

### **Effect of drought on soil properties and grain quality**

No significant difference in the total soil nitrogen under drought treatments resembles that drought may not hamper the total nitrogen content in the soil, though our study revealed that impaired transformation of different forms may occur. The observed changes in ammoniacal form of nitrogen in the drought exposed soil can be attributed to the sensitivity of the soil microbial community structure and functioning. The shifts in the ammoniacal N and nitrate N with respect to soil organic N under the exposure of drought delineates the mobilization of nitrogen from one form to another (Fig. 4.1-4.2). The alterations in the soil urease activity under drought exposure (Fig. 4.21-4.22) support our findings as urease is the key enzyme for conversion of urea to ammonium<sup>[1]</sup>. Differential impact of drought on soil ammonium content was also recorded. This can be attributed to the potential enhancement of denitrification enzyme activity under drought<sup>[2]</sup>, leading to extractable ammonium (NH<sub>4</sub><sup>+</sup>) accumulation<sup>[2,3]</sup>. Differential impact of the applied drought under the cultivation of both the crops represent a change in microbial community structure which might be due to the fact that some microbes are drought-tolerant or may be low water potentials kill some microbes. This restricts plant nitrogen uptake and reduces competition of N for the surviving microbes<sup>[4]</sup>. Nitrogen transformation in soil is governed by the activity as well as the community structure of the microorganisms present in the soil as they play crucial role in decomposition, nutrient uptake, and nitrogen (N) cycling<sup>[5,6]</sup>.

Higher accumulation of both microbial biomass nitrogen and microbial biomass carbon under drought signifies positive impact of drought in microbial population. Ample evidences exist that support our results and report that the pool of soil microbial biomass may increase under the influence of drought<sup>[2,3,7,8]</sup>. However, their effectiveness in nutrient transformations cannot be precisely determined. Observed enhancement of fungal and actinobacterial colony forming units under drought (Table. 4.3-4.4), along with a decrease in bacterial colony forming units (CFU) delineates the change in microbial community

structure which directly affects their functioning. Microbial biomass carbon (MBC) and nitrogen (MBN) are considered an important marker of soil biological characteristics<sup>[9]</sup> and make up 1–7% and 5%, respectively<sup>[10]</sup>, of the total soil C and N. The microbial biomass nutrient pool is thought to be the main factor regulating nutrient availability<sup>[11]</sup>. Moreover, drought induced changes in stability of the soil organic carbon (SOC) content alters the physical and chemical characteristics of macroaggregates as well as their chemical composition<sup>[12]</sup>. This influences the composition of microbial communities and their activities, favoring those with stronger recalcitrant C conversion capacities<sup>[13]</sup>. Enhanced SOC in drought treated soils in the current study can be attributed to lower actinobacterial CFU as compared to the treatment with reduced SOC. This might be due to higher degrading ability of soil organic matter, including cellulose and chitin by actinobacteria<sup>[14,15]</sup>. Further, moisture stress generally triggers the ribosomal synthesis in Actinobacteria, which could explain their increased abundance following drought.

The impact of drought in our study is also observed in the phosphorus fractions of the soil (Fig. 4.5-4.6). The enhanced labile P observed under drought is supported by increased activity of enzyme acid phosphomonoesterase in the soil (Fig. 4.17-4.18) This findings are in line with the studies previously done by Fan et al. (2018)<sup>[16]</sup> where they documented that the enzyme activities in the forest can convert the immobilized P to labile P. Fe and Al bound P are available for plant uptake and hence the differential crop based response documented in our tested crops might be due to variation in Fe and Al bound P under the cultivation of both the crops. Higher uptake of P by *Vigna radiata* crops compared to *Lathyrus sativus* may have resulted in lower accumulation of Fe-P in *Vigna radiata* cultivated soils As documented by Amirbahman et al. (2013)<sup>[17]</sup>, reductant soluble phosphorus are exchangeable and can be released under anoxic conditions during the reduction and dissolution of iron hydroxide (FeOH<sub>3</sub>). A decrease in reductant soluble P observed under drought can be attributed to the oxidation of the same due to soil aeration and transformation to other forms. Since calcium bound phosphorus (Ca-P) can only be liberated from soil in acidic environments (pH<6), it is typically referred to as a non-bioavailable fraction<sup>[18,19]</sup>. The acidic sandy loam soil in the present study, however, has a pH (Table. 3.1) lower than the aforementioned, suggesting that it may be available for plant uptake and the reduction of the same observed under drought confirms it.

