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Predicting soil microbial responses to drought: a laboratory analysis

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A chi ogni giorno si batte per lasciare questo mondo
migliore di come l’ha trovato.
Con amore e rabbia.

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Abstract

Soils are fundamental components of terrestrial ecosystems, sustaining plant productivity, nutrient cycling, and carbon storage. Microbial communities play a central role in these processes through decomposition and nutrient mineralization, but their activity is highly sensitive to drought, an increasingly frequent climatic stressor.

This study aims to assess how soil microbial activity responds to varying degrees of water stress across sites differing in soil properties and climatic regimes. Soil samples were collected from five Italian grassland sites spanning a gradient of mean annual precipitation (MAP), organic matter content, and pH. Controlled laboratory incubations were conducted at five moisture levels (10 - 80% water holding capacity) to measure soil respiration, eight extracellular enzymatic activities, microbial biomass carbon (MBC) and nitrogen (MBN), and available nutrients.

We hypothesized that microbial communities from drier sites would be more resistant to water stress than those from wetter regions. In agreement to this expectation, sites with higher MAP showed stronger reductions in respiration under low moisture, but all sites converged to similar relative respiration rates at the lowest moisture levels, even after normalization by soil organic matter. Microbial communities exhibited two contrasting nutrient-acquisition strategies under drought - either increasing or decreasing enzyme production - yet these shifts did not translate in to differences in CO_2 efflux, indicating that drought imposes a dominant physiological constraint regardless of community strategy or climatic origin. Respiration rates were strongly and positively associated with dissolved nitrogen, revealing a tight coupling of microbial C and N cycling and a potential role for available N in mediating drought effects on soil metabolic activity.

Overall, our results demonstrate that microbial functional responses to drought are governed more by intrinsic moisture limitation than by long-term climatic adaptation, providing mechanistic insights into how soil carbon losses may accelerate in a drying world.

Introduction

Soils are the foundation of life on land, storing vast amounts of carbon and nutrients and sustaining nearly all terrestrial ecosystems. They also represent the most diverse ecosystems, home to 59% of all life on the planet[1] and they produce 98.8% of the calories consumed by humans[2].

Microbial communities are central in regulating key biogeochemical processes, such as carbon and nitrogen cycling, which are critical for regulating mineralization of nutrients as well as soil C storage. However, climate change is altering the frequency and intensity of drought events, which could have large consequences for central processes controlled by soil microbial communities.

Soils microbial communities as key drivers of ecosystem functioning

Soil is the largest C pool among terrestrial ecosystems, storing approximately 2500 Gt of C, more than vegetation and atmosphere combined[3]. Grasslands, which cover almost 40% of terrestrial biomes, significantly contribute to the global carbon pool, representing 25-34% of the total carbon stock[4].

The soil C pool contains both organic and inorganic C, both playing crucial roles in contributing to the global C pool.

Soil organic carbon (SOC) is mainly composed of plant litter, microbial biomass and their metabolic byproducts, with plant-derived biomass from primary production constituting the major organic carbon input.

Soil microorganisms critically regulate SOC balance and contribute directly to its formation, with their necromass accounting for 35–54% of SOC[5]. Soil microbes break down dead organic material (decomposition) and form new microbial biomass from organic matter (secondary production), which eventually stabilize in soil as dead microbial biomass (necromass).

Although soil acts as a sink for carbon through sequestration processes, it simultaneously emits carbon to the atmosphere, playing a critical role in the regulation of atmospheric trace gas dynamics. In fact, about a fifth of atmospheric CO_2 originates from soils (~110 Gt per year), which is about ten times more than anthropogenic CO_2 emissions.

Soil heterotrophic respiration (HR) is one of the primary mechanisms through which terrestrial ecosystems release CO_2 into the atmosphere[6] and it is driven by microbial mineralization and decomposition of SOM.

During decomposition, microorganisms assimilate organic compounds to sustain growth, maintenance, and energy production. Low-molecular-weight compounds are directly utilized, whereas macromolecular substances require prior depolymerization via extracellular enzymatic activity (EEA).

Microbial decomposition of soil organic matter (SOM) accounts for a large proportion of soil C emissions and depends on extra- and intracellular enzymatic activities[7]. The extracellular steps of SOM decomposition include the microbial release of enzymes to convert SOM into dissolved organic carbon (DOC) and nutrients, which are directly metabolizable. This microbial activity is strongly influenced by carbon inputs from plant roots (rhizodeposition)[8]. When SOM is accessible for microbial decomposers, the activity of extracellular enzymes represents the rate limiting step of SOM decomposition and regulates the C balance between ecosystems and the atmosphere[9].

Nutrients in excess for microbial biomass requirements are released, potentially contributing to nutrient availability for plants.

The reciprocal interaction between plants and soil microbial communities - whereby plants supply energy-rich compounds that stimulate microbial decomposition, and microbes, in turn, release nutrients essential for plant growth - underpins the functional integrity of terrestrial ecosystems[10].

As anthropogenic activities related to soil use and affecting microbial processes contribute approximately 15% to the total global warming[2], understanding and assessing microbial activity rates under climate change is crucial.

Anthropogenic factors leading to drought

Atmospheric carbon dioxide concentrations have increased remarkedly from approximately 278 ppm[3] during the pre-industrial period (circa 1750) to over 430 ppm recorded in March 2025 (NOAA, 2025). The principal drivers of this increase are deforestation, other types of land-use changes such as drainage, and fossil fuels use. If the current reliance of fossil energy sources persists, this upward trajectory in atmospheric CO_2 levels is expected to continue (IPCC, 2022), causing significant alterations to carbon cycling balance. In relation to this, climate models predict that average annual air temperatures will increase by an additional 2.7–4°C[11].

Together with the increase of mean global temperature, the frequency and intensity of climate extremes such as floodings and drought events will notably escalate (IPCC 2014). Mean summer precipitations are expected to decrease by approximately 25% across extensive regions of the globe during the 21 st century[11].

