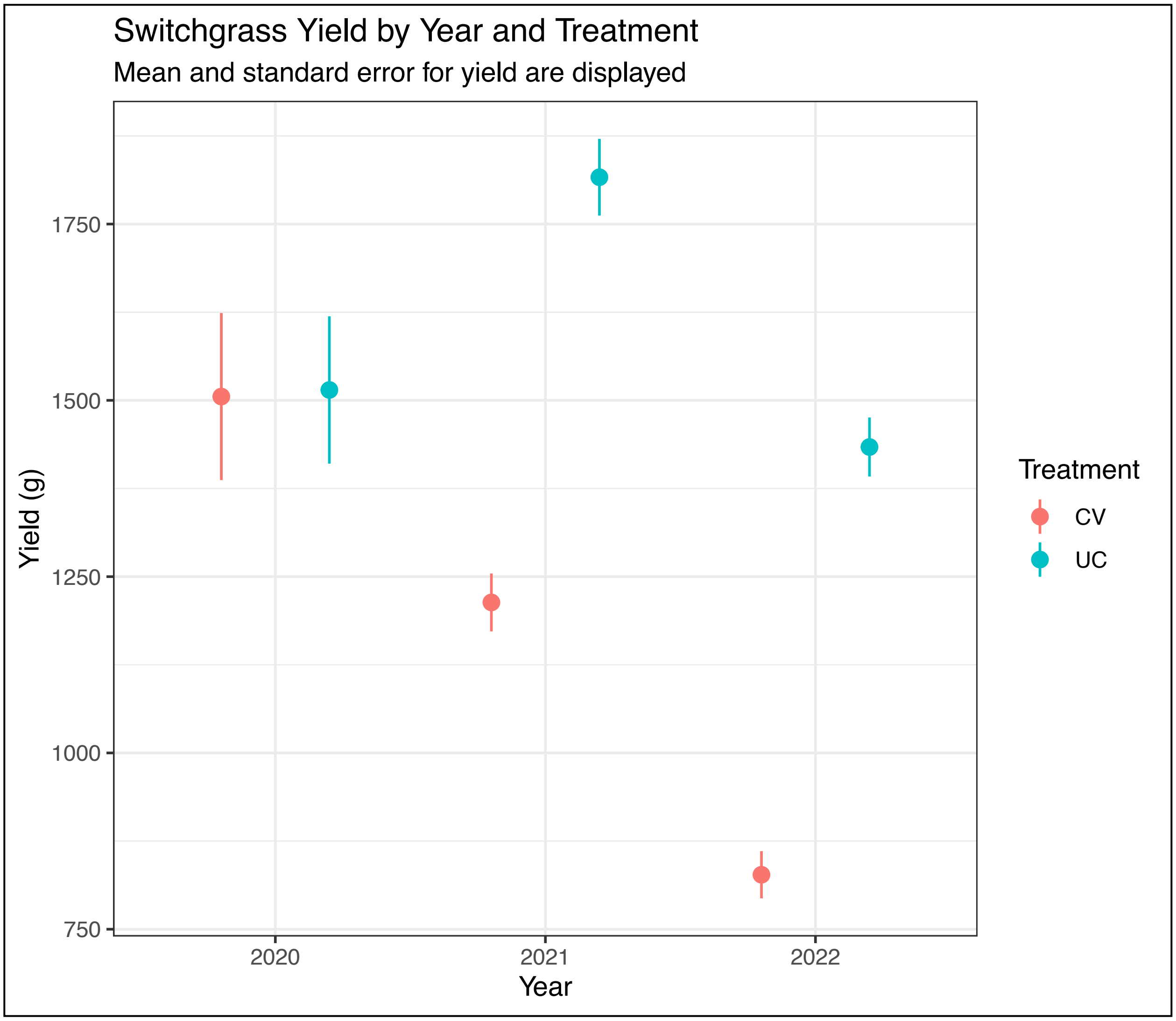


Figure 2.11: Average Switchgrass yield (±se) by treatment and year. Different colors indicate different treatments. Yield in 2020 is comparable, whereas in the following years average yield in the control (UC) is much higher than the average yield in the plot under drought treatment (CV).

Table 2.2: Summary of the type II analysis of deviance carried out in R software. All of the fixed effects included in the model and their interactions are significant to the 0.001 significance level.

| Source | Chisq | df | p-value |
|---|---|---|---|
| Treatment | 731.336 | 1 | <0.001 |
| Year | 412.165 | 2 | <0.001 |
| Depth | 169.692 | 2 | <0.001 |
| Treatment:Year | 48.923 | 2 | <0.001 |
| Treatment:Depth | 43.797 | 2 | <0.001 |
| Year:Depth | 19.131 | 2 | <0.001 |
| Treatment:Year:Depth | 19.293 | 2 | <0.001 |

Table 2.3: Post-hoc pairwise comparisons carried out through the *emmeans()* function in the emmeans package in R software. P-values were Tukey-adjusted. P-values in bold are considered significant. There is no difference in the average POXC level observed at 0-15 cm depth and 15-30 cm depth, exception made for the control in 2022, where the average POXC level at 15-30 cm depth is significantly lower than the average POXC at 0-15 cm depth.

| Treatment | Year | Contrast | Estimate | SE | Z ratio | p-value |
|---|---|---|---|---|---|---|
| CV | 2021 | 0-15 vs 15-30 | -4.11 | 7.91 | -0.520 | 0.8617 |
| | | 0-15 vs 30-60 | 27.09 | 7.96 | 3.401 | **0.0019** |
| | | 15-30 vs 30-60 | 31.20 | 7.93 | 3.936 | **0.0002** |
| | 2022 | 0-15 vs 15-30 | 0.70 | 7.89 | 0.089 | 0.9957 |
| | | 0-15 vs 30-60 | 18.88 | 7.90 | 2.388 | **0.0446** |
| | | 15-30 vs 30-60 | 18.18 | 7.90 | 2.300 | 0.0558 |
| UC | 2021 | 0-15 vs 15-30 | -13.78 | 8.19 | -1.683 | 0.2118 |
| | | 0-15 vs 30-60 | 48.68 | 8.20 | 5.938 | **<0.0001** |
| | | 15-30 vs 30-60 | 61.73 | 8.22 | 7.505 | **<0.0001** |
| | 2022 | 0-15 vs 15-30 | 51.74 | 7.95 | 6.509 | **<0.0001** |
| | | 0-15 vs 30-60 | 101.41 | 7.95 | 12.758 | **<0.0001** |
| | | 15-30 vs 30-60 | 49.67 | 7.95 | 6.249 | **<0.0001** |

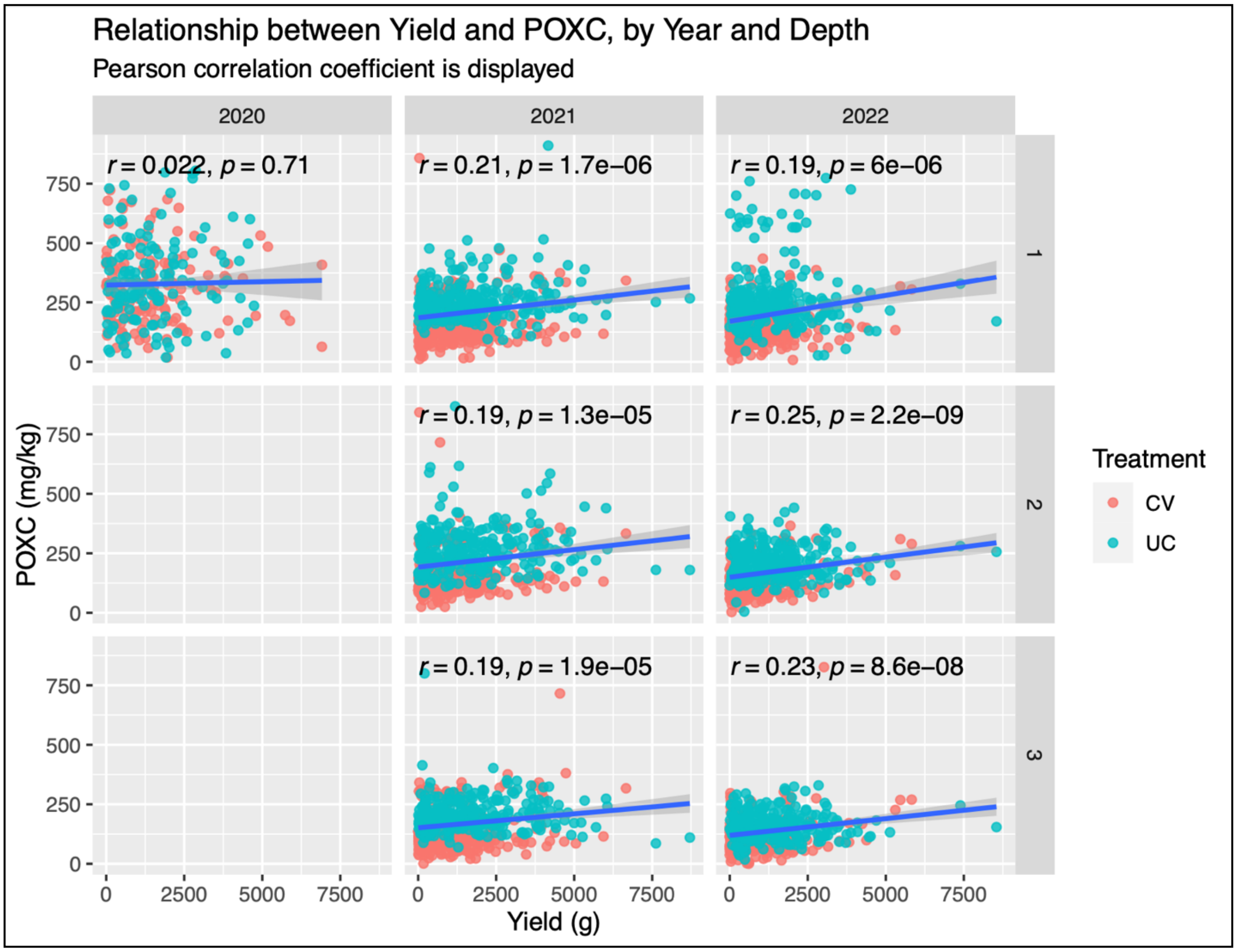


Figure 2.12: Correlation between yield and POXC for every combination of year and depth under study. Depth 1 is 0- to 15 cm, depth 2 is 15- to 30 cm and depth 3 is 30- to 60 cm. In 2020 (baseline) there is no correlation. In the next years, there is a slightly positive, consistent correlation between yield and POXC, which becomes higher and strongly significant in 2022 and as soil sampling depth increases.

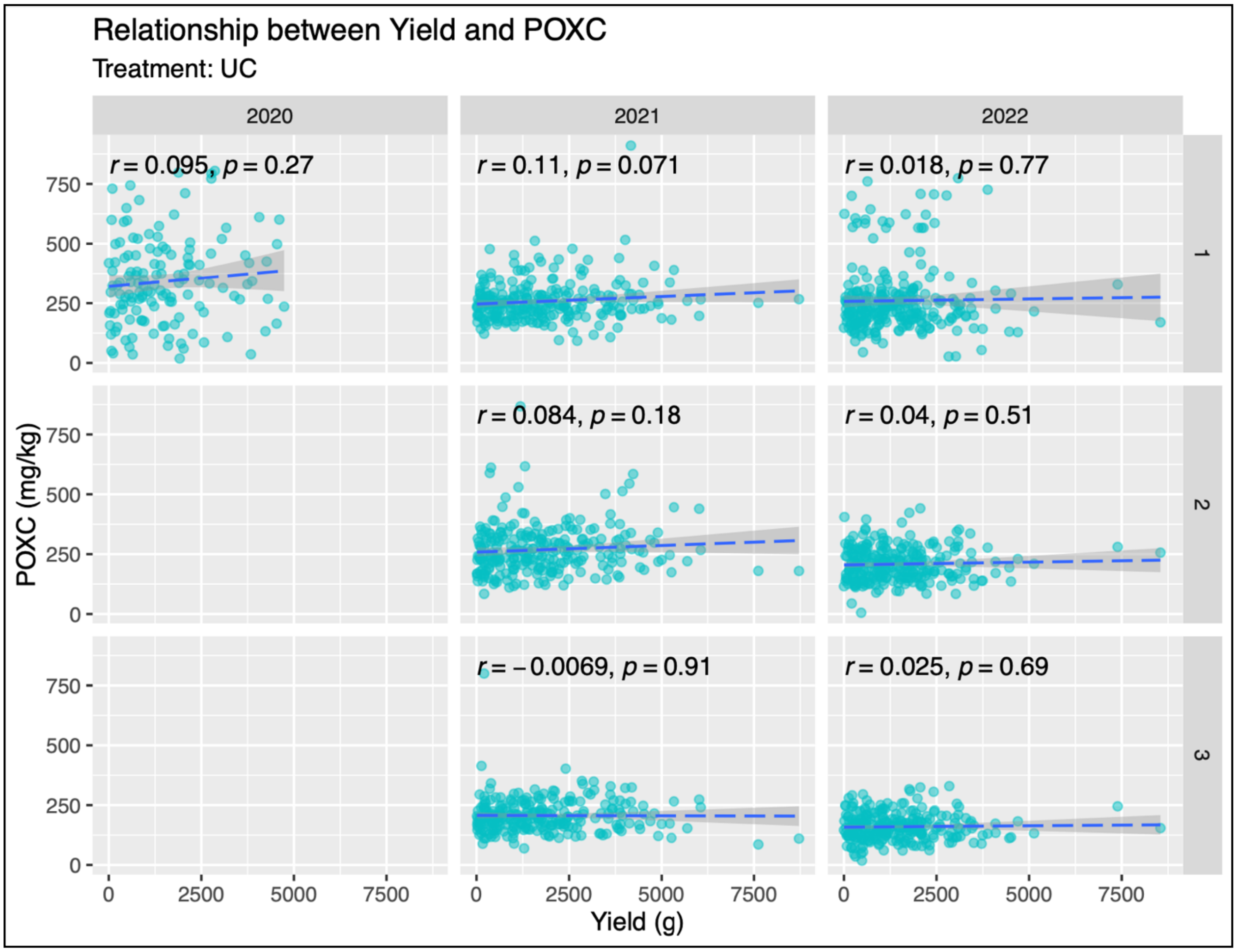


Figure 2.13: Correlation between yield and POXC for every combination of year and depth under study, UC (control) treatment. Depth 1 is 0- to 15 cm, depth 2 is 15- to 30 cm and depth 3 is 30- to 60 cm. There doesn't seem to be any correlation between yield and POXC for any of the years and depths under study.