The recorded decline in arylsulfatase activity of the studied soils under cultivation of both the crops are in line with the previous studies of Staszal et al. (2022)<sup>[20]</sup> where a

significant decline of arylsulphatase activity was reported on *Quercus pertaea* seedlings exposed to drought treatments. However, the exact mechanisms behind this decline feebly known<sup>[21]</sup>. Difference in crop specific response on  $\beta$ -glucosidase activity indicates the variations in root exudate characteristics of both the crops as the activity of  $\beta$ -glucosidase indicates the transformation of carbohydrates and, consequently, of energy production in the soil microbial population<sup>[21]</sup>. This is in correspondence with the previous studies done by Walker et al. (2003)<sup>[22]</sup> where they reported that the composition of the soil microbial community may be affected by the differences in the root exudates of two species of *Sonneratia*. The behavior of biomarkers of general microbial activity like DHA, connected to oxidative phosphorylation processes and basal respiration, also supported the negative impact of drought on soil microbial activity of both the crops<sup>[23]</sup>. Enhanced FDA hydrolysis activity of the soil under drought indicate increased hydrolysis of FDA in the soil matrix. However, FDA hydrolysis activity is not bacteria-specific<sup>[24]</sup>. Therefore, the observed increase in overall activity may have been caused by additional soil organisms like lichens and fungi<sup>[25,26]</sup>. Drought induced reduction in soil alkaline phosphomonoesterase activity decreased P mineralization and the ratio of the immediately/short-term available-P concentration<sup>[27]</sup>. This might be primarily as a result of a direct impact on the soil microbial population caused by decreased soil water content.

The effect of drought on accumulation of crude protein in grains of both the crops can be observed in our study. Lower crude protein content due to moisture deficit stress at vegetative phase of the crop can be attributed to lower production of protein in vegetative parts leading to lesser translocation in grains<sup>[28]</sup>. However, the documented higher content of grain crude protein due to water stress at reproductive stage can be a result of higher production of the same during the vegetative stage. Increased production of the drought stress induced proteins such as late embryogenesis abundant (LEA) protein, chloroplastic drought-induced stress protein (CDSP 32) may also attributed to this hike<sup>[29]</sup>. Crop specific responses in P uptake under drought as indicated by the accumulation of grain phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate, InsP6) content as it is the major source of P in the seeds<sup>[30]</sup>. Documented differential response in phytic acid accumulation in the grains of both the crops under drought indicate either disruptions in the P metabolic pathway or lesser uptake of P from the soil. This is also supported by our observations in the differences in the plant available P in the soil indicating disruptions in the P uptake of the crops (Fig.4.5-4.6).

The observed higher globulin fractions under drought is in the agreement with the findings by Sehgal et al. (2019)<sup>[31]</sup> who recorded higher globulin in drought tolerant lentil genotypes. Similarly, drought-induced hike in glutelin and albumin might be due to impaired protein synthesis as a result of disruption in protein biosynthetic pathway or insufficient precursors<sup>[32]</sup>. The irregularities observed in the ammoniacal and nitrate nitrogen availability in the soil under drought justifies impaired N uptake and ultimately the disturbed protein synthesis as N uptake is directly responsible for the protein synthesis in the plants<sup>[33]</sup>. Similar results were also documented by Konopka et al. (2007) and Sehgal et al. (2019)<sup>[31,34]</sup>. However, our study suggests that this increase in globulin, glutelin, and albumin under drought is at the cost of prolamins and residual fractions of protein. Increased protein digestibility due to drought indicates accumulation of higher digestible protein fractions such as albumin<sup>[35,36]</sup>. Crop specific response to drought was also documented in grain mineral contents. However, it can be inferred that higher grain mineral (Mg, K, Ca, and P) content recorded under drought is possibly due to the 'concentration effect' as reported in earlier studies<sup>[37-39]</sup>. Disruptions in Na and Fe uptake due to drought has also been reported in earlier studies<sup>[39]</sup>.

#### **Effect of drought and soil amendment on soil properties and grain quality**

Under application of both the soil amendments, the increment of ammoniacal N indicates the additional N input to the soil, especially under FYM application and also the enhanced potential of the microorganisms to mobilize urea into ammonium N<sup>[3]</sup>. Enhanced nitrate content under drought and application of FYM as soil amendment is due to the additional nitrogen in the FYM that is being released slowly and made available to the soil<sup>[40]</sup>. Similarly, biochar increases the mineral nitrogen content of soils by retaining ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub>) and reduced their losses to the environment through leaching and gaseous emissions<sup>[41,42]</sup>. Given the absence of nitrogen or at very low nitrogen composition of biochar in general, there are already established records of biochar additions to soils that stimulate net nitrification<sup>[43,44]</sup>. Crop specific response on soil organic nitrogen content under biochar or FYM application can be attributed to the activity of the soil microbial community. A build-up of organic nitrogen in biochar or FYM amended soils in *Vigna radiata* cultivated soil exposed to drought at vegetative stage might be a result of additional N input from the amendments that may not have been mobilized. However, a reduction of the same during the reproductive stage may indicate higher transformation of organic N by the microbial communities at the later stages of crop growth. Moreover, a reduced

