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High temperature microbial activity in upper soil layers

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Running headline: Soil thermophiles

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Abstract

Biomineralization at high temperatures in upper soil layers has been largely ignored, although desertification and global warming have led to increasing areas of soils exposed to high temperatures. Recent publications evidenced thermophilic bacteria ubiquity in soils as viable cells, their role on nutrient cycling and seedling development. High temperature events, frequently observed at medium and low latitudes, locate temporal niches for thermophiles to grow in soils. There, at temperatures inhibitory for common mesophiles, thermophilic bacteria could perform biogeochemical reactions with importance to soil food web. Nutrient cycling analyses in soils at medium and low latitudes would benefit from considering the potential role of thermophiles.

Keywords: soil thermophiles; biomineralization; C, N, and S-cycles; plant growth; global warming

The ubiquitous presence of thermophilic bacteria in soils

Microorganisms are known to control the turnover of elements through the immobilization and mineralization of nutrients, i.e. through biogeochemical reactions, (Coleman DC & Crossley, 1996; Crawford *et al*., 2005; Torsvik *et al*., 2002) but how these processes occur in nature is not fully understood. Plant growth is largely dependent on the availability of inorganic nutrients provided by biomineralization, so understanding nutrient cycling in soils under a variety of conditions is of major importance.

Current climate changes are leading to an increase of high temperature events (Stocker *et al*., 2013) and misuse of soil capabilities and poor management of vegetation can lead towards desertification and formation of arid or semi-arid soils. Soils with poor or highly reduced plant cover are exposed to intense solar radiation which leads to increasing temperatures at the upper soil layers. Altogether, these factors lead to soil temperature values well above the optimum for commonly studied mesophilic soil bacteria; values above 40ºC are frequently observed with measurements reaching 75ºC (Portillo *et al.*, 2012; Gonzalez *et al.*, 2014) and some investigators have reported temperatures higher than 90ºC in deserts (McCalley & Sparks, 2009). As a consequence, microbial activity at upper soil layers (i.e., the outermost layer of soil, usually the top 5 cm in this study) has been suggested to be highly reduced during such extreme temperature events similar to animals and plants inhabiting these soil zones (Townsend *et al.*, 1992; Conant *et al.*, 2011).

Some cases of potential activity by mesophilic bacteria have been reported at temperatures above 40ºC (Gonzalez *et al.*, 2014) or after exposure to these temperatures as a result of metabolic stimulation (Ho & Frenzel, 2012) or germination of resting cells (Whittenbury et al., 1970). In general, mesophilic bacteria undergo a decline in activity and survival period under high temperature events although other microorganisms, adapted to these high temperatures, may succeed in finding suitable temporal conditions to develop. Recent reports have highlighted the occurrence of peaks of enzymatic activity at high soil temperatures (in the 55ºC to 75ºC range) (Gonzalez *et al*., 2014) and the ubiquitous presence in all tested soils of thermophilic bacteria, specifically species belonging to *Geobacillus* and related genera (Marchant *et al*., 2002; Marchant *et al*., 2008; Portillo *et al*., 2012; Santana *et al*., 2013). These thermophiles exhibit optimal growth between 50ºC and 70ºC under laboratory conditions. In addition, these thermophilic bacteria are mostly present in temperate soils as vegetative viable cells (Marchant *et al*., 2008; Portillo *et al*., 2012) which strongly suggest that they can be potential participants of soil biogeochemical reactions (Gonzalez *et al*., 2014). Therefore, the study of these soil thermophilic bacteria and their role in soil ecosystems is an aspect deserving further consideration.

Soil thermophiles contribute to carbon, nitrogen and sulfur cycling

The largest fractions of nitrogen (N) and sulfur (S) in soils are in the form of organic matter (Anandham *et al*., 2008; Gonzalez-Prieto & Carballas, 1991; Schlesinger, 1997). The mineralization of these organic compounds contributes to CO_2 release to the atmosphere and to make inorganic N and S readily available to soil primary producers. For instance, in non-fertilized soils, organic S released as sulfate is considered the major contribution for plant S nutrition, as sulfate is the dominant form of sulfur used by plants. Organic sulfur is estimated to represent more than 90% of soil total sulfur (Anandham *et al*., 2008; Schlesinger, 1997). In spite of this large pool of organic sulfur in soils, under temperate conditions, around 20ºC, organic S biotransformation to sulfate has been reported to be very limited (Eriksen, 1996; Ghani *et al*., 1993). On the other hand, recent research has demonstrated that thermophilic gram-positive bacteria of the Firmicutes phylum, mainly the genera *Geobacillus*, *Ureibacillus* and *Brevibacillus*, were able to release significant quantities of sulfate under high temperature conditions as a product of their metabolism (Portillo *et al*., 2012; Santana *et al*., 2013). This represents a process involving a dissimilatory organic-sulfur mineralization pathway recently described (Santana *et al*., 2014) which suggests that soil thermophilic bacteria can be actively involved in C and S cycling in soil upper layers and refutes