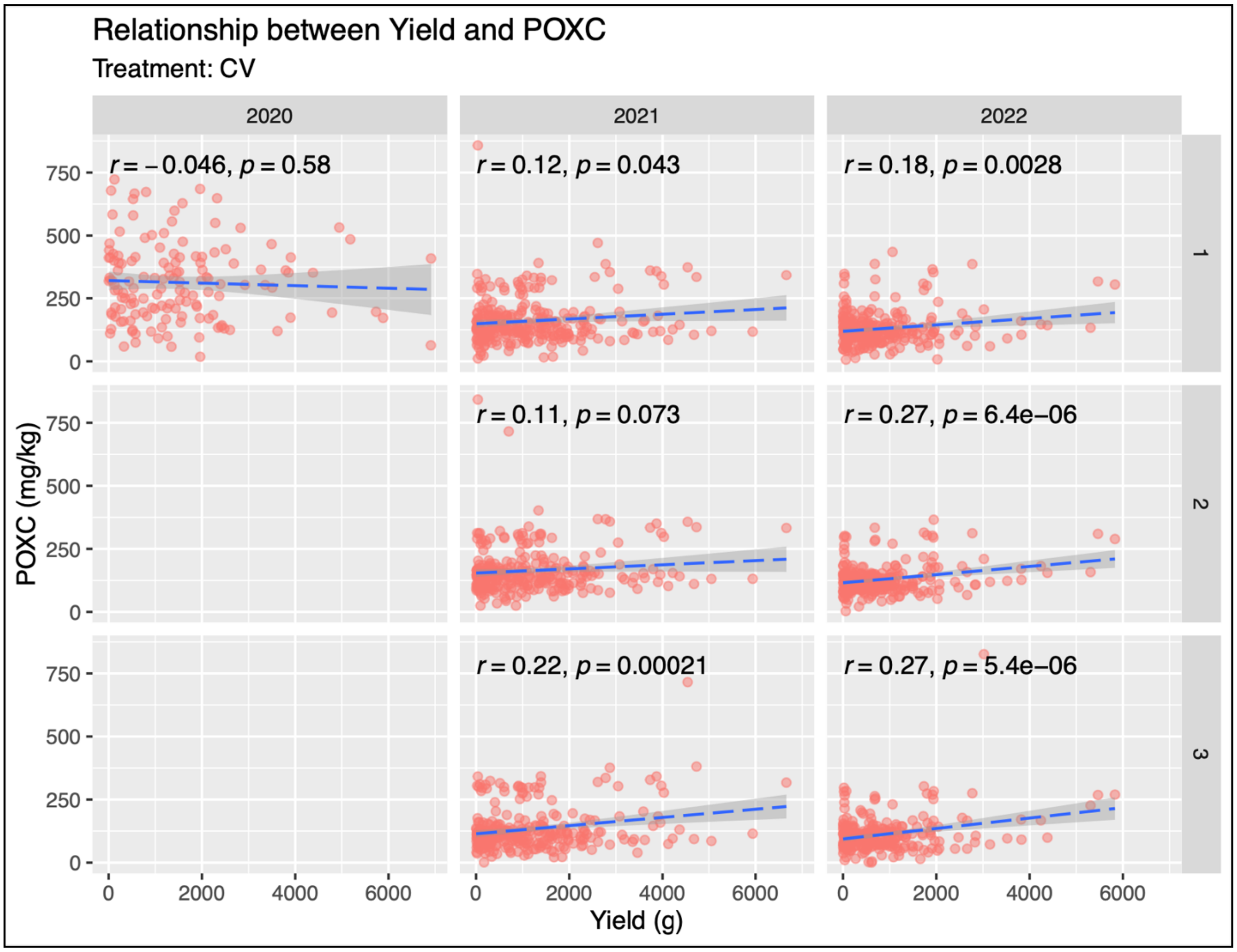


Figure 2.14: Correlation between yield and POXC for every combination of year and depth under study, CV (drought) treatment. Depth 1 is 0- to 15 cm, depth 2 is 15- to 30 cm and depth 3 is 30- to 60 cm. The slightly positive correlation between yield and POXC becomes particularly evident and significant in 2022, as sampling depth increases.

Table 2.4: Pearson and Spearman rank correlation coefficients between yield and POXC for every combination of year, treatment, and depth of interest. The color indicates a stronger (yellow) or weaker (violet) correlation. The strongest values are observed under the CV treatment in 2022, for the two higher sampling depths (15-30 and 30-60 cm).

**Pearson and Spearman Rank Correlation Coefficients**

Between POXC and Yield, by Year and Depth

| | Pearson (2021) | | Spearman (2021) | | Pearson (2022) | | Spearman (2022) | |
|---|---|---|---|---|---|---|---|---|
| Depth | UC | CV | UC | CV | UC | CV | UC | CV |
| 0-15 | 0.113 | 0.122 | 0.0820 | 0.048 | 0.018 | 0.181 | 0.047 | 0.038 |
| 15-30 | 0.084 | 0.107 | 0.1230 | 0.075 | 0.040 | 0.271 | 0.023 | 0.111 |
| 30-60 | -0.007 | 0.223 | 0.0734 | 0.098 | 0.025 | 0.274 | 0.009 | 0.107 |

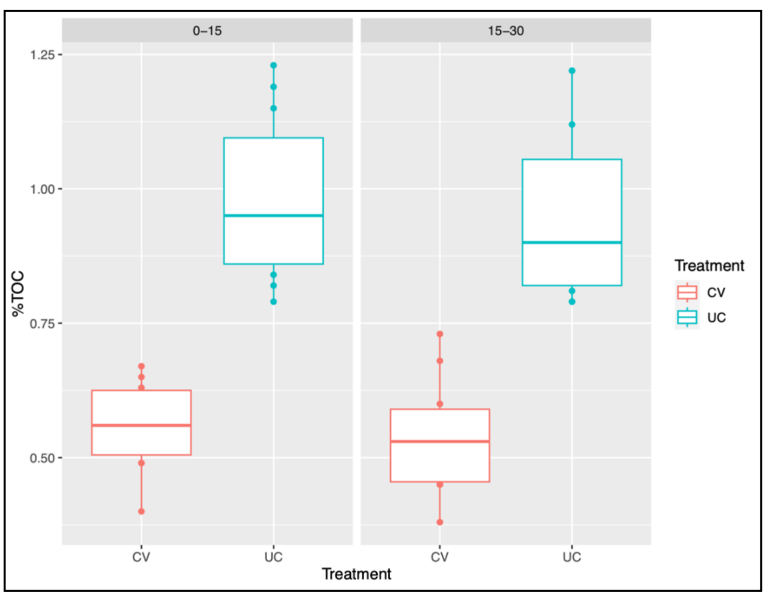


Fig. 2.15: % total organic carbon (%TOC) by treatment and depth. Samples taken in April 2023.

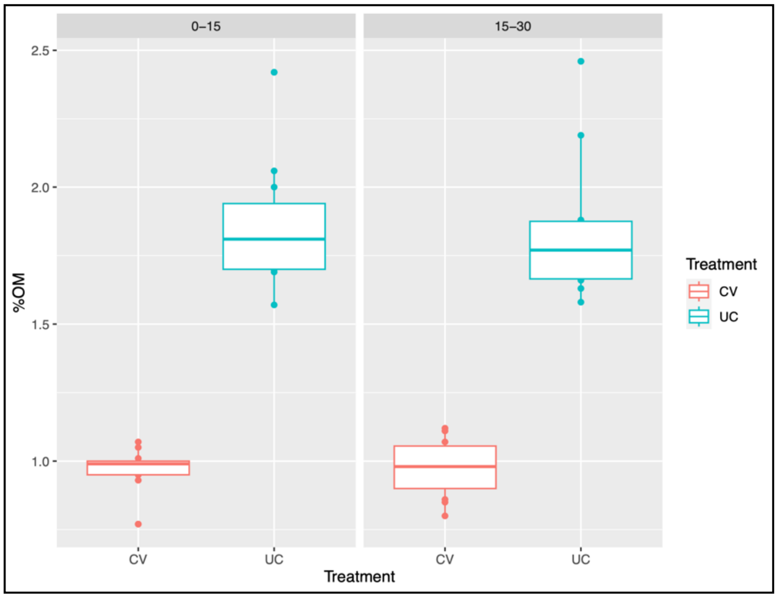


Fig. 2.16: % organic matter (%OM) by treatment and depth. Samples taken in April 2023. Organic matter is determined by the "loss on ignition" method (LOI).

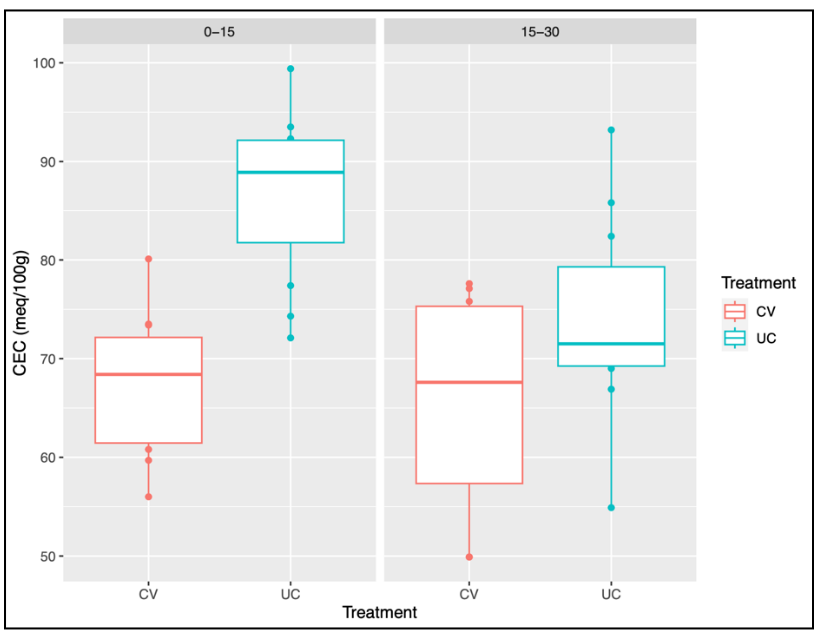


Fig. 2.17: Cation exchange capacity (meq/100g) by treatment and depth. Samples taken in April 2023.

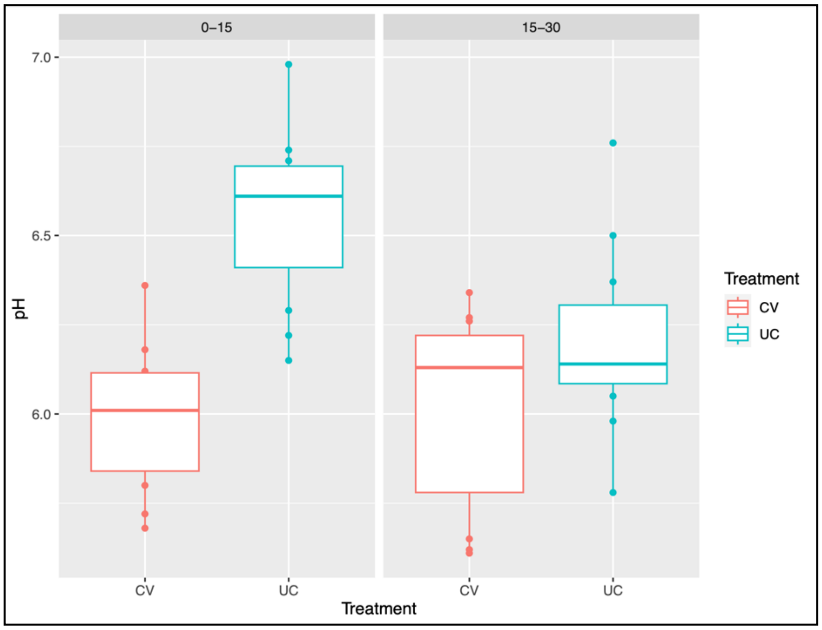


Fig. 2.18: pH by treatment and depth. Samples taken in April 2023.

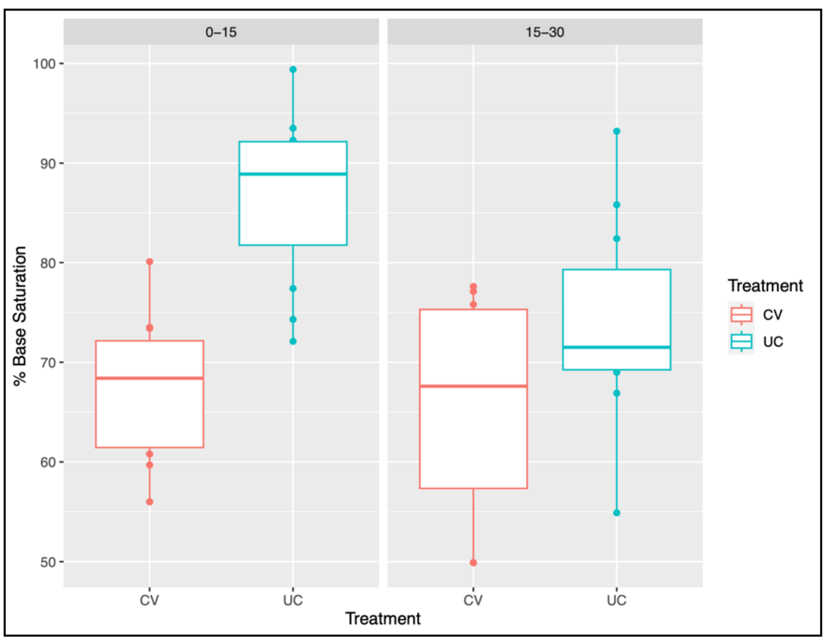


Fig. 2.19: % base saturation by treatment and depth. Samples taken in April 2023.