organic N in *Lathyrus sativus* cultivated field exposed to drought delineate higher mobilization of the organic N to the mineral forms (Fig. 4.1-4.2) by the microbial communities even at the early growth stages. Enhancement in microbial biomass carbon and microbial biomass nitrogen in biochar or FYM amended soils even under drought indicate their ability to support growth of the microbial communities under stress. Biochar boosts the activity of agriculturally important beneficial microorganisms and soil enzymes<sup>[45]</sup>, which can have a significant impact on the soil microbial environment. The increased total nitrogen observed in biochar and FYM amended soils is due to the biochar and FYM containing a certain amount of nitrogen (Fig. 4.1-4.2). Incorporating carbon rich biochar into the soil results in a significant increase in SOC. Similarly, enhanced SOC under FYM application is possibly due to the fertilization induced C accumulation by stimulating biomass production <sup>[46]</sup>.

Acidic soil caused an increase in Al-P and Fe-P relative to labile P and the findings of Bapat et al. (1965) <sup>[47]</sup> supports our results. Sesquioxides, which convert a portion of native or added P into these fractions, can be held responsible for the presence of Fe-P and Al-P in significant amounts. With the addition of organic amendments, all inorganic P fractions increased. This finding is in line with Lee et al. (2004)<sup>[48]</sup>, who observed a notable rise in all inorganic P fractions following the application of organic amendments. Since biochar or FYM is a potential source of P, the increased availability of Ca-P on their incorporation is due to the addition of P to the soil during mineralization. In addition, the produced humic, fulvic, and other organic acids on decomposition of organic amendments compete with solution P for clay mineral sorption sites at low pH levels. This results conversion of majority of applied inorganic P to Ca-P<sup>[49]</sup>. The lessening of reductant soluble P in biochar or FYM amended soils exposed to drought is possibly due to the aeration in the soil resulting in oxidation of this reduced form of P<sup>[50]</sup> and ultimately its transformation.

The impact of drought and application of soil amendments on various soil biological activities were also documented. Crop specific response to the arylsulphatase activity, beta glucosidase activity, bacterial, and fungal CFU delineate differential response of the crops on the microbial community structure and functioning arising from the application of biochar or FYM on drought exposure. It can be inferred from our study that supplementation of nutrients and increasing water holding capacity of the soil by the applied soil amendments promote change in microbial community structure which

ultimately affects the nutrient cycling by the virtue of the changes in enzyme activities. An increase in arylsulphatase activity, dehydrogenase activity, FDA hydrolysis activity, acid phosphomonoesterase activity, alkaline phosphomonoesterase activity, urease activity, along with actinobacterial CFU indicates a positive impact of the applied soil amendments on soil biological properties, even under drought. This increase in soil enzymatic activities can be attributed to the presence of higher labile C fractions (Fig. 4.7-4.8) and inherent microorganisms (Table. 4.3-4.4) in the applied soil amendments<sup>[51]</sup>. The obtained results are in line with the studies by Sarma et al. (2017)<sup>[52]</sup> where an increase in soil enzymatic activities viz. urease, phosphatase, dehydrogenase and FDA hydrolysis activity was documented with the use of organic amendments.

Lesser content of grain crude protein observed due to application of soil amendments as compared to drought treatments is possibly due to the lesser productions of the stress proteins such as late embryogenesis abundant protein (LEA protein) and chloroplastic drought induced stress protein (CDSP 32) under drought<sup>[29]</sup>. The enhanced grain carbohydrate in the grains could be a result of increased photosynthesis as observed due to the application of biochar or FYM (Fig. 7.3-7.4). This lead to better carbohydrate accumulation in the leaves and ultimately their translocation to the grains. Higher availability of soil P under application of biochar and FYM (Fig. 4.5-4.6) as well as documented from our previous study<sup>[52,53]</sup> along with reduction of drought intensity (as evident from LWP data) is possibly responsible for the perceived higher phytic acid content under application of both the amendments. Crop specific responses to the accumulation of different protein fractions as a response to application of biochar or FYM and exposure to drought at vegetative or reproductive stage delineates differential mechanism in storage protein accumulation and composition. In *Vigna radiata* grains, drought induced reductions in albumin, prolamin and glutelin were mitigated by biochar or FYM and an enhancement of the respective proteins were documented. Whereas, in *Lathyrus sativus* grains, an increase in globulin, albumin, prolamin fractions was documented at the expense of glutelin and residual fractions of protein.