Recently, a high-resolution global drought analysis (1901–2022) reported a marked increase in drought severity, driven largely by rising atmospheric evaporative demand[12]. In 2022, 82% of Europe's land area experienced drought, with half under moderate to severe conditions. Both traditionally dry and wet regions are exhibiting drying trends, with drought expansion accelerating sharply over the past five years, leading to depletion of water reserves, lowering of crop yields and decrease of livestock production.

Another recent study predicts that under a high emissions scenario, almost all of Europe, except for northern Norway and Sweden, is expected to become drought hotspot regions, with at least 40 more events than in the past, representing an increase of over 44 %[13].

Drought is one of the most common and complex natural hazards affecting the environment, economies and populations globally.

For this reason, studying how microbial communities react to such events has become a central priority, particularly in the light of accelerating impacts of climate change on life-supporting systems.

Microbial responses to water stress

Drought leads to reductions in soil microbial metabolic activity, microbial biomass, nutrient mineralisation (especially carbon and nitrogen) and respiration rates, having cascading effects on soil ecosystem functions and services, such as soil fertility and plant productivity[14].

The duration of drought, the number of drying and rewetting cycles, and microbial community composition are key factors[11] that should be taken into account when analyzing microbial activities. For example, soil microbial communities with lower organic matter content are demonstrated to be more strongly affected by drought stress[15]. Instead, communities with higher organic matter are more resistant, as organic matter helps to retain moisture and provides a more stable environment for microbial communities.

However, regarding respiration rates, carbon-rich soils are more vulnerable to rapid carbon loss when a rewetting event follows a drying period[16].

Heterotrophic respiration (one of the largest CO_2 contribution to the atmosphere C balance) primary climatic drivers are soil temperature and moisture[6].
Soil moisture shows a non-linear relationship: low soil moisture reduces heterotrophic respiration rates by limiting solute flux in soil pores, while high moisture reduces heterotrophic respiration by limiting oxygen supply. As a result, the response of heterotrophic respiration to soil water saturation shows a bell-shaped response curve, with optimal conditions for respiration at intermediate soil moisture content.
However, to the best of our knowledge, there are no investigations on the response of enzymatic activities to different water stress levels, leaving a potential knowledge gap, linking soil microbial nutrient acquisition strategies with respiration rates.

At the global scale, soil enzyme activities are linked to microbial biomass stoichiometry and their nutrient demand[9], so this is a significant parameter to consider when analyzing drought response.
Notably, the nutrient storage capacity of soil microbes becomes particularly significant during drought, when the mobility of nutrients and of dissolved organic carbon (DOC) is reduced and organisms are disconnected from substrates. Therefore, linking dynamics of microbial biomass stoichiometry and enzymatic activities could reveal mechanistic explanation of effects of climate change on terrestrial biogeochemical cycles.

Furthermore, there is evidence of microbial community adaptation to drought, via shift in composition, or resistance strategies such as dormancy, spore formation or investment in osmolytes[15].
It was shown that soil microbial communities that have previously been exposed to drought may change less in response to subsequent drought events, due to the selection of drought-resistant taxa, with multiple studies showing that fungi are more resistant than bacteria under water stress conditions[15,17].
Therefore, soils with prior drought exposure or historical aridity may harbour microbial communities that are already adapted to drought stress compared to historically wet sites.

Aim of this study

The aim of this study was to evaluate the response of microbial activity to different levels of water stress. To achieve this aim, we examined five different sites varying in soil parameters (e.g. organic matter, pH) and historical precipitation regime (from 616 to 2100 mm of mean annual precipitations). We hypothesize that sites with lower mean annual precipitation contain communities that are more adapted to drought conditions, and therefore displaying different shape responses to water stress. We thus conducted a laboratory experiment at 5 different water levels and measured: (i) soil respiration; (ii) 8 different enzymatic activities; (iii) microbial biomass carbon and nitrogen, and (iv) available nutrients (C and N).

Achieving a full understanding of the conditions and variables predicting microbial response to water stress is essential to improve our understanding and forecasting ability of future climate change impacts on ecosystems.

Methods

Study area

Soils samples were collected from five distinct sites located in Italy, selected to represent a wide range of soil with different levels of organic matter, pH, vegetation and historical precipitation regime (*Table 1*). These sites are grassland ecosystems, managed by cutting the vegetation once a year to harvest the biomass except one site that is a managed natural park (Boschi della Fagiana), in which the forest had been removed to maintain the natural grassland species.

Table 1: Summary of site characteristics, including mean annual precipitation (MAP) soil pH, soil organic matter (SOM) and their GPS coordinates

Site name	MAP (mm)	Soil pH	SOM (%)	Coordinates
Boschi della Fagiana	843	4.44	6.4%	45°25′46.49″ N, 8°49′31.01″ E
Prati di Tribil	1587	4.45	17.9%	46°6′55.84″ N, 13°34′38.14″ E
Montagna di Torricchio	1098	6.07	19.3%	42°57′22.97″ N, 13°01′04.55″ E
Val Venosta	616	5.27	9.3%	46°37′48.79″ N, 10°42′00.83″ E
Prealpi Giulie	2100	6.81	29.1%	46°19′46.63″ N, 13°25′39.97″ E

Soil samples collection and preparation

Within each site 24 samples were collected in an area of approximately 600 m^2. Soil samples were obtained using a soil corer of 10 by 2.5 cm (*Figure 1*).

Samples were brought to the laboratory where they were sieved using metal sieves (2mm pore size) to remove coarse debris including stones, grass filaments and plant roots and then they were combined.

Following this preparation, soils were left to air-dry until no loss of water could be observed and 15 grams of soil were weighed into 15 glass jars (125 ml size) per site.

The jar lids had one hole that was filled with sterile cotton to ensure gas exchanges without contamination.

The prepared samples were then subjected to five different water treatments corresponding to 10%, 20%, 40%, 60% and 80% of the water holding capacity

Figure 1: Soil samples collection in Boschi della Fagiana.