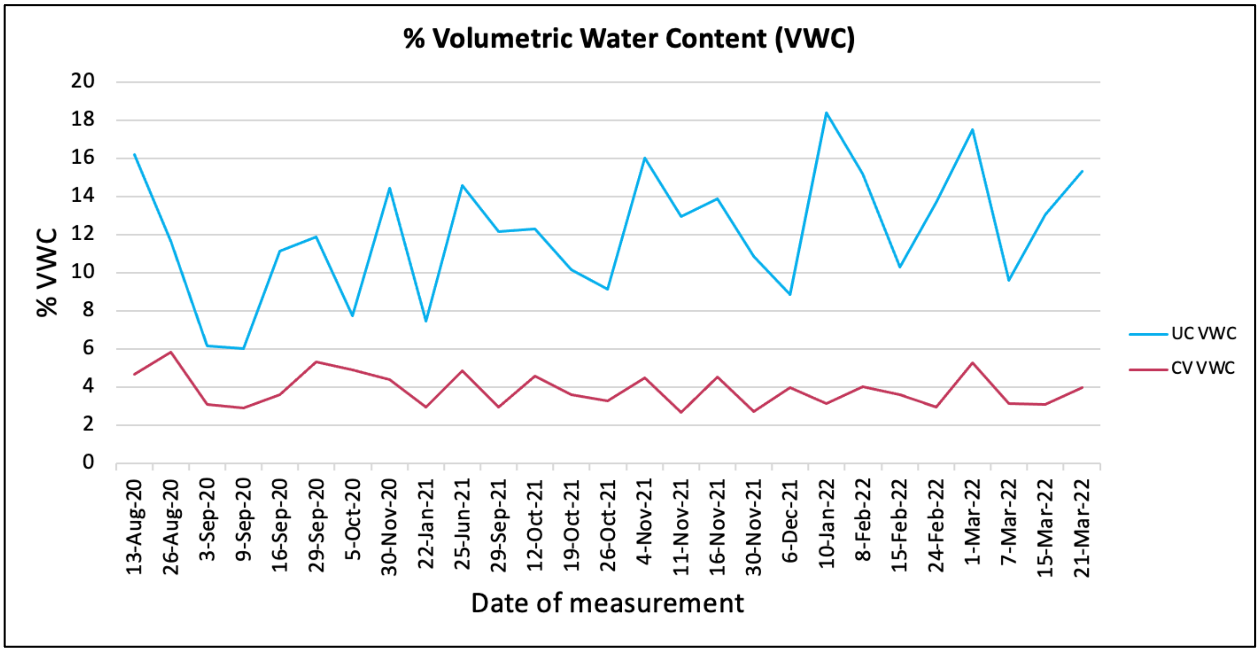


Fig. 2.20: Soil volumetric water content within the control (UC) and the drought-stressed (CV) plot.

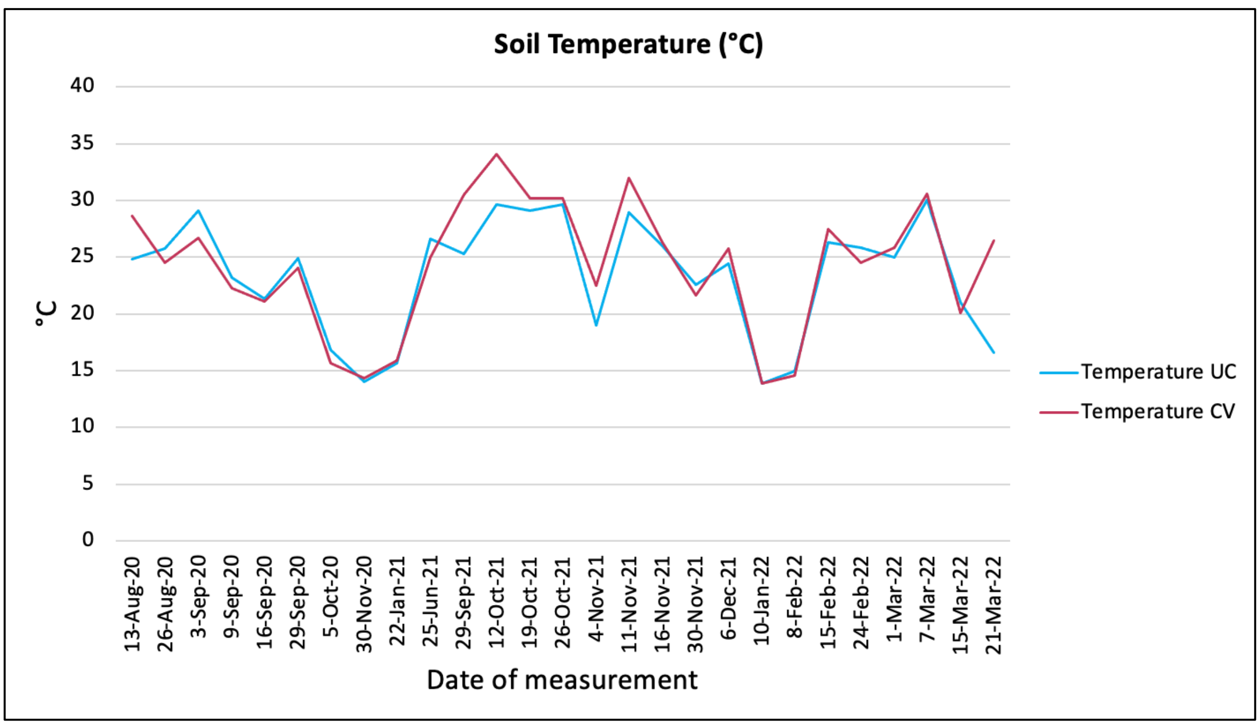


Fig. 2.21: Soil temperature (°C) within the control (UC) and the drought-stressed (CV) plot.

## Discussion

Switchgrass is a candidate biofuel feedstock crop. The potential contribution of Switchgrass to climate change mitigation is determined by its ability to serve as a GHG sink (Field et al., 2018, Bai et al., 2022). This depends on the biogeochemical impacts of switchgrass cultivation over time in terms of carbon neutrality and water and nutrient conservation (Robertson et al., 2011). Both climatic and soil characteristics affect SOC sequestration of switchgrass on marginal lands (Martinez-Feria and Basso, 2020).

Previous studies have demonstrated that Switchgrass can increase soil C storage, although with considerable variation depending on sites (Liebig et al., 2008) and management practices (Follett et al., 2012, Collins et al., 2020). The assessment of Switchgrass as a GHG sink requires data on soil organic carbon change in different environments. POXC has been indicated as a measure of the biologically active soil carbon fraction, which is a component of the total organic carbon pool. It was suggested as a sensitive tool to monitor active soil C content in response to management in various cropping systems (Culman et al., 2012). In this study, POXC was used to measure the variability in soil carbon sequestration capability of 150 Switchgrass genotypes at three different depths and to investigate the effect of water limitation over three growing seasons. The lack of significant differences in soil POXC content at baseline between the two study sites indicates that the difference in previous management did not have any impact on the soil carbon pool measured by POXC, providing a suitable soil environment for the experiment.

When looking at the difference in average POXC between the two treatments in 2021, it appears that the water limitation treatment significantly lowered soil POXC. This is likely caused by the detrimental impact of water limitation on both switchgrass growth and soil quality. Overall, POXC continued decreasing in the covered section in 2022.

Moreover, we were able to observe that in 2021 POXC measured at 0-15 cm was not significantly different from POXC measured at 15-30 cm under both treatments. In 2022, in the control plot, there was a significant difference in average POXC content between 0-15 and 15-30 cm: mean POXC content at 15-30 cm depth was substantially lower. Whether this is due to seasonal variation or is part of a consistent phenotypic behavior, it will have to be evaluated through more sampling rounds. Under both treatments, POXC was significantly lower at 30- to 60 cm depth, possibly due to the combination of two factors: (i) the lower switchgrass root biomass at deeper depths; (ii) the lack of water in deeper soil layers, especially under drought treatment.

The correlation between yield and POXC was statistically significant ($p < 0.05$). The magnitude of this correlation becomes more prominent over time and at deeper depths. We did not find any statistically significant correlation between yield and POXC content in the UC plot. However, this was not the case for the CV treatment, where we found a positive correlation in both 2021 and 2022. It is possible that the POXC content was partially influenced by the behavior of Switchgrass under drought conditions, as suggested by the positive correlation found in the CV plot. In 2022, the two deeper soil layers (15- to 30 and 30- to 60 cm) showed a stronger correlation between yield and POXC content. In other words, the data showed that the positive correlation between yield and POXC was only detectable under drought conditions and became more evident at deeper soil layers, similarly to what was observed by Liebig et al. (2005).

**Future Directions**

This study provides a framework for the evaluation of Switchgrass as a sustainable biofuel crop in that it addresses both soil health and soil carbon content through POXC measurements over time. If Switchgrass becomes a relevant, sustainable source of biofuel feedstock, it will depend on the ability to develop and commercialize a cultivar that meets economic and sustainability standards (Field et al., 2018). While economic standards are mainly influenced by yield, yield stability, and agronomic practices, sustainability standards depend on the resources that are necessary to efficiently cultivate Switchgrass and on the overall carbon balance of switchgrass-based biofuel production. This study addresses all of these issues, investigating the relationship between yield and POXC, also in relation to water limitation.

In addition, the data presented here constitute useful information in the definition of soil sampling protocols for future sampling rounds and experiments involving the evaluation of the impact of Switchgrass on soil characteristics within deep soil layers. Future developments of this study should be (1) further exploration of the database to investigate the relationship between POXC and other morphological traits; (2) further monitoring of the effect of water limitation on POXC content, through repeated measurements over time.

**References**

BAI, J., LUO, L., LI, A., LAI, X., ZHANG, X., YU, Y., WANG, H., WU, N., ZHANG, L. 2022. Effects of Biofuel Crop Switchgrass (Panicum virgatum) Cultivation on Soil Carbon Sequestration and Greenhouse Gas Emissions: A Review. *Life*, 12**,** 2015-2027.

BATES, D., MAECHLER, M., BOLKER, B. & WALKER, S. 2015. lme4: Linear Mixed-Effect Models Using Eigen and S4. R package version 1.1-10.

BLAIR, G. J., LEFROY, R. D. B. & LISLE, L. 1995. Soil Carbon Fractions Based on their Degree of Oxidation, and the Development of a Carbon Management Index for Agricultural Systems. *Australian Journal of Agricultural Research,* 46**,** 1459-1466.

BRANSBY, D. I., MCLAUGHLIN, S. B. & PARRISH, D. J. 1998. A Review of Carbon and Nitrogen Balances in Switchgrass Grown for Energy. *Biomass and Bioenergy,* 14**,** 379-384.

COLLINS, H. P., KIMURA, E., POLLEY, W., FAY, P. A., FRANSEN, S. 2020. *Biomass and Bioenergy*, 138**,** 105558.

CULMAN, S., FREEMAN, M. & SNAPP, S. Procedure for the Determination of Permanganate Oxidizable Carbon [Online]. Available: https://lter.kbs.msu.edu/protocols/133 [Accessed May 2023].

CULMAN, S. W., SNAPP, S. S., FREEMAN, M. A., SCHIPANSKI, M. E., BENISTON, J., LAL, R., DRINKWATER, L. E., FRANZLUEBBERS, A. J., GLOVER, J. D., GRANDY, A. S., LEE, J., SIX, J., MAUL, J. E., MIRSKY, S. B., SPARGO, J. T., WANDER, M. M. 2012. Permanganate Oxidizable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. Soil Science Society of America Journal, 76**,** 494-504.

FAN, P., HAO, M., DING, F., JIANG, D. & DONG, D. 2020. Quantifying Global Potential Marginal Land Resources for Switchgrass. *Energies,* 13.

FENG, Q., CHAUBEY, I., ENGEL, B., CIBIN, R., SUDHEER, K. P. & VOLENEC, J. 2017. Marginal Land Suitability for Switchgrass, Miscanthus and Hybrid Poplar in the Upper Mississippi River Basin (UMRB). *Environmental Modelling & Software,* 93**,** 356-365.

FIELD, J. L., EVANS, S. G., MARX, E., EASTER, M., ADLER, P. R., DINH, T., WILLSON, B., PAUSTIAN, K. 2018. High-resolution Techno-ecological Modelling of a Bioenergy Landscape to Identify Climate Mitigation Opportinities in Cellulosic Ethanol Production. *Nature Energy*, 3**,** 211-219.

FOLLETT, R. F., VOGEL, K. P., VARVEL, G. E., MITCHELL, R. B., KIMBLE, J. 2012. Soil Carbon Sequestration by Switchgrass and No-Till Maize Grown for Bioenergy. *Bioenergy Research*, 5**,** 866-875.

FRANK, A. B., BERDAHL, J. D., HANSON, J. D., LIEBIG, M. A. & JOHNSON, H. A. 2004. Biomass and Carbon Partitioning in Switchgrass. *Crop Science,* 44**,** 1391-1396.

LAL, R. & FOLLETT, R. F. 2009. *Soil Carbon Sequestration and the Greenhouse Effect*, Soil Science Society of America.

LEFROY, R. D. B., BLAIR, G. J. & STRONG, W. M. 1993. Changes in Soil Organic Matter with Cropping as Measured by Organic Carbon Fractions and 13C Natural Isotope Abundance. *Plant and Soil,* 155/156**,** 399-402.