Crop specific responses were also documented in mineral accumulation of grains. In *Vigna radiata* crops, reduction of minerals like Fe, K, Na, Mg, and Ca indicates the importance of water in this stage of growth even under the application of both the soil amendments. However, these reductions are relative to the enhanced mineral contents recorded under drought. Therefore, it may be inferred that this reduction could be at par

with the normal mineral accumulation by the grains. Under biochar and FYM addition and exposure to drought at either stage, *Lathyrus sativus* grains were documented to be particularly sensitive to Fe and Ca accumulation in grains. Relatively lower Fe and Ca content observed in biochar amended crops is possibly due to ability of biochar to bind metals in its surfaces <sup>[53,54]</sup>, making unavailable for plant uptake. Likewise, the reduced Fe content observed in FYM amended crops can be attributed to the formation of  $\text{Fe}_2(\text{CO}_3)_3$  through the carbonates reaction, which are released during decomposition of the FYM, with soluble Fe <sup>[55]</sup>.

### **Effect of drought and soil amendments on soil properties at harvest**

At harvest, a decrease in ammoniacal or nitrate N may indicate greater N uptake by the plants as both are the major N fractions accumulated by the crops. However, higher ammoniacal N at harvest (after 105 days) under the application of FYM is possibly a result of slow N release mechanism of FYM. No significant difference in soil organic nitrogen content among drought treatments and control at harvest of *Vigna radiata* indicates that over its cultivation period, rewetting may play a major role in microbial community structure and accelerate the mineralization process of nitrogen. A shift in microbial biomass carbon, microbial biomass nitrogen, urease activity, bacterial CFU, fungal CFU, and actinobacterial CFU at harvest also support this. Crop specific differential responses in soil organic nitrogen content delineates that the benefits of the different soil amendments may depend upon the plant root exudates, rhizospheric community structure, available nutrients, rewetting period, etc., which may shape the nutrient cycling. This is also supported by our findings where crop specific responses in aforementioned soil biological properties are observed. An increased total N in drought treated soils indicates a higher atmospheric nitrogen fixation by the legumes in the later stages of growth, which is in line with the studies done by Seuss et al. (2022)<sup>[56]</sup> where results show that in more arid climate zones, the rate of  $\text{N}_2$  fixation increases more quickly in response to soil rewetting. Crop specific response to the accumulation of SOC after rewetting was observed in our study. This can be attributed to the microbial responses to the drying-rewetting of the soil. Contrasting results are also documented by previous studies as Xu et al. (2019)<sup>[57]</sup> reported strong positive correlation between drying-rewetting cycles with formation of soil organic matter, favoring SOC mineralization. However, Lal et al. (2015)<sup>[58]</sup> reported that cycles of drying and rewetting may also accelerate the decomposition of SOC by exposing physically protected SOC in aggregate fractions.

Rewetting of soil after completion of the drought in pulse crop can result in higher available P in the surface soil. According to Kieft et al. (1987)<sup>[59]</sup>, the high temperature that follows the wetting of soils by rainfall causes the death of 58% of the microbial biomass during rapid drying. The documented reduction in MBC in drought treated soils at harvest also supports the findings. In biochar or FYM amended soils, the increase in fungal CFU along with the reduction of bacterial and actinobacterial CFU indicate the sensitivity of bacterial and actinobacterial populations to the drying-rewetting phenomenon. This is in line with the studies by Cosentino et al. (2006)<sup>[60]</sup>, who found that fungi are less impacted by drying and rewetting than bacteria, despite Butterly et al. (2009)<sup>[61]</sup> and Gordon et al. (2008)<sup>[62]</sup> found the opposite. However, the underlying mechanisms that cause P to be released from the drying and rewetting of arid soils are weakly understood. An increment in plant available P fractions, viz. Al-P and Fe-P even at the end of cultivation period delineates the slow P release of the tested soil amendments. However, a reduction of Ca-P at harvest in soils amended with biochar or FYM may indicate its transformation to other forms due to acidic environment<sup>[18,19]</sup>. Active phosphatases can release P from P-rich microbial cells after rewetting soil since bacteria typically have higher P quantities than fungi<sup>[63]</sup>. Mineralization of photodegraded biomass, mineralization of dead microbial cells (due to drying), and mineralization of solutes made available upon wetting are additional biotic pathways responsible for the release of P<sup>[64]</sup>.

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