(WHC), to simulate varying soil moisture conditions. Because WHC expresses moisture relative to each soil's own physical properties, using %WHC makes moisture levels physiologically comparable across soils - a given percentage reflects a similar balance of air-filled vs water-filled pores, and therefore a comparable level of microbial water stress, even if the soils differ in texture. The right amount of water was added with respect to the air-dried soil weight. The combined weight of soil and water was recorded for each treatment.

To maintain the designed moisture levels the water content was adjusted daily, compensating for losses due to absorption and evaporation.

All samples were incubated at 20°C for a duration of two weeks under controlled conditions. In total we used 5 treatments, in 5 sites with 3 replicates per water level, with a total of 75 samples.

Water holding capacity

Water holding capacity (WHC) was measure by weighing around 20 grams of soil on a filter paper placed on a funnel. Soil was soaked with water multiple times to completely saturate soil pores. Saturated soils were left to drain for 48 hours while the funnel was closed using aluminium foil to avoid loss via evaporation. A subsample of soil was taken after 48 hours and its gravimetric water content was measured via drying the soil at 105 C° for 24 hours. The water content obtained was used to calculate the 100% WHC.

Loss of Ignition method (LOI) to calculate SOM

LOI estimates soil organic matter (SOM) content by measuring the mass loss of oven-dried soil samples after combustion at high temperatures. Organic matter is oxidized and volatilized, leaving behind the mineral fraction.

Three samples per site were randomly chosen and 20 g per sample were selected and placed on an aluminium tray of known weight (W1). The weight with the added soil was recorded (W2).

Subsequently, the trays were placed evenly in an analytic ashing furnace at 550°C and rotated 180° after 1.5 hours to reduce door-effect heat variability. The total time of ignition was 6 hours.

The samples were let cool in the desiccator and the new weight was recorded (W3).

The formula used to calculate SOM (%) is: $\text{SOM}(\%) = [(W2 - W3) / (W2 - W1)] \times 100$

Soil pH determination

Soil pH was determined by using a 0.01 M calcium chloride ($CaCl_2$) solution.

Three samples per site were randomly chosen and 2 g per sample were selected and put in 15 mL vials. 10 mL of 0.01 calcium chloride solution were added and vials were shaken for 30 minutes in a horizontal shaker. The mixture was then allowed to equilibrate for an additional 30 minutes.

Finally, pH measurements were performed on the supernatant using a calibrated pH meter equipped with a glass electrode. Calibration was conducted using standard pH buffers.

Soil respiration measurement

To measure soil respiration we incubated soil in a closed environment and measured the accumulation of CO_2 over time. Around 1 gram of soil was weighted in 2 ml cryovials and inserted inside 50 ml falcon tubes, sealed with suba seal to maintain a closed environment. CO_2 concentration was measured at the beginning of the incubation and after 48 hours. Soil respiration was quantified using an EGM-5 CO_2 Gas Analyzer (PP System), a flow-through infrared gas analyzer (IRGA) designed for CO_2 measurement. Respiration rates were calculated by converting the measures in ppm obtained from EGM-5 CO_2 Gas Analyzer to nanograms of C per gram of dry soil per hour, using the ideal gas law.

We calculated the “Drought effect”, as the difference between mean microbial respiration at optimal soil moisture levels (80%) and mean respiration at low soil moisture levels (10%). We used this parameter to make comparisons between sites and assess their different responses to water stress. Remaining activity under drought was measured by diving the mean respiration rate at 10% treatment to the mean respiration rate at 80%, and multiplying by 100 to get the percentage.

Fluorometric method to assess hydrolytic enzyme activities

To assess soil enzyme activity, fluorometric assays employing substrates labelled with MUF (4-Methylumbelliferone) or AMC (7-Amino-4-methylcoumarin) were conducted, assessing the activity of 8 different enzymes involved in the carbon, nitrogen and phosphorous cycle (*Table 2*).

In the presence of the corresponding enzymes in the soil samples, enzymatic hydrolysis of these substrates releases a fluorescent product, the intensity of which can be quantified using a microplate reader. Enzyme activity was subsequently determined by reference to standard curves, generated from known concentrations of MUF or AMC standards.

Table 2: Summary of the enzyme targeted, their respective function and the substrate used in our assay.

Cycle	Enzyme	Function	Fluorescent substrate
C Cycle	β-Cellobiosidase	Cellulose degradation	4-Methylumbelliferyl-β-D-cellobioside
	β-Glucosidase	Cellulose degradation	4-Methylumbelliferyl-β-D-glucopyranoside
	β-Xylosidase	Hemicellulose degradation	4-Methylumbelliferyl-β-D-xylopyranoside
	Lipase	Lipid degradation	4-Methylumbelliferyl oleate
N Cycle	N-acetyl-β-glucosaminidase	Chitin degradation	4-Methylumbelliferyl-N-acetyl-β-D-glucosaminide
	Leucine-aminopeptidase	Protein degradation	L-Leucine(4-methyl-7-coumarinylamide) hydrochloride
	Tyrosine-aminopeptidase	Protein degradation	L-Tyrosine 7-amido-4-methylcoumarin
P Cycle	Phosphatase	Organic phosphorus mineralization	4-Methylumbelliferyl phosphate free acid

For the assay workflow, soil suspensions were prepared by mixing approximately 1 g of soil with 50 mL of 50 mM acetate buffer in individually numbered vials corresponding to each sample.

To ensure accurate dispensing of the buffer, a calibrated dispenser verified with milliQ water was used to determine the precise volume required.

Afterwards, the soil suspensions were sonicated at 350 ± 30 J to facilitate the dispersion of microbes and the release of extracellular enzymes into the solution.

Following preparation, 96-well microplates were labelled with the eight substrate identifiers, resulting in a total of 24 plates per incubation.

Soil suspensions were prepared by placing them into a glass bowl, adding 50ml of acetate buffer and by removing coarse material with a sieve. Continuous homogenization was maintained with a magnetic stirrer. After this setup, large pore tips were used to pipette 200 µL of samples in all the wells to initiate the enzymatic reactions.

Standard series and substrate were added to the appropriate wells in the amount of 50 µL.