LEMUS, R. & LAL, R. 2005. Bioenergy Crops and Carbon Sequestration. *Critical Reviews in Plant Sciences,* 24**,** 1-21.

LENTH, R. 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means.

LIEBIG, M. A., JOHNSON, H. A., HANSON, J. D. & FRANK, A. B. 2005. Soil Carbon Under Switchgrass Stands and Cultivated Cropland. *Biomass and Bioenergy,* 28**,** 347-354.

LIEBIG, M. A., SCHMER, M. R., VOGEL, K. P., MITCHELL, R. B. 2008. Soil Carbon Storage by Switchgrass Grown for Bioenergy. *Bioenergy Research*, 1**,** 215-222.

MA, Z., WOOD, C. W. & BRANSBY, D. I. 2000. Soil Management Impacts On Soil Carbon Sequestration by Switchgrass. *Biomass and Bioenergy,* 18**,** 469-477.

MAKAJU, S. O., WU, Y. Q., ZANG, H., KAKANI, V. G., TALIAFERRO, C. M. & ANDERSON, M. P. 2013. Switchgrass Winter Yield, Year-Round Elemental Concentrations, and Associated Soil Nutrients in a Zero Input Environment. *Agronomy Journal,* 105**,** 463-470.

MARTINEZ-FERIA, R. & BASSO, B. 2020. Predicting Soil Carbon Changes in Switchgrass Grown on Marginal Lands Under Climate Change and Adaptation Strategies. *GCB-Bioenergy*, 12**,** 742-755.

MCLAUGHLIN, S. B. & KSZOS, L. A. 2005. Development of Switchgrass (Panicum virgatum) as a Bioenergy Feedstock in the United States. *Biomass and Bioenergy,* 28**,** 515-535.

MITCHELL, R. B., VOGEL, K. P. & UDEN, D. R. 2012. The Feasibility of Switchgrass for Biofuel Production. *Biofuels*, 3**,** 47-59.

MUNSHOWER, F. F. 1994. Practical Handbook of Disturbed Land Revegetation. CRC Press, Boca Raton, FL, USA.

PARRISH, D. J. & FIKE, J. H. 2005. The Biology and Agronomy of Switchgrass for Biofuels. *Critical Reviews in Plant Sciences,* 24**,** 423-459.

R CORE TEAM 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. R package version 1.8.5 ed. Vienna, Austria.

SCHMER, M. R., VOGEL, K. P., MITCHELL, R. B. & PERRIN, R. K. 2008. Net energy of cellulosic ethanol from Switchgrass. *PNAS,* 105**,** 464-469.

TAN, K. H. 2005. *Soil Sampling, preparation and analysis,* CRC Press, Boca Raton, FL, USA.

TIROL-PADRE, A. & LADHA, J. K. 2004. Assessing the reliability of Permanganate-Oxidiazable Carbon as an index of Soil Labile Carbon. *Soil Science Society of America Journal,* 68**,** 969-978.

UGA WEATHER NETWORK. [Online]. Available: www.georgiaweather.net [Accessed May 2023].

U. S. DEPARTMENT OF AGRICULTURE. 2011. Plant Fact Sheet for Switchgrass (*Panicum virgatum* L.). USDA - Natural Resources Conservation Service [Online]. Available: https://plants.sc.egov.usda.gov [Accessed May 2023].

U. S. DEPARTMENT OF ENERGY. 2011. U. S. Billion-ton update - Biomass Supply for a Bioenergy and Bioproducts Industry. PERLACK, R. D. & STOKES, B. J. (Leads), ORNL/TM/-2011/224. Oak Ridge National Laboratory, Oak Ridge, TN.

WANG, E., CRUSE, R. M., SHARMA-ACHARYA, B., HERZMANN, D. E., GELDER, B. K., JAMES, D. E., FLANAGAN, D. C., BLANCO-CANQUI, H., MITCHELL, R. B. & LAIRD, D. A. 2020. Strategic Switchgrass (Panicum virgatum) Production within Row Cropping Systems: Regional-scale Assessment of Soil Erosion Loss and Water Runoff Impacts *GCB Bioenergy,* 12**,** 955-967.

WEIL, R. R., ISLAM, K. R., STINE, M. A., GRUVER, J. B. & SAMSON-LIEBIG, S. E. 2003. Estimating Active Carbon for Soil Quality Assessment: a Simplified Method for Laboratory and Field Use. *American Journal of Alternative Agriculture,* 18**,** 3-17.

WERLING, B. P., DICKSON, T. L., ISAACS, R., GAINES, H., GRATTON, C., GROSS, K. L., LIERE, H., MALMSTROM, C. M., MEEHAN, T. D., RUAN, L., ROBERTSON, B. A., ROBERTSON, P. G., SCHMIDT, T. M., SCHROTENBOER, A. C., TEAL, T. K., WILSON, J. K. & LANDIS, D. A. 2014. Perennial Grasslands Enhance Biodiversity and Multiple Ecosystem Services in Bioenergy Landscapes. *PNAS,* 111**,** 1652-7.

WRIGHT, L. & TURHOLLOW, A. 2010. Switchgrass Selection as a “Model” Bioenergy Crop: A History of the Process. Biomass and Bioenergy, 34:6, 851-868.

ZAN, C., FYLES, J. M., GIROURARD, P., SAMSON, R. & DOAN, M. 1997. Carbon Storage in Switchgrass and Short-rotation Willow Plantations. Making a Business from Biomass in Energy, Environment, Chemicals, Fibers, and Materials. Montréal, Québec, Canada. New York: Pergamon, 355-361.

# CHAPTER 3

# THE DROUGHT ADAPTATION INDEX AS A TOOL TO IDENTIFY DROUGHT-TOLERANT SWITCHGRASS GENOTYPES

[1]Anita Giabardo and Ali Missaoui. To be submitted to *Biomass and Bioenergy*

**Abstract**

Drought adaptation is an increasingly important characteristic in cultivar development. In the case of Switchgrass (*P. virgatum*), a candidate biofuel crop, drought tolerance is of particular interest. In this study, we focus on a diverse panel of Switchgrass genotypes and evaluate their drought adaptation in terms of yield and Permanganate Oxidizable Carbon (POXC), a measure of soil health, by means of a Drought Adaptation Index. A diverse switchgrass panel was planted in Tifton, GA, in 2018. Drought was simulated by means of a rain exclusion shelter. Yield was measured yearly, as well as POXC. In addition, POXC was measured at three different depths (0-15, 15-30 and 30-60 cm). We calculate the DAI for 150 genotypes involved in the study. We were able to tell apart tolerant and non-tolerant genotypes based on the DAI, for each of the traits under study. We ranked the genotypes based on their DAI and selected three genotypes that scored a DAI < 1 for both traits and years involved in the study. These four genotypes are all lowland tetraploid. In addition, we did not find any significant effect of ecotype for any of the traits under study.

**Keywords:** Switchgrass, yield, soil carbon, drought tolerance, POXC, DAI

## Introduction

Agricultural drought is defined by the National Drought Mitigation Center (2023) as a type of drought that "links various characteristics of meteorological (or hydrological) drought to agricultural impacts, focusing on precipitation shortages, differences between actual and potential evapotranspiration, soil water deficits, reduced groundwater or reservoir levels, and so forth". The impact of drought on agriculture is a growing concern in the agricultural sector worldwide (Ciais et al., 2005). With the increase of drought severity in many regions, developing cultivars adapted to drought conditions (or xerophytic) is one of the elements that could contribute to the achievement of sufficient agricultural yield, alongside the improvement of agricultural practices.

Switchgrass (*Panicum virgatum*) has been identified as a promising source of feedstock for the production of sustainable aviation biofuel by the USDA (Mclaughlin and Kszos, 2005, USDA, 2011, Wright and Turhollow, 2010). Among the reasons for this choice, there are the adaptation to a variety of environments in the Unites States and high yields under low management and harsh abiotic conditions. For biofuel production to be compatible with land use needs from the agri-food sector, Switchgrass would have to be cropped in marginal lands (Khanna et al., 2021).

Lewis *et al.* (2014) used a spatial model to identify potentially suitable geographic areas for the maximization of sustainable switchgrass cultivation. The results suggest the Great Plain region of the United States as a potentially suitable area. The researchers developed a dryness index that they used to generate a measure of drought severity for Switchgrass in Kansas. Depending on the chosen dryness threshold, the model allows for the identification of areas where Switchgrass may thrive. Clearly, a switchgrass cultivar that would be able to keep yield high while reducing the amount of water needed would increase the extension of suitable areas in the United States. The development of high-yielding, drought-tolerant cultivars will most likely benefit from a variety of plant breeding tools, from traditional breeding methods to more advanced techniques such as QTL detection and Marker-Assisted Selection, genomics, and rising biotechnological strategies such as genome editing.

*Drought Tolerance in $C_4$ Grasses and Switchgrass*

Although there is evidence that there might be some increase in photorespiration in proportion to photosynthesis in $C_4$ plants exposed to drought stress (Carmo-Silva et al., 2008), $C_4$ species are generally considered to be particularly adapted to drought conditions. In fact, drought tolerance is a characteristic that determined their evolution and geographical distribution over time (Ehleringer et al., 1997). Switchgrass, in particular, shows great environmental tolerance and ability to thrive in soil moisture stress conditions, both drought and flooding (Barney et al., 2009). Nevertheless, severe drought can significantly reduce leaf biomass, plant height, and overall aboveground biomass (Hui et al., 2018). There is great phenotypic variation among switchgrass genotypes: Liu et al. (2015) assessed drought tolerance of 49 switchgrass genotypes and ranked them based on PCA analysis of physiological and morphological data measured after 30 days of drought stress conditions. The authors divided the genotypes under study into three groups based on their performance. Drought can also influence seedlings establishment. Ye et al. (2016) investigated the proteome of seedlings as they were subjected to simulated drought conditions.

Lopes *et al.* (2011) highlighted the importance of focusing on root-related traits in order to advance drought tolerance in $C_4$ species and found a correlation between wheat rootstock size and yield under drought management conditions. Clearly, in the case of Switchgrass, any breeding effort towards increased drought tolerance would have to be combined with high yield and other morphological traits that would allow for the economic sustainability of Switchgrass as a source of biofuel feedstock. The transcriptome and metabolome profiles of Switchgrass in response to drought revealed an accumulation of specialized root diterpenoids, suggesting that they might have a role in drought stress tolerance (Tiedge et al., 2022).

Rhizosphere interactions constitute an important element to understand the biological nature of drought stress tolerance (Vurukonda et al., 2016, Hestrin et al., 2021). Liu et al. (2021) characterized the microbiome of two switchgrass varieties, Alamo and Kanlow, also in response to simulated drought. Stewart et al. (2017) found that microbial communities are ecotype-specific and can affect carbon accumulation in the soil profile and highlighted their importance in the adaptation to drought stress. Emery et al. (2022) studied arbuscular mycorrhizal fungi (AMF) richness in Switchgrass in relation to drought treatment and Nitrogen fertilization. Surprisingly, they found a 15% increase in AMF species richness in fertilized plots under drought stress. Research conducted at the Noble Foundation by Ghimire and Craven (2011) showed that switchgrass plants co-cultivated with the ectomycorrhizal fungus *Sebacina vermifera* produced higher biomass yield under both drought and non-drought conditions.

Lovell et al. (2016) investigated the physiological and genetic basis of drought tolerance of Switchgrass by means of field experiments. They found that a diverse gene expression response was responsible for similar physiological responses observed in the field and highlighted the genetic complexity of drought response. They identified hundreds of genes that were differentially expressed depending on water availability, including genes related to photosynthesis, water status, and reactive oxygen species (ROSs) responsive genes. Several genomic studies have highlighted the pivotal role of miRNAs in the adaptation to abiotic stress conditions (Xie et al., 2014, Hivrale et al., 2016).

Meyer et al.(2014) investigated the genetic mechanisms driving the response to drought and drought recovery in AP13, a lowland switchgrass cultivar (also known as Alamo). They demonstrated that drought-responsive gene expression is tightly linked with time of day and changes within the first few hours of drought recovery. Hawkes and Kiniry (2017) investigated how rainfall prior to a drought event constrained plant growth and biomass yield. They found that the larger the plant during the drought event, the more likely they were to survive.

*The Drought Adaptation Index*

There is tremendous variation in Switchgrass, with high-yielding genotypes coexisting with low-yielding genotypes within the same ecotype group. In addition, different genotypes may adapt differently to yield-limiting environments such as drought. The introduction of a drought adaptation index can help in the evaluation and selection of switchgrass genotypes that are able to thrive in drought conditions without sacrificing yield.

Howeler (1991) compared three different indexes used to calculate Soil Acidity Tolerance Indices (SATI) or Acid Soil Adaptation Indices (ASAI) of individual cultivars. He found that the formula that would allow for the selection of high-yielding varieties under both stress and non-stress conditions was the ratio of the product of yields under stress and non-stress conditions and the product of the overall average yield under stress and non-stress conditions. In this way, it is possible to tell apart "adapted" accessions from "non-adapted" ones, with the denominator serving as a correction factor for differences in overall yield levels between years (or, more in general, sites).

**Objectives**

The objective of the present study is to evaluate a diverse panel of switchgrass genotypes based on their adaptation to drought in field conditions, measured through a Drought Adaptation Index elaborated from the one proposed by Howeler (1991). In addition, we are interested in testing whether there is any effect of ecotype on drought adaptation.

For this purpose, two traits are taken into account: yield and Permanganate Oxidizable Carbon (POXC). Yield is considered probably the most important trait for a candidate biofuel crop such as Switchgrass, whereas POXC is a measure of the “active” soil carbon pool and soil health that allows to assess the effect of switchgrass’ rootstock on the soil.

**Materials and Methods**

*Experimental Design*

The experimental site is located within the University of Georgia Gibbs farm in Tifton, GA. A randomized complete block design involving around 400 different genotypes was set up in 2018. For the purpose of this study, 150 of the 400 genotypes available were considered. There are three replications for each of the two treatments under study – drought stress (covered, or “CV”, Fig. 3.1) and control (uncovered, or “UC”, Fig. 3.2). Two of the three replications are considered in this study. Data collection started in 2019.

Data from the 2021 and 2022 growing seasons were analyzed. Hydrological drought was simulated through a rain exclusion shelter, which is depicted in Fig. 2. Switchgrass plants were harvested every year in the Fall. Soil sampling was carried out after harvest, from January onwards, by means of a drill (bid diameter: 2 cm) and a perforated soil bucket (Fig. 3.3). Soil samples were stored in paper bags and air-dried. With regards to soil analysis, the protocol followed for POXC (Culman et al., 2012) analysis can be found at: https://lter.kbs.msu.edu/protocols/133 (consulted on 15th April 2023). Before performing POXC analysis, they were ground and sieved to exclude any residues that could affect the analysis.

Fresh biomass was measured directly in the field. A sample of each plant was weighed and put in a paper bag. Later, it was dried in order to estimate the amount of dry biomass each plant produced. If the amount of fresh biomass of the individual plant was able fit in one of the paper bags, fresh biomass was weighed in the field, stored in a paper bag, and then weighed once dried to measure dry biomass production.

*Drought Adaptation Index Calculation*

In order to rank and tell apart genotypes that were able to maintain a high level of yield and POXC under both treatments, we used a Drought Adaptation Index based on the Acid Soil Adaptation Index (ASAI) adopted by Howeler to identify plants tolerant to low pH conditions.

The index was calculated as follows:

$$\text{Drought Adaptation Index (DAI)} = \frac{Y_{CV} \times Y_{UC}}{\bar{Y}_{CV} \times \bar{Y}_{UC}}$$

For the purpose of this study, the stress condition is identified with the drought condition (CV) and the non-stress condition with the control (UC). Y and $\bar{Y}$ are individual performance and grand mean, respectively, for both treatments under study. The traits considered are yield and POXC; therefore, two different DAIs are calculated (one for yield and one for POXC) for every genotype and for every year under study. A genotype with a DAI higher than one may be considered adapted to drought conditions, whereas a genotype with a DAI lower than one may be considered non-adapted.

*Heritability Calculation*

Broad-sense heritability for POXC was calculated separately within each of the two treatments (CV and UC). Following an adaptation of the "standard" method from the CGIAR Excellence in Breeding manual (Covarrubias-Pazaran, 2019), the formulas used for the calculation of broad-sense heritability were the following.

$$H^2_{Standard} = \frac{\sigma^2_g}{\sigma^2_p}$$

With: $\sigma^2_p = \sigma^2_g + \frac{\sigma^2_{ga}}{n_a} + \frac{\sigma^2_{res}}{n_{ar}}$

Where: $\sigma^2$ refers to variance, "n" to the number of, "g" to genotype, "p" to phenotype, "a" to years, "r" to replications, and "res" to the residual variance.

To estimate the variance components of the response variable (POXC), a linear model using the restricted maximum likelihood (REML) method was fitted in JMP® PRO 16.0.0 (SAS Institute Inc., 2023). The model included the random effect of genotype, year, and their interaction.

Figure 3.1: Picture of the control (UC) plot, April 2023.

Figure 3.2: Picture of the covered (CV) plot, April 2023.

Figure 3.3: Soil sampling, April 2023. Soil sampling is carried out by means of an automatic drill. A bucket collects the soil that is then stored in a soil paper bag.

**Results**

The DAI was calculated for the 150 genotypes under study, for both traits (yield and POXC), and for both years (2021 and 2022) under study. Following the representation proposed by Howeler, we plotted each cultivar on a graph relating observed yields in the covered (x-axis) and uncovered (y-axis) sections. It is possible to tell apart adapted and non-adapted genotypes by plotting a line of DAI = 1. The observations above the line correspond to individuals with a DAI > 1, *ergo* to individuals that are adapted to drought. Vice versa, the individuals below the line have a DAI < 1, and are classified as non-adapted to drought conditions.

Figures 3.4 to 3.9 represents the relationship between Switchgrass POXC observed under drought and well-watered conditions for all depths and all years under study. The DAI = 1 line separates the adapted genotypes from the non-adapted. In other words, the DAI = 1 line represents all the possible combinations of POXC under drought and under well-watered conditions that would result in a DAI equal to 1. Figures 3.10 and 3.11 represent the relation between switchgrass yield under drought and well-watered conditions for both years under study.

Fig. 3.12 to 3.15 represent the DAI for all genotypes involved in the study by trait and ecotype. There doesn't seem to be a clear difference between ecotypes in terms of DAI, although this might be due to different sample sizes (most of the genotypes are either lowland or coastal).

We also calculated the average DAI for POXC and yield for each genotype and the overall average DAI (including both traits in the calculation). Fig. 3.16 to 3.18 show the distribution of the average DAI for yield, POXC, and both, respectively.

The Venn diagrams in Figures 3.19 and 3.20 group together genotypes scoring a DAI >1 for the different traits, depths and years under study. With regard to yield, in 2021, there were 60 genotypes with DAI >1. In 2022, there were 57. There were 50 genotypes with a yield DAI >1 in both 2021 and 2022. With regards to POXC, in 2021, there were 23 genotypes with DAI >1 at each depth. In 2022, there were 21. Taking into account both yield and POXC, among the genotypes under study, there were four that scored a DAI > 1 for both traits and both years under study. Figure 3.18 summarizes this finding.

Fig. 3.19 and 3.20 show the distribution of average DAI for yield and POXC. In the case of yield, DAI from 2021 and 2022 were averaged. In the case of yield, POXC from the three depths under study in 2021 and 2022 were averaged. Fig. 3.21 shows the distribution of overall average DAI, calculated including all DAIs for both traits and years.

Broad-sense heritability values were 0.16 within the CV treatment and 0.23 within the UC treatment. These values indicate a low heritability of POXC. Tables 3.1 and 3.2 summarize the results of the variance components analysis.

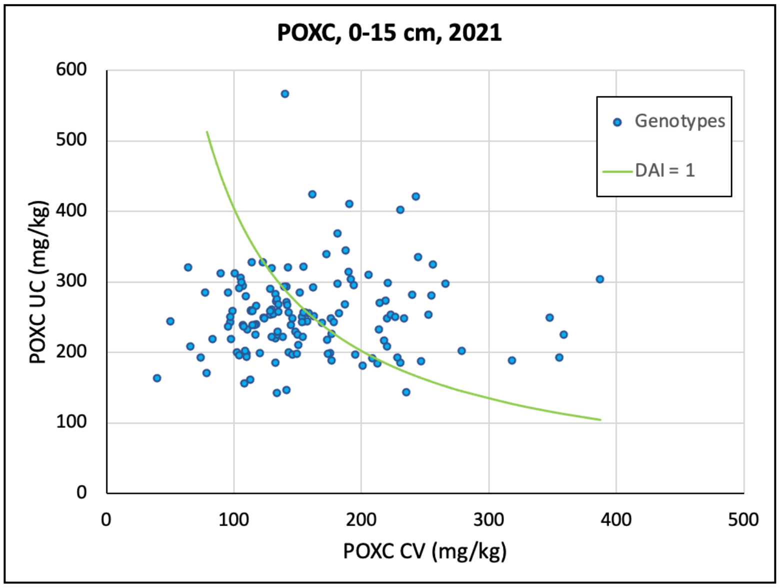


Figure 3.4: Relation between soil POXC (0- to 15 cm depth) observed under drought and well-watered conditions (2021). Experimental plots are located in Tifton, GA. The DAI =1 line separates the adapted genotypes from the non-adapted.

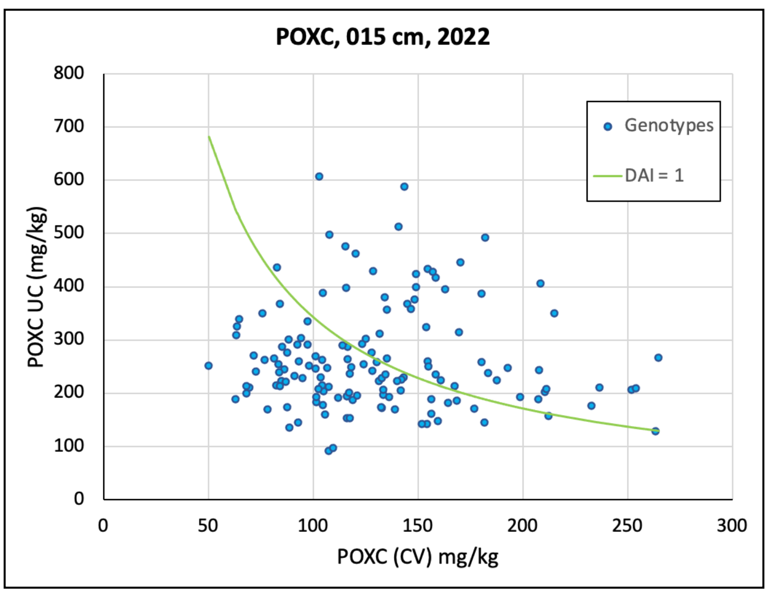


Figure 3.5: Relation between soil POXC (0- to 15 cm depth) observed under drought and well-watered conditions (2022). Experimental plots are located in Tifton, GA (2022). The DAI =1 line separates the adapted genotypes from the non-adapted.

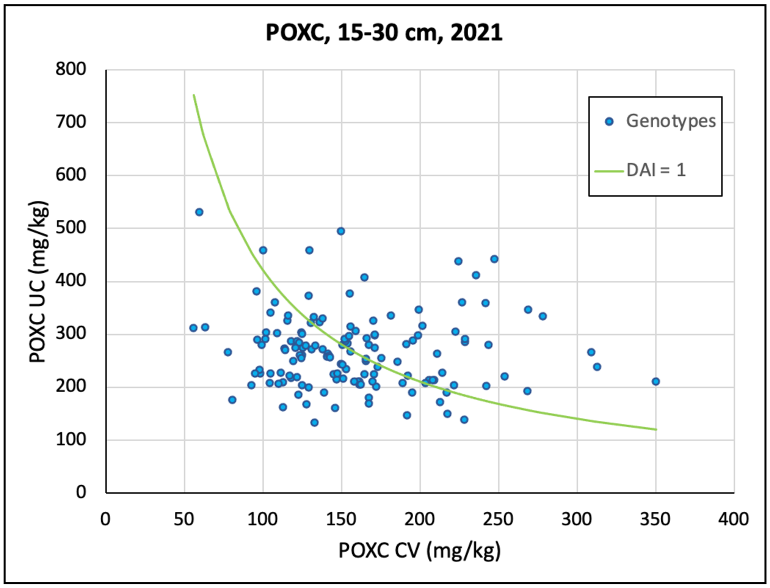


Figure 3.6: Relation between soil POXC (15- to 30 cm depth) observed under drought and well-watered conditions (2021). Experimental plots are located in Tifton, GA. The DAI =1 line separates the adapted genotypes from the non-adapted.

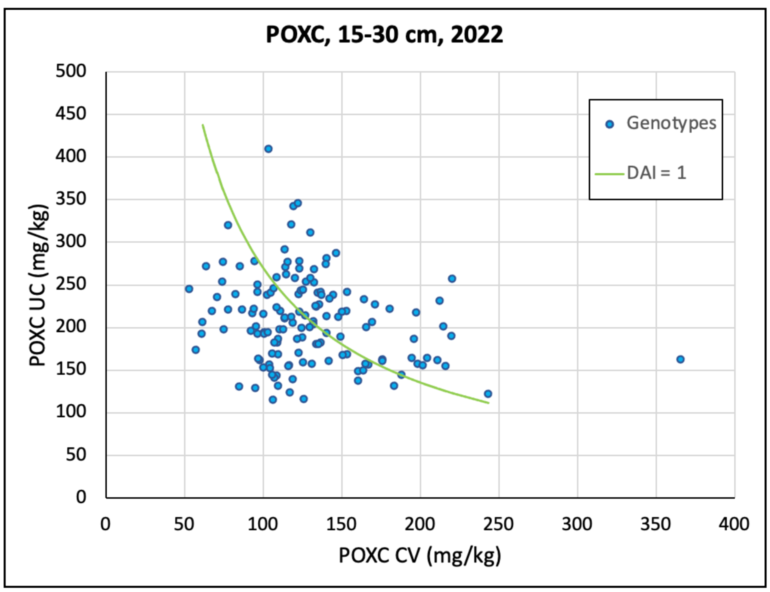


Figure 3.7: Relation between soil POXC (15- to 30 cm depth) observed under drought and well-watered conditions (2022). Experimental plots are located in Tifton, GA. The DAI =1 line separates the adapted genotypes from the non-adapted.

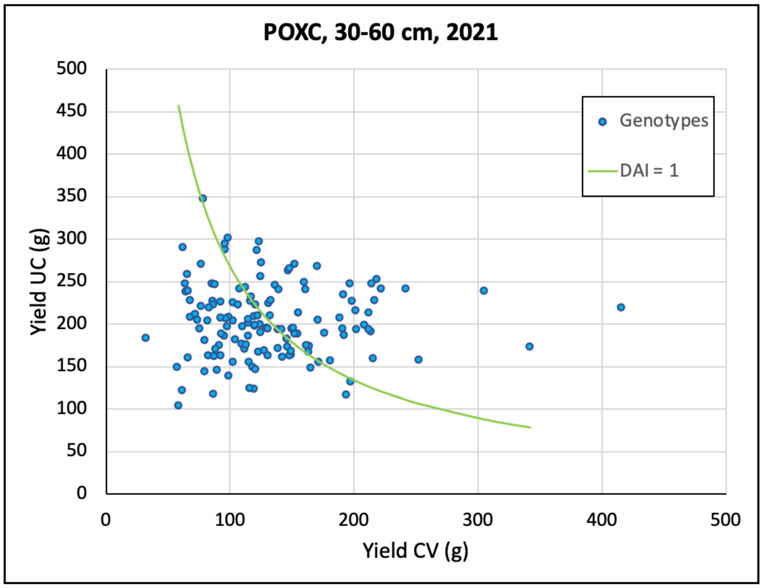


Figure 3.8: Relation between soil POXC (30- to 60 cm depth) observed under drought and well-watered conditions (2021). Experimental plots are located in Tifton, GA. The DAI =1 line separates the adapted genotypes from the non-adapted.