Finally, plates were placed in the fluorometric plate reader (Molecular Devices Spectramax iD3S) and analyzed under automatic gain settings, with excitation and emission wavelengths set to 365 nm and 450 nm respectively.

Each plate was measured at four time points at about 20 minutes apart. Enzymatic activity was calculated as increase in fluorescence over time.

The final results were converted in nmol/h/g of dry soil and extracellular enzymes acting on the same substrate in terms of nutrients (carbon, nitrogen or phosphorous) were associated to plot the graphs (*Figure 7*). To do so, β-Cellobiosidase, β-Glucosidase, β-Xylosidase and lipase activities were summed to represent carbon-related enzymes, N-acetyl-β-glucosaminidase, leucine-aminopeptidase and tyrosine-aminopeptidase were summed to represent nitrogen-related enzymes and phosphatase was considered for phosphorous.

Microbial biomass analysis using chloroform fumigation

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using a modification of the chloroform fumigation extraction method.

For each soil sample two vials of approximately 2 g of soil were prepared in either a plastic or glass 20 ml vial.

The non-fumigated vials (plastic) were extracted with 15 ml of 1 M potassium chloride (KCl) by shaking for 30 minutes. The resulting suspensions were filtered through ashless paper filters (Whatmann) and frozen at -20°C until further analyses.

The fumigated vials (glass) were exposed to chloroform vapour by placing an open glass vial containing 1 ml chloroform inside each vial and sealed with a Teflon coated lid. This setup prevented direct contact between the liquid chloroform and the soil. The vials were then sealed airtight and incubated at room temperature for 48 hours to allow fumigation.

Following fumigation, the soils samples were extracted in the same manner as the non-fumigated ones, using 15 ml of 1M KCl.

Total organic carbon (TOC) and total organic nitrogen (TON) concentrations in the extracts were determined using a TOC/TN analyser (Elementar vario TOC cube).

Microbial biomass carbon (MBC) was calculated as the difference in TOC concentrations between the fumigated and the non-fumigated extracts and corrected using a conversion factor of 0.45[18].

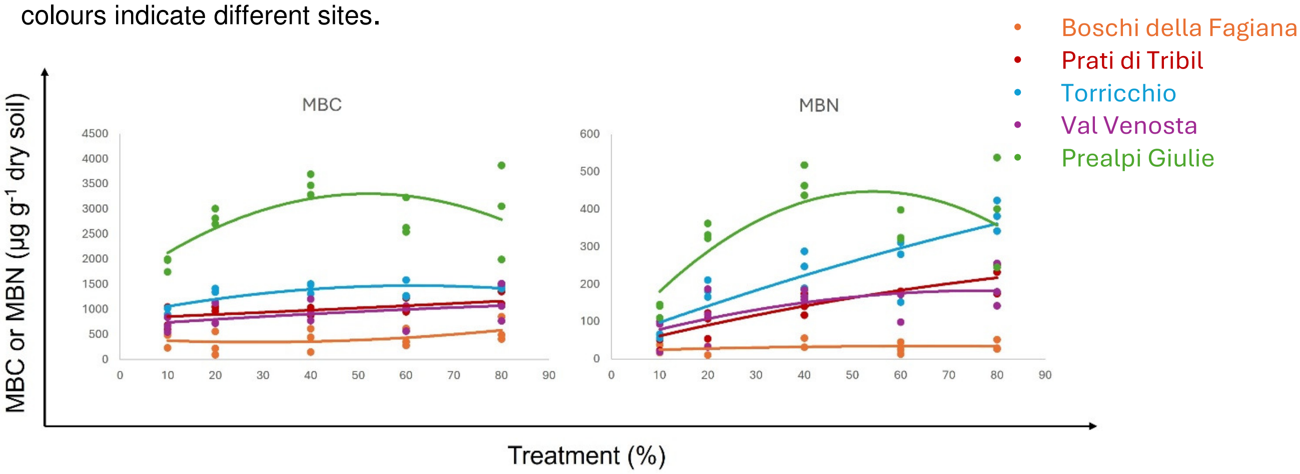
Similarly, microbial biomass nitrogen (MBN) was obtained from the difference in TON concentrations between fumigated and non-fumigated extracts, using a factor of 0.54[19].

Discussion of the results

Microbial biomass carbon and nitrogen

MBC and MBN show a positive relationship with increasing soil moisture levels (*Figure 2*). This shows that drought has a pronounced effect on microbial biomass carbon and nitrogen, since microbial growth (cell division) slows down due to limited water availability and nutrient diffusion. Because the production of new biomass is critical for the formation of stable SOC, limiting soil microbial biomass production could have long term implication for formation and stabilization of soil C in terrestrial ecosystems.