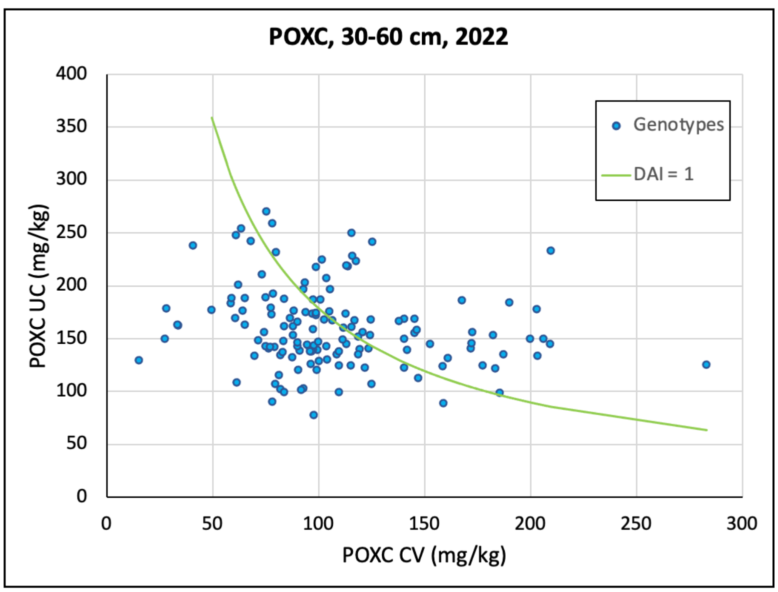


Figure 3.9: Relation between soil POXC (30- to 60 cm depth) observed under drought and well-watered conditions (2022). Experimental plots are located in Tifton, GA. The DAI =1 line separates the adapted genotypes from the non-adapted.

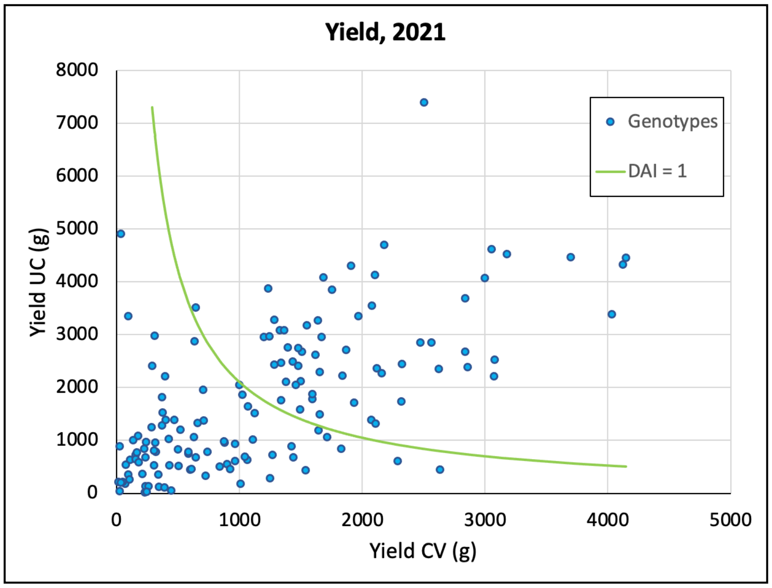


Figure 3.10: Relation between switchgrass yield observed under drought and well-watered conditions (2021). Experimental plots are located in Tifton, GA. The DAI =1 line separates the adapted genotypes from the non-adapted.

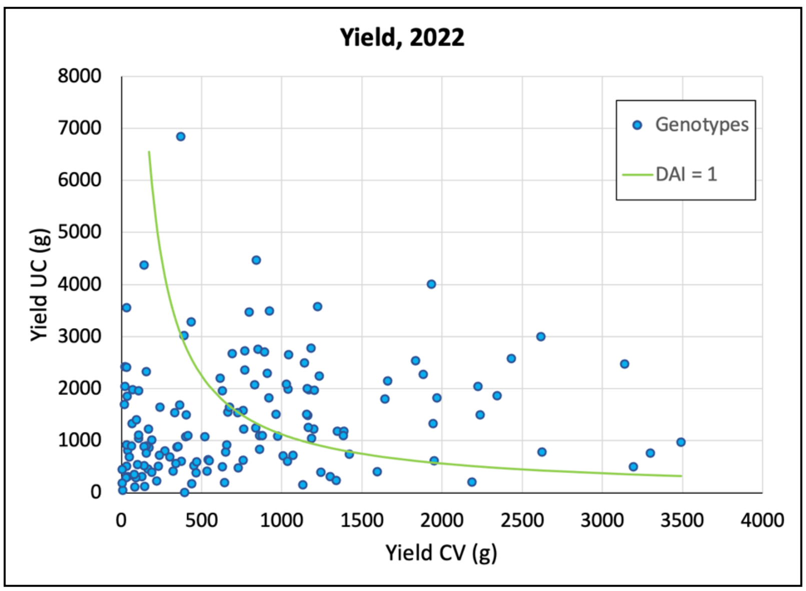


Figure 3.11: Relation between switchgrass yield observed under drought and well-watered conditions (2022). Experimental plots are located in Tifton, GA. The DAI =1 line separates the adapted genotypes from the non-adapted.

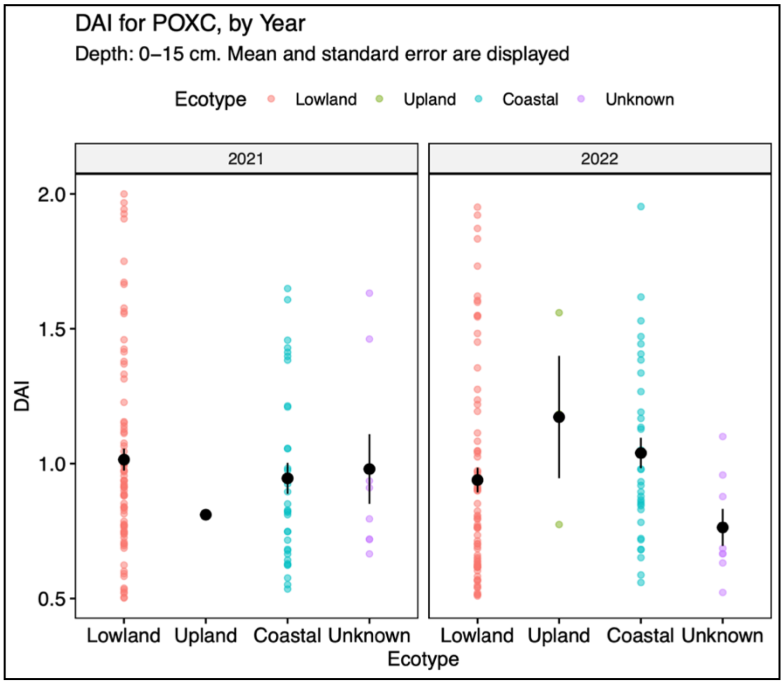


Figure 3.12: DAI for POXC, 0- to 15 cm depth, by year and ecotype.

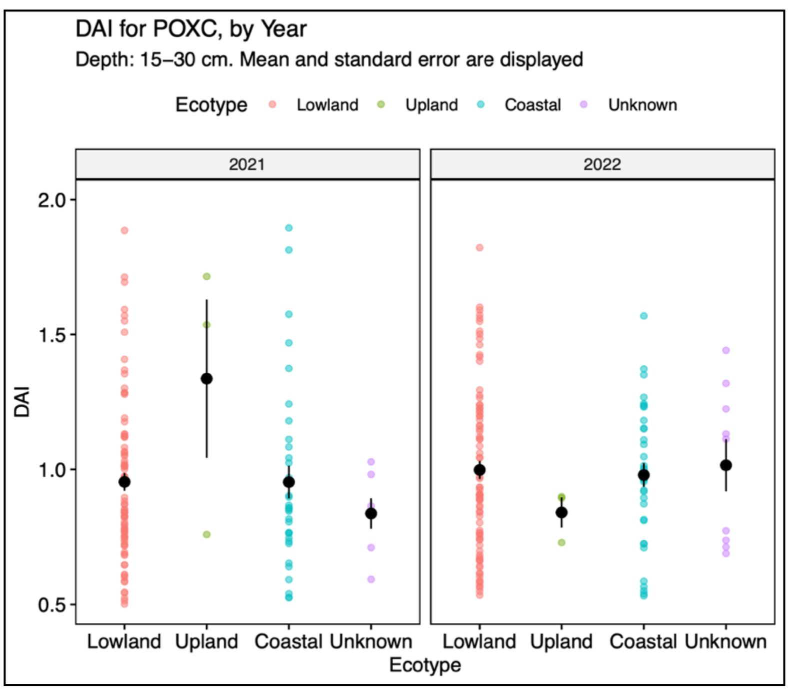


Figure 3.13: DAI for POXC, 15- to 30 cm depth, by year and ecotype.

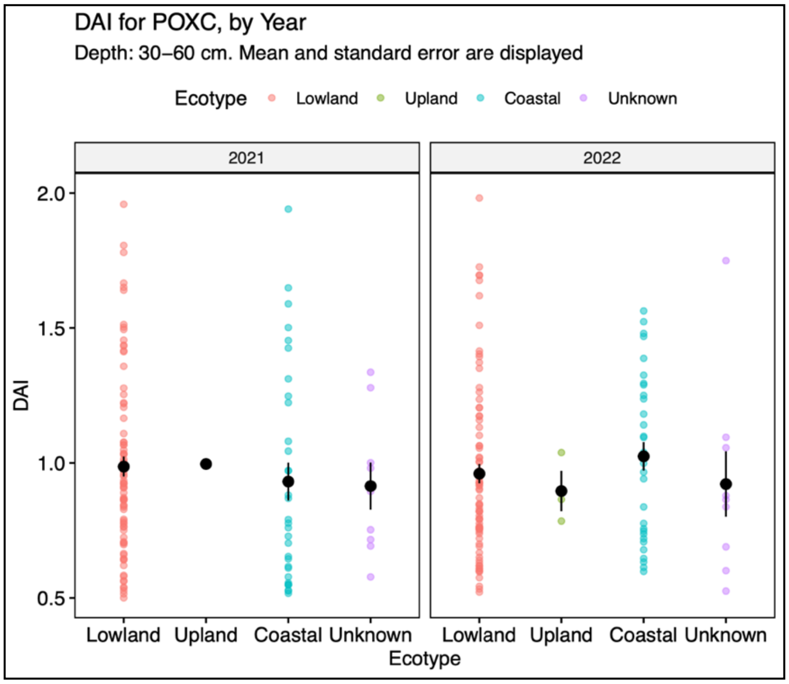


Figure 3.14: DAI for POXC, 30 to -60 cm depth, by year and ecotype.

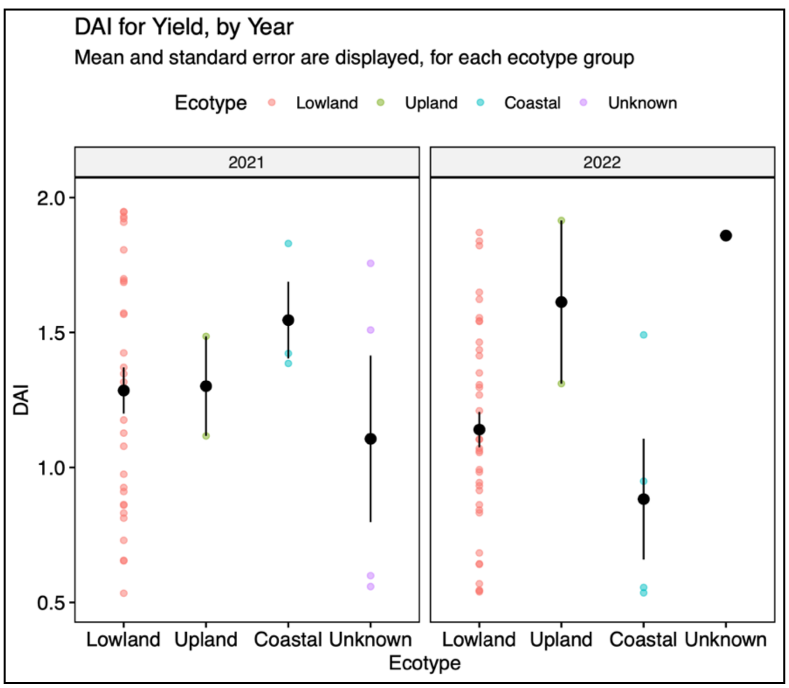


Figure 3.15: DAI for yield, by year and ecotype.

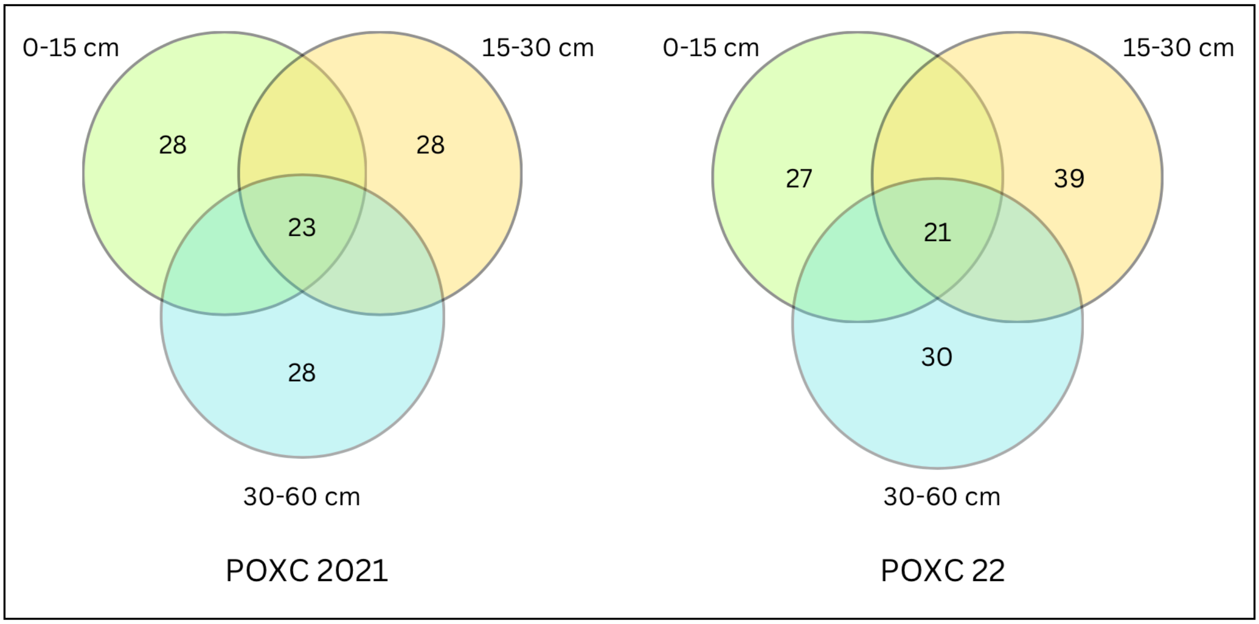


Figure 3.16: Venn diagram showing the number of genotypes that scored a POXC DAI higher than 1. In 2021, there were 23 genotypes scoring a DAI higher than 1 at all depths. In 2022, there

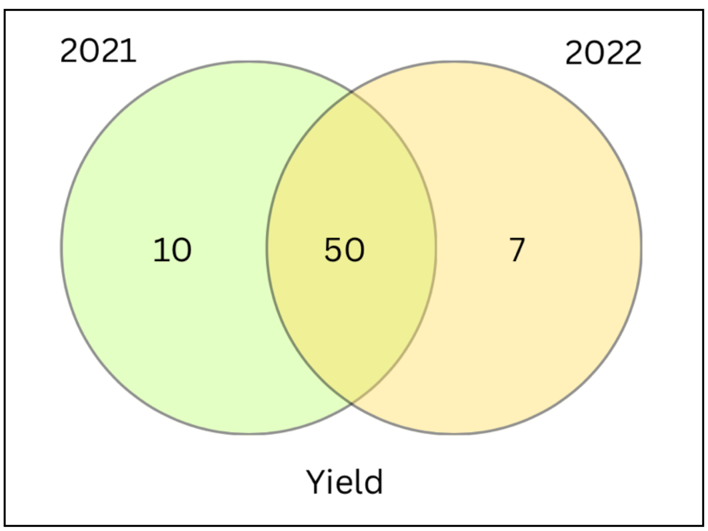


Figure 3.17: Venn diagram showing the number of genotypes that scored a yield DAI higher than 1 in both 2021 and 2022.

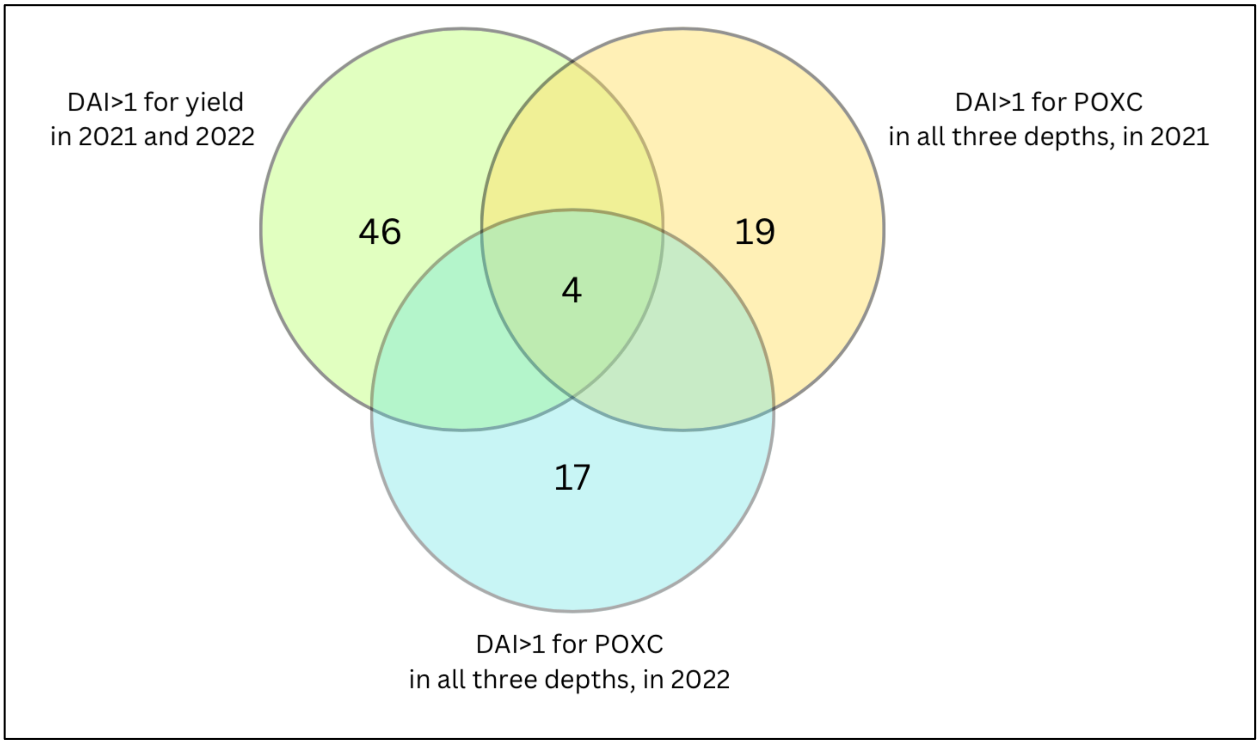


Figure 3.18: Venn diagram for yield and POXC DAI, for 2021 and 2022. There are 4 genotypes that score a DAI higher than 1 across all years and both traits under study.

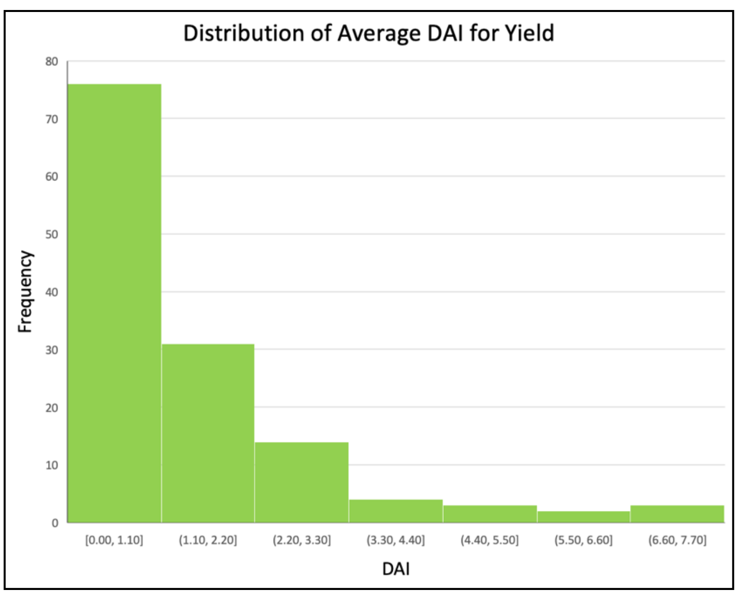


Figure 3.19: Distribution of average yield DAI in 2021 and 2022.

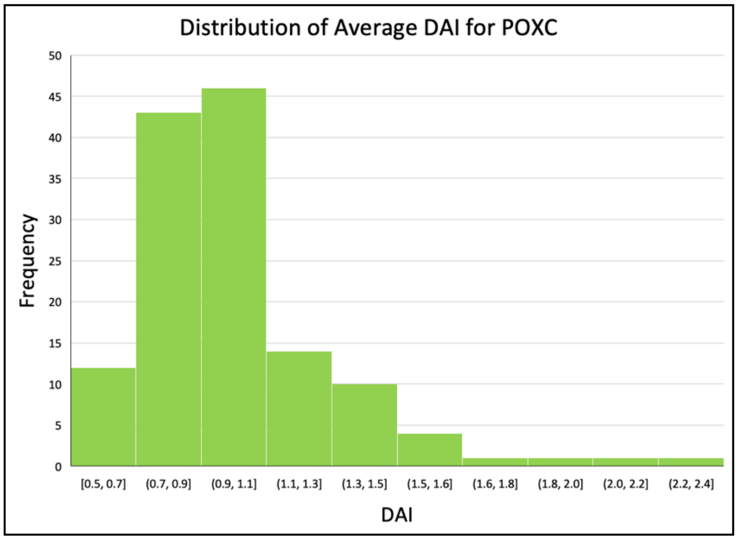


Figure 3.20: Distribution of average DAI for POXC in 2021 and 2022, including all three depths under study (0- to 15, 15- to 30 and 30- to 60 cm depth).

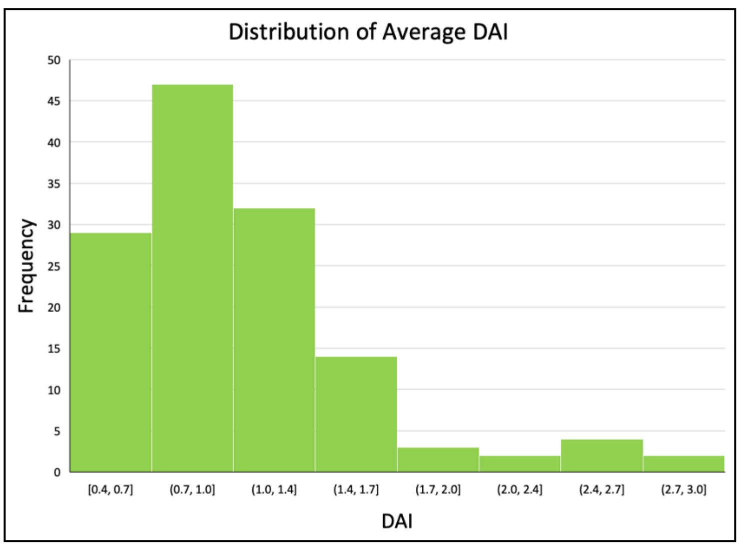


Figure 3.21: Distribution of average DAI.

Table 3.1: REML Variance Component Estimates for POXC within the CV plot. p-values in bold are considered statistically significant.

| Random Effect | Variance Component | Std Error | Wald p-value | Pct of Total |
|---|---|---|---|---|
| Genotype | 341.64 | 235.88 | 0.1475 | 1.81 |
| Year | 10541.05 | 10586.8 | 0.3194 | 55.75 |
| Genotype*Year | 2643.88 | 489.95 | **<.0001** | 13.98 |
| Residual | 5381.61 | 232.14 | | 28.46 |
| Total | 18908.18 | 10594.88 | | 100 |

Table 3.2: REML Variance Component Estimates for POXC within the UC plot. p-values in bold are considered statistically significant.

| Random Effect | Variance Component | Std Error | Wald p-value | Pct of Total |
|---|---|---|---|---|
| Genotype | 520.81 | 199.21 | **0.0089** | 3.34 |
| Year | 4678.19 | 4732.31 | 0.3229 | 30 |
| Genotype*Year | 266.34 | 307.51 | 0.3864 | 1.71 |
| Residual | 10128.76 | 416.54 | | 64.95 |
| Total | 15594.1 | 4747.79 | | 100 |

**Discussion**

Rootstock traits are difficult to observe and measure, making phenotyping particularly challenging, expensive, and labor-intensive. In this study, we used POXC as a method to evaluate the impact of different switchgrass genotypes on soil carbon over time, also in relation to drought conditions.

Then, we used a drought adaptation index (DAI) to rank and evaluate the genotypes under study based on their performance under drought conditions for both yield and POXC. Thanks to the index, we were able to identify genotypes within the panel that were performing consistently above average for both traits and both growing seasons under study. This constitutes useful background information for future switchgrass breeding efforts. In addition, as suggested by Howeler, the calculated DAI values may be integrated further, including data from more growing seasons and more traits of interest, such as morphological traits and flowering time, making the DAI more comprehensive. DAI data may also be used in combination with genotypic data in the framework of genome-wide association and genomic selection studies, which would allow unraveling the genetic nature of drought tolerance and point toward its biological explanation.

As the DAI captures the adaptability to a certain environment characterized by abiotic stress, in this case by drought stress, the expectation would be to find differences in how different ecotypes score. In this study, no significant differences between ecotypes could be detected. This could be due to the composition of the switchgrass panel: most of the genotypes are lowland or coastal, making it difficult to capture any effect due to the ecotype.

In the evaluation of the most drought-adapted genotypes within the panel, the ones scoring a DAI > 1 for both traits and both years under study were considered to be the best performers.

These genotypes are J065.B, J247.A, J330.A, and J587.B. They are all tetraploid lowland and were originally collected in Texas.

Broad-sense heritability may be defined as "the extent to which a phenotype is genetically determined" (Lourenço et al., 2017). Heritability has important consequences in the genetic gain attainable through selection, as it represents the proportion of the selection differential that is possible to realize as a response to the selection (Falconer and Mackay, 2005). The broad-sense heritabilities calculated in this study were low within both treatments, but especially within the CV treatment (0.16), suggesting the scarce possibility for genetic improvement through selection.

In conclusion, these results provide both useful reference information for future studies and breeding programs involving Switchgrass and its adaptation to water-limited environments and a framework to combine yield-related with sustainability-related traits in switchgrass breeding for sustainable biofuel production.