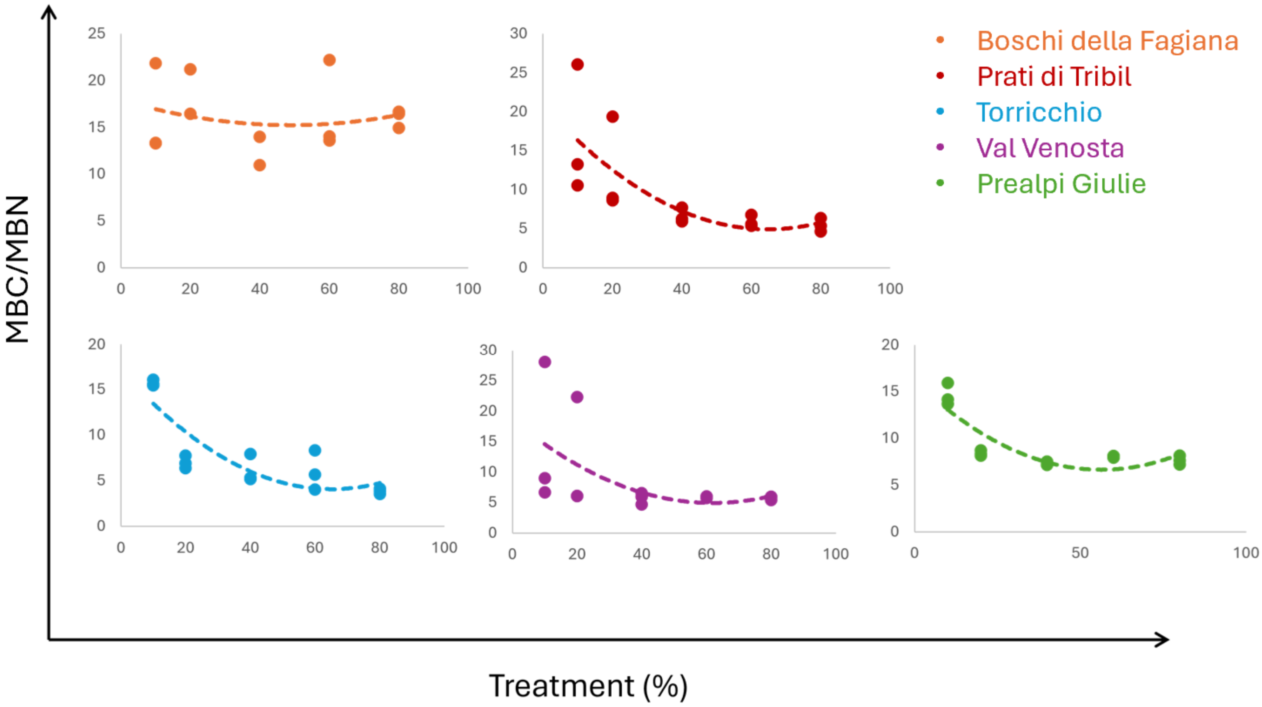
Figure 2. MBC and MBN expressed in µg C or N per gram of dry soil for each water treatment (%). Different colours indicate different sites.



The ratio of MBC to MBN increased at low soil moisture level at all sites (*Figure 3*). This could be due to a shift in microbial community composition, as better surviving organisms such as fungi may be selected (fungal cells have a higher C to N ratio than prokaryotes), or due to carbon allocation into C-rich storage compounds (such as triglycerides or polyhydroxyalkanoates)[17]. During stress conditions microbes can produce and store energy-rich compounds into their cells to ensure a survival advantage when environmental conditions improve.

Our results thus indicate a strong effect of drought on biomass and stoichiometry, pointing at either a shift in community or a physiological response to drought (or both).

Figure 3: MBC to MBN ratio for each water treatment (%). Different colours indicate different sites.



Available nutrients and stoichiometric imbalance

Soil carbon and nitrogen transformations are mainly mediated by microbial processes. During drought dissolved organic carbon (DOC) increases its concentration in the remaining water (*Figure 4*) but might not be accessible to build new biomass. Therefore, microbes might invest in storage compounds, allowing them to conserve energy and matter that allow them a fast regrow during the rewetting phase.

As moisture rises DOC is more rapidly utilized, so its concentration in the soil drops. Microbes might convert it to other forms, such as biomass (*Figure 2*) or CO_2 (*Figure 8*). This aligns with microbial biomass carbon and respiration rates which both increase with increasing soil moisture levels.

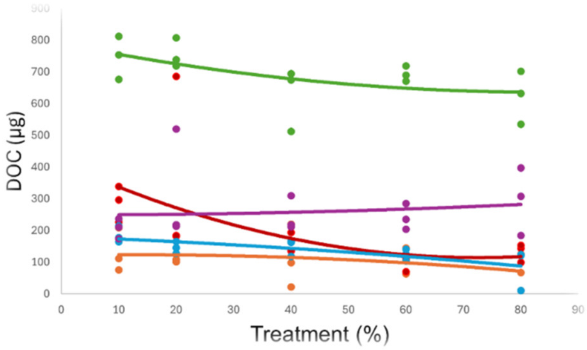


Figure 4: DOC (ugC/g of soil) for each water treatment (%). Different colours indicate different sites.

On the contrary, dissolved nitrogen (DN) increases with increasing soil moisture displaying a bell-shaped relationship similar to CO_2, either reaching a plateau or declining at high soil moisture levels (*Figure 5*). This suggests that microbial activity under variable water availability is tightly coupled between C and N cycles[20].

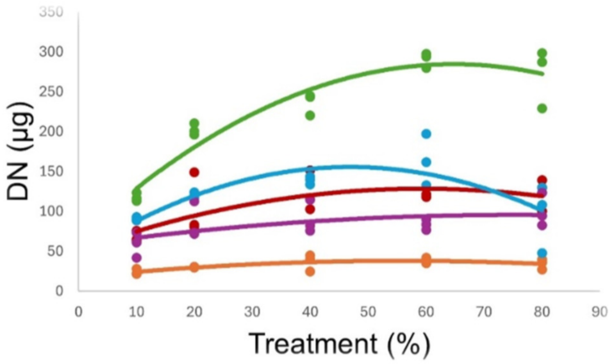


Figure 5: DN (µgN/g of soil) for each water treatment (%). Different colours indicate different sites.

- Boschi della Fagiana
- Prati di Tribil
- Torricchio
- Val Venosta
- Prealpi Giulie

The stoichiometric imbalance refers to a mismatch between the ratio of chemical elements required by microbes for growth and metabolism, and the ratio of those same elements available in their environment.

It is calculated with the formula:

Imbalance = (DOC/DN) / (MBC/MBN)

If the stoichiometry of available resources diverges from that of soil microbes (i.e. stoichiometric imbalance), microbial activity will be limited by a specific nutrient.

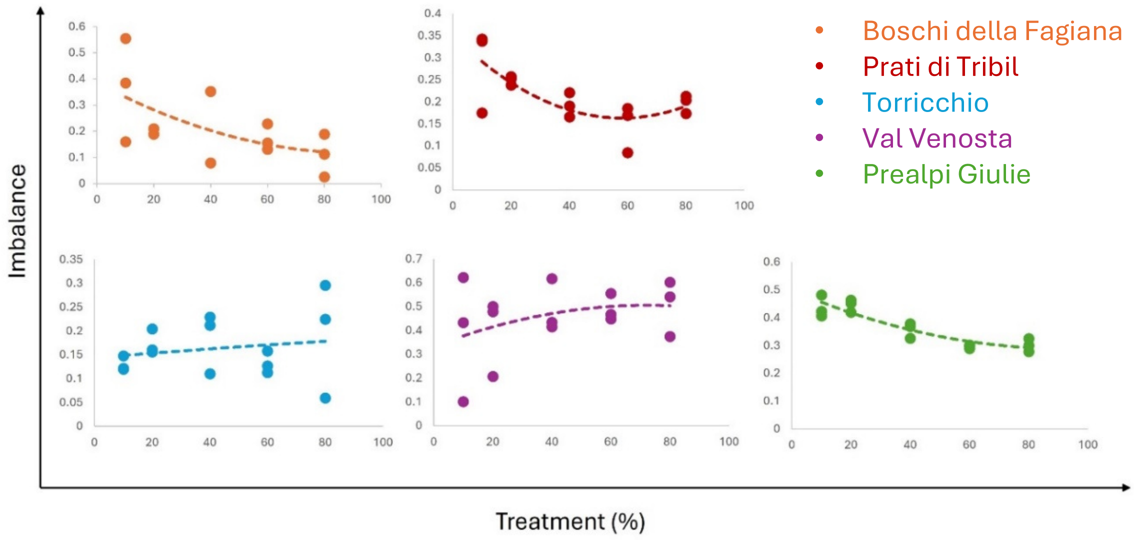
A decrease in the stoichiometric imbalance represents a higher microbial demand for C (in C:N), whereas an increase indicates a higher demand for N (in C:N) (*Figure 6*).

Some sites show a negative slope so a higher demand for nitrogen at low moisture levels. This reflects limited nutrient motility and increased carbon storage during drought conditions.

Others, show a slightly positive slope and a general more stable imbalance across water treatment. Probably these microbial communities adopt different survival strategies or have different nutrient limitations, leading to less sensitivity to soil moisture. Moreover, wetter conditions may increase DOC inputs or mineralization rates, leading to an excess of carbon and temporarily increasing the imbalance.

Overall, these results highlight that drought tends to change the stoichiometric imbalance, but the direction of this effect is site dependent.

Figure 6: Stoichiometric imbalances for each water treatment (%). Different colours indicate different sites.



Enzyme activities

Soil enzyme activities serve as functional indicators for the breakdown of organic matter, which is ultimately determined by the microbial demand for energy and nutrients.

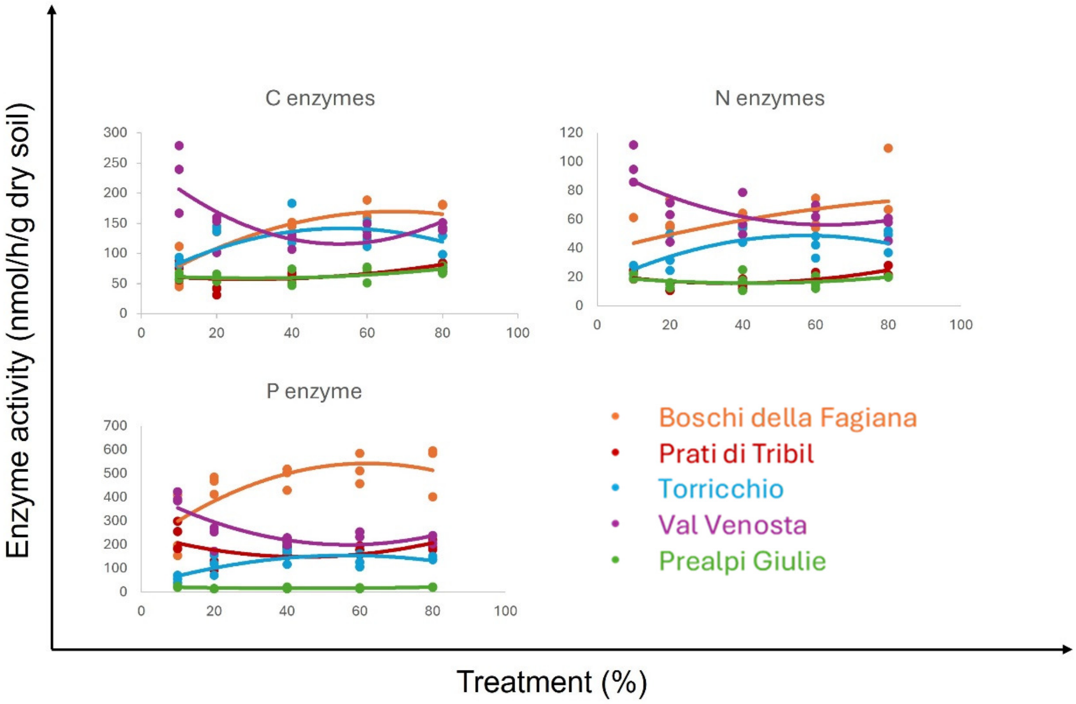
At all sites, soil microorganisms invested more on phosphatase and then carbon related enzymes, except for Prealpi Giulie where they invested more on carbon related enzymes. In relation to this, it is interesting to note that Prealpi Giulie has the lowest overall enzyme activity, probably due to high soil organic matter (*Table 1 and Figure 4*).

The different sites presented two different strategies in terms of enzymatic responses to water levels: either increase or decrease (*Figure 7*).

Both strategies could make sense and reflect different ecological and survival modes, with both benefits and costs. Reducing enzyme production during water stress can save energy and lead to longer survival under stressful conditions, but limiting potential nutrient acquisition; increasing enzyme production can be helpful if the substrate can be reached and the products of the enzymatic reaction are close to microbes, but the metabolic costs of this strategy can be high if drought lasts long.

We did not find a clear correlation between these different strategies and MAP, since sites with distinct MAP can adopt the same strategy and vice versa.