**Future directions**

This study evaluated a diverse panel of 150 genotypes and selected four on the basis of a drought adaptation calculated using yield and POXC data collected over two years. Both yield and POXC at three different depths (o- to 15, 15- to 30, and 30- to 60 cm depth) were taken into account as to select genotypes that are able to produce high biomass yield and have a positive impact on soil properties in both drought and non-drought conditions. The four genotypes selected are all lowland tetraploid. Future studies should investigate the nature of drought tolerance in these four genotypes, addressing their biology and genetic basis. Several root-associated traits should be the subject of future research, such as root biomass in relation to aboveground biomass and root-associated microbiome.

The four genotypes (J065.B, J247.A, J330.A, and J587.B) were selected based on their DAI. They scored a DAI > 1 for both yield and POXC in 2021 and 2022. Depending on the selection criterion, different groups of genotypes can be selected depending on the breeding goal or research objective. For example, it's possible to select the genotypes that outperformed the others in terms of biomass yield at different degrees of intensity. This study provides rich background information for future research oriented at developing drought-tolerant switchgrass cultivars.

**References**

BARNEY, J. N., MANN, J. J., KYSER, G. B., BLUMWALD, E., VAN DEYNZE, A. & DITOMASO, J. M. 2009. Tolerance of Switchgrass to Extreme Soil Moisture Stress: Ecological Implications. *Plant Science,* 6**,** 724-732.

CARMO-SILVA, A. E., POWERS, S. J., KEYS, A. J., ARRABACA, M. C. & PARRY, M. A. 2008. Photorespiration in $C_4$ Grasses Remains Slow under Drought Conditions. *Plant Cell Environ,* 31**,** 925-40.

CIAIS, P., REICHSTEIN, M., VIOVY, N., GRANIER, A., OGEE, J., ALLARD, V., AUBINET, M., BUCHMANN, N., BERNHOFER, C., CARRARA, A., CHEVALLIER, F., DE NOBLET, N., FRIEND, A. D., FRIEDLINGSTEIN, P., GRUNWALD, T., HEINESCH, B., KERONEN, P., KNOHL, A., KRINNER, G., LOUSTAU, D., MANCA, G., MATTEUCCI, G., MIGLIETTA, F., OURCIVAL, J. M., PAPALE, D., PILEGAARD, K., RAMBAL, S., SEUFERT, G., SOUSSANA, J. F., SANZ, M. J., SCHULZE, E. D., VESALA, T. & VALENTINI, R. 2005. Europe-wide Reduction in Primary Productivity Caused by the Heat and Drought in 2003. *Nature,* 437**,** 529-33.

COVARRUBIAS-PAZARAN, G. E. 2019. Heritability: Meaning and Computation. CGIAR Excellence in Breeding Plarform [Online]. Available: https://excellenceinbreeding.org/sites/default/files/manual/EiB-M2_Heritability_18-02-20.pdf [Accessed May 2023].

CULMAN, S., FREEMAN, M. & SNAPP, S. Procedure for the Determination of Permanganate Oxidizable Carbon [Online]. Available: https://lter.kbs.msu.edu/protocols/133 [Accessed May 2023].

CULMAN, S. W., SNAPP, S. S., FREEMAN, M. A., SCHIPANSKI, M. E., BENISTON, J., LAL, R., DRINKWATER, L. E., FRANZLUEBBERS, A. J., GLOVER, J. D., GRANDY, A. S., LEE, J., SIX, J., MAUL, J. E., MIRSKY, S. B., SPARGO, J. T. & WANDER, M. M. 2012. Permanganate Oxidiazable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. *Soil Science Society of America Journal,* 76.

EHLERINGER, J. R., CERLING, T. E. & HELLIKER, B. R. 1997. $C_4$ Photosynthesis, Athmospheric CO2, and Climate. *Oecologia***,** 285-299.

EMERY, S. M., BELL-DERESKE, L., STAHLHEBER, K. A. & GROSS, K. L. 2022. Arbuscular Mycorrhizal Fungal Community Responses to Drought and Nitrogen Fertilization in Switchgrass Stands. *Applied Soil Ecology,* 169.

FALCONER, D. S., & MACKAY, T. F. C. 2005. Introduction to Quantitative Genetics, 4th ed. Pearson Prentice Hall, Upper Saddle River, NJ.

GHIMIRE, S. R. & CRAVEN, K. D. 2011. Enhancement of Switchgrass (Panicum virgatum L.) Biomass Production under Drought Conditions by the Ectomycorrhizal Fungus *Sebacina vermifera*. *Appl Environ Microbiol,* 77**,** 7063-7.

HAWKES, C. V. & KINIRY, J. R. 2017. Legacies in Switchgrass Resistance to and Recovery from Drought Suggest That Good Years Can Sustain Plants Through Bad Years. *BioEnergy Research,* 11**,** 86-94.

HESTRIN, R., LEE, M. R., WHITAKER, B. K. & PETT-RIDGE, J. 2021. The Switchgrass Microbiome: A Review of Structure, Function, and Taxonomic Distribution. *Phytobiomes Journal,* 5**,** 14-28.

HIVRALE, V., ZHENG, Y., PULI, C. O. R., JAGADEESWARAN, G., GOWDU, K., KAKANI, V. G., BARAKAT, A. & SUNKAR, R. 2016. Characterization of Drought- and Heat-Responsive MicroRNAs in Switchgrass. *Plant Sci,* 242**,** 214-223.

HOWELER, R. H. Identifying Plants Adaptable to Low pH Conditions. *In:* WRIGHT, R. J., BALIGAR, V. C. & MURRMAN, R. P., eds. Second International Symposium on Plant-Soil Interactions, 1991 Beckley West Virginia, USA. Springer, 885-904.

HUI, D., YU, C.-L., DENG, Q., DZANTOR, E. K., ZHOU, S., DENNIS, S., SAUVE, R., JOHNSON, T. L., FAY, P. A., SHEN, W. & LUO, Y. 2018. Effects of Precipitation Changes on Switchgrass Photosynthesis, Growth, and Biomass: a Mesocosm Experiment. *PLos ONE,* 13.

JMP®, Version *16.0.0*. SAS Institute Inc., Cary, NC, 1989–2023.

LOURENÇO, V.M., RODRIGUES, P. C., PIRES, A. A. M., PIEPHO, H-P. 2017. A Robust DF-REML Framework for Variance Components Estimation in Genetic Studies. *Bioinformatics*, 33(2)**,** 3584-3594.

LIU, T.-Y., YE, N., WANG, X., DAS, D., TAN, Y., YOU, X., LONG, M., HU, T., DAI, L., ZHANG, J. & CHEN, M.-X. 2021. Drought Stress and Plant Ecotype Drive Microbiome Recruitment in Switchgrass Rhizoshealth. *Journal of Integrative Plant Biology,* 63**,** 1753-1774.

LIU, Y., ZHANG, X., TRAN, H., SHAN, L., KIM, J., CHILDS, K., ERVIN, E. H., FRAZIER, T. & ZHAO, B. 2015. Assessment of Drought Tolerance of 49 Switchgrass (Panicum virgatum) Genotypes Using Physiological and Morphological Parameters. *Biotechnol Biofuels,* 8**,** 152.

LOPES, M. S., ARAUS, J. L., VAN HEERDEN, P. D. & FOYER, C. H. 2011. Enhancing Drought Tolerance in C(4) Crops. *J Exp Bot,* 62**,** 3135-53.

LOPES, M. S. & REYNOLDS, M. P. 2011. Drought Adaptive Traits and Wide Adaptation in Elite Lines Derived from Resynthesized Hexaploid Wheat. *Crop Science,* 51**,** 1617-1626.

LOVELL, J. T., SHAKIROV, E. V., SCHWARTZ, S., LOWRY, D. B., ASPINWALL, M. J., TAYLOR, S. H., BONNETTE, J., PALACIO-MEJIA, J. D., HAWKES, C. V., FAY, P. A. & JUENGER, T. E. 2016. Promises and Challenges of Eco-Physiological Genomics in the Field: Tests of Drought Responses in Switchgrass. *Plant Physiology,* 172**,** 734-748.

MCLAUGHLIN, S. B. & KSZOS, L. A. 2005. Development of Switchgrass (Panicum virgatum) as a Bioenergy Feedstock in the United States. *Biomass and Bioenergy,* 28**,** 515-535.

MEYER, E., ASPINWALL, M. J., LOWRY, D. B., PALACIO-MEJÍA, J. D., LOGAN, T. L., FAY, P. A. & JUENGER, T. E. 2014. Integrating Transcriptional, Metabolomic, and Physiological Responses to Drought Stress and Recovery in Switchgrass (*Panicum virgatum* L.). *BMC Genomics,* 15.

NATIONAL DROUGHT MITIGATION CENTER. 2023. *Types of Drought* [Online]. Available: https://drought.unl.edu/Education/DroughtIn-depth/TypesofDrought.aspx [Accessed May 2023].

STEWART, C. E., ROOSENDAAL, D., DENEF, K., PRUESSNER, E., COMAS, L. H., SARATH, G., JIN, V. L., SCHMER, M. R. & SOUNDARARAJAN, M. 2017. Seasonal Switchgrass Ecotype Contributions to Soil Organic Carbon, Deep Soil Microbial Community Composition and Rhizodeposit Uptake During an Extreme Drought. *Soil Biology and Biochemistry,* 112**,** 191-203.

TIEDGE, K., LI, X., MERRILL, A. T., DAVISSON, D., CHEN, Y., YU, P., TANTILLO, D. J., LAST, R. L. & ZERBE, P. 2022. Comparative Transcriptomics and Metabolomics Reveal Specialized Metabolite Drought Stress Responses in Switchgrass (Panicum virgatum). *New Phytol,* 236**,** 1393-1408.

USDA 2011. Plant fact sheet for Switchgrass (*Panicum virgatum* L.). USDA - Natural Resources Conservation Service.

VURUKONDA, S. S., VARDHARAJULA, S., SHRIVASTAVA, M. & SK, Z. A. 2016. Enhancement of Drought Stress Tolerance in Crops by Plant Growth Promoting Rhizobacteria. *Microbiol Res,* 184**,** 13-24.

WRIGHT, L. & TURHOLLOW, A. 2010. Switchgrass Selection as a “Model” Bionenergy Crop: A History of the Process. *Biomass and Bioenergy,* 34**,** 851-868.

XIE, F., STEWART, C. N., JR., TAKI, F. A., HE, Q., LIU, H. & ZHANG, B. 2014. High-throughput Deep Sequencing Shows that MicroRNAs Play Important Roles in Switchgrass Responses to Drought and Salinity Stress. *Plant Biotechnol J,* 12**,** 354-66.

YE, Z., SANGIREDDY, S., OKEKEOGBU, I., ZHOU, S., YU, C. L., HUI, D., HOWE, K. J., FISH, T. & THANNHAUSER, T. W. 2016. Drought-Induced Leaf Proteome Changes in Switchgrass Seedlings. *Int J Mol Sci,* 17.

# CHAPTER 4

# SUMMARY

Switchgrass is a promising second-generation biofuel crop. It is adapted to a wide range of climates in Nothern America, it provides high yield under low-input management and it can be grown on marginal land. There is rich genetic and phenotypic variation in wild switchgrass germplasm, which is generally classified into lowland, upland and coastal. Among switchgrass' breeding objectives there are both yield and tolerance to water-stressed conditions. In addition, root-related traits are becoming increasingly important in the evaluation of switchgrass' sustainability as a potential source of lignocellulosic biomass for the production of bioethanol. Phenotyping root-related traits is costly, labor-intensive and time-consuming. Yet, studying them is fundamental in order to understand the effect of switchgrass cropping on soil health

The objectives of this study were to: i) measure soil active C under drought treatment withing a diverse panel of 150 switchgrass genotypes, ii) investigate the relationship between soil active C and yield, and iii) evaluate a diverse panel of switchgrass genotypes based on their adaptation to drought in field conditions, taking into account both yield and soil active C. Permanganate Oxidizable Carbon (POXC) as a measure of the “active” soil carbon pool allowed to assess the effect of switchgrass’ rootstock on the soil. This soil analysis technique was selected for its simplicity and convenience, factors that allowed the collection and analysis of around 4000 samples over three years. A diverse panel of 150 genotypes was involved in this study. In-field drought was simulated by means of a rain exclusion shelter that prevented rainwater from reaching the plants treated. Data was collected yearly from 2020 to 2022.

The results of this study showed that treatment, year and depth had a significant effect on soil POXC content. Starting from 2021, soil POXC was higher in the control plot compared to the drought-treated. In addition, POXC was lower at deeper depths, under both the control and the drought-treated plot. When looking at the relationship between POXC and yield, we found that there was a slightly positive significant correlation between POXC and yield under the drought treatment. The correlation was stronger in 2022 and at deeper depths, whereas there was no correlation in the control plot. Overall, these results provide rich background information on the potential of Switchgrass as a carbon sink, and a framework for the evaluation of the soil active carbon pool in switchgrass' cropping. Further research is required to elucidate the nature of the relationship between biomass yield and rootstock, as well as studies oriented at investigating the genetic basis of soil carbon sequestration in Switchgrass.

The evaluation of the genotypes involved in the study based on their drought adaptation in terms of yield and POXC under drought conditions was carried out through the calculation of a drought adaptation index (DAI). The DAI allowed to discriminate between genotypes adapted and non-adapted to drought based on their performance relative to the average performance of the panel under both the control and the drought treatment. A genotype with a DAI higher than 1 was considered adapted to drought conditions, whereas a genotype with a DAI lower than 1 was considered non-adapted. DAI calculation and analysis brought to the identification of four genotypes that consistently scored above 1 across both years and traits under study. These genotypes should be subject of research in future studies, as they represent a valuable resource as plants that are able to deliver both high yield and high soil POXC under drought conditions. Further research on the root architecture and microbiome associated with these individuals could contribute to the understanding of the biology switchgrass' performance in drought-stressed environments.