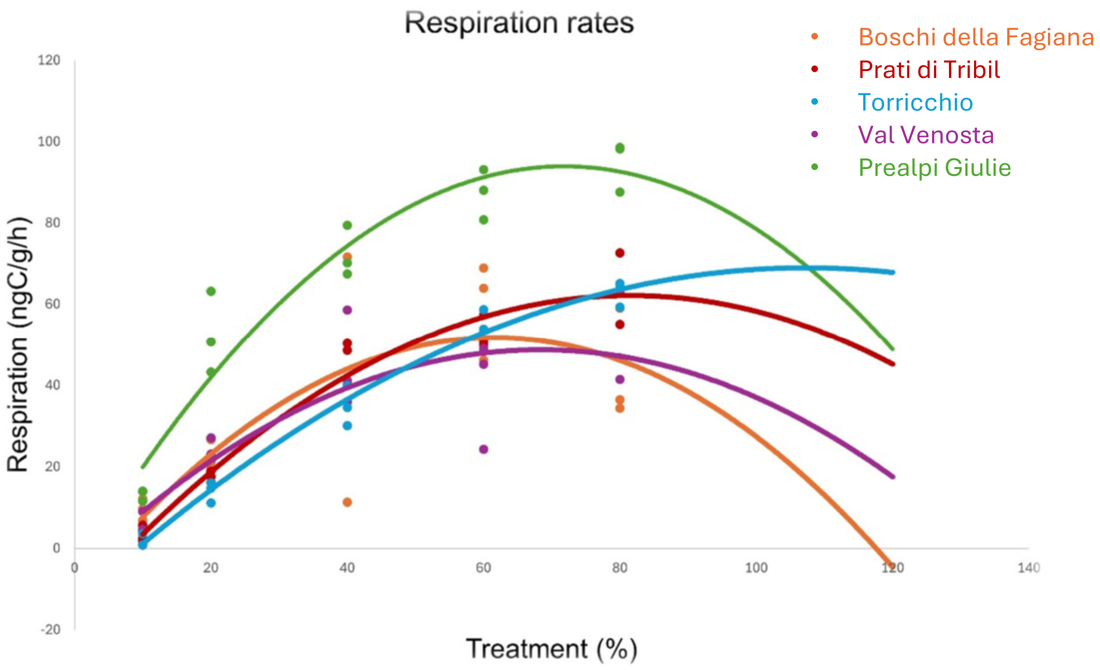
Figure 7: Enzyme activity for C, N and P related enzymes expressed in nmol/h/ g of dry soil (normalized to SOM) for each water treatment (%). Different colours indicate different sites.



Respiration

Respiration rates display bell-shaped curves for all sites, meaning that respiration is optimal at 60/80% of water holding capacity and it decreases when soils experience water stress conditions, either drought or flooding (*Figure 8*). This pattern is consistent with the idea that drought limits microbial metabolism by restricting substrate diffusion and enzyme activity, whereas adequate moisture promotes decomposition and carbon mineralization. Respiration rates were normalized for soil organic matter to reflect metabolic activity intensity (respiration per unit of soil organic matter), regardless of the amount of substrate in the soil: soils with more organic matter will naturally have higher respiration rates because more substrate is available, not necessarily because microbes are more active. Prealpi Giulie has the highest respiration rate between sites, even when normalized by unit of soil organic matter.

Figure 8: Respiration rates expressed in nanograms of carbon per grams of soil per time (hours) normalized for soil organic matter for each site, over water treatment (%). Different colours indicate different sites.



We calculated two indexes to assess the hypothesis that MAP modulates the effects of drought on soil microbial community functions. The first is the “Drought effect” which refers to how much microbial activity decreases under drought. The second is the remaining activity under the most extreme drought level, and it is expressed as a percentage of the highest moisture level, which indicates what is the fraction of activity under the most intense drought level.

We found that MAP correlates with the “Drought effect”, indicating the sites with higher total respiration have a stronger decrease of total MAP (*Figure 9*). While we show a positive correlation, we also observed a positive correlation with soil organic matter (SOM) with a higher R^2. The weaker correlation with MAP suggests that precipitation effects on respiration are primarily mediated by SOM content. MAP influences respiration indirectly through its effects on SOM accumulation: a wetter soil has more plant biomass and more litter input and SOM.

We further normalized respiration rates by SOM in order to account for differences between sites due to higher C content. Even after normalization we found the same correlations (*Figure 10*). This shows that sites with higher organic matter (and higher MAP) have higher rates of C release per unit of organic material in soil and that similarly to total respiration rates, they will decrease more the total activity of C release from soil.

Figure 9: Drought effect calculated considering non normalized respiration rates plotted with MAP (mm) and SOM (%). Different colours indicate different sites.

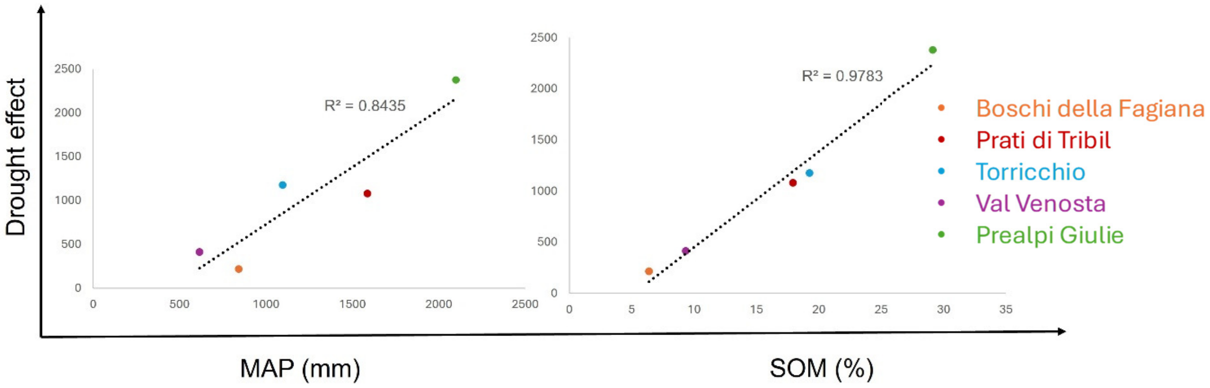
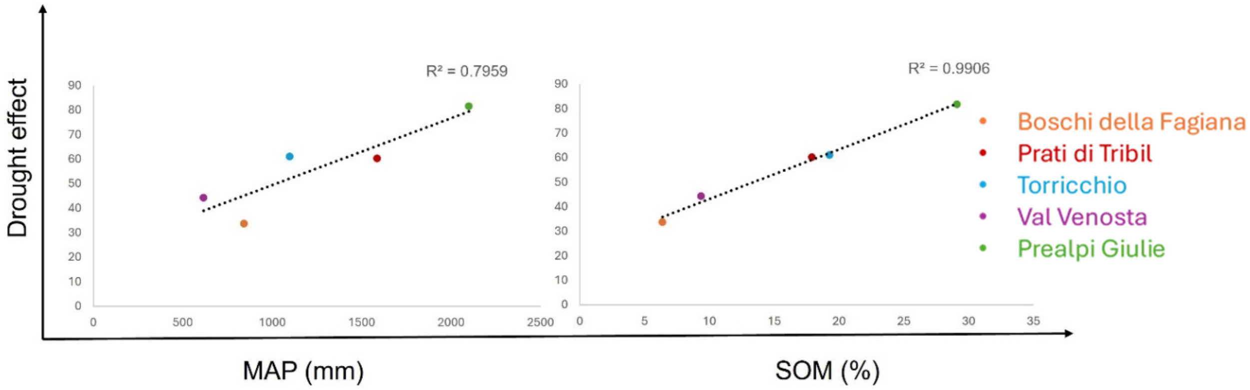


Figure 10: Drought effect calculated considering normalized respiration rates plotted with MAP (mm) and SOM (%). Different colours indicate different sites.



The remaining activity at 10% WHC was plotted with MAP (and SOM< Figure not shown) showing to no correlation (*Figure 11*).

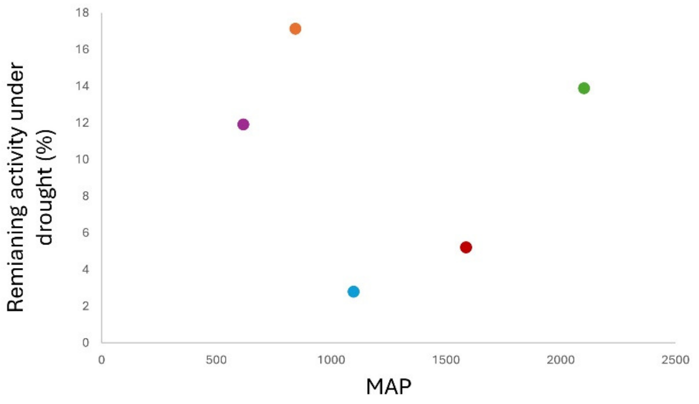


Figure 11: remaining activity under drought (%) over MAP (mm). Different colours indicate different sites.

This suggests that soils from different climatic regimes retain a similar proportion of their baseline activity under drought, thus rejecting our initial hypothesis. However, the absolute reduction in respiration is greater in sites with higher MAP and SOM content. This indicates that soils richer in organic matter and developed under wetter conditions experience a stronger relative suppression of C release during drying, but this is due to higher respiration rates (even when normalized by SOM), while displaying similar relative remaining activity.

We further checked for correlation among different variables to explain respiration rates and found a correlation with dissolved nitrogen (DN) (*Figure 12*). This shows a tight coupling between C and N cycling, potentially suggesting that CO_2 production is controlled by N availability.

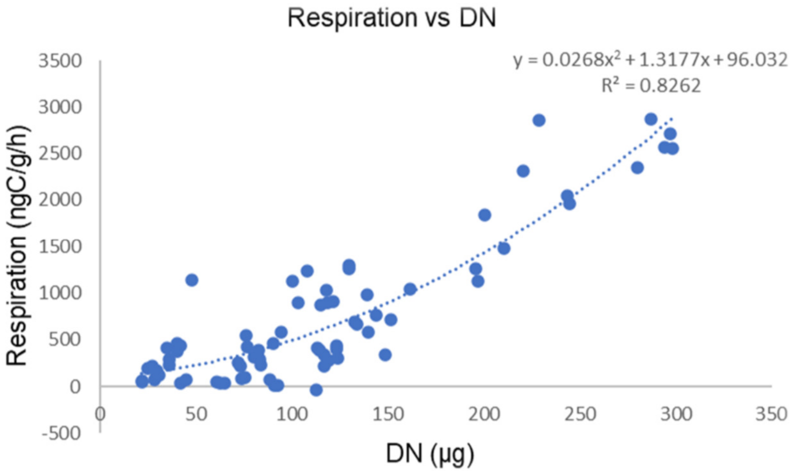


Figure 12: Correlation between respiration rates (non-normalized) and DN.

Conclusion

In this laboratory analysis we assessed for the first time the response of microbial stoichiometry and enzymatic activity to different levels of soil moisture and analyzed their relationship with soil CO_2 emissions. We hypothesized that sites with lower MAP have soil microbial communities that are more adapted to drought than wetter sites, displaying higher resistance to water stress. Our results indicate that while sites with higher MAP have a stronger reduction of respiration rates (even when normalized by SOM), the remaining relative activity at low soil moisture level is similar across sites, rejecting our initial hypothesis.

We found that communities displayed two types of strategies in nutrient acquisition at low moisture level, either by increasing or decreasing the release of enzymes, but this did not reflect changes in respiration rates, indicating that drought is an extremely strong modulator of microbial activity, despite the strategy used. We found a positive relationship between respiration rates and the available N in soil. This indicates a tight coupling between C and N cycling, and a possible role of available nitrogen in mediating drought effects on respiration rates.

Overall, our results highlight that drought sensitivity of microbial processes is fundamentally constrained by water limitation itself, rather than by site-specific climatic history, with important implications for predicting soil carbon losses in a drying world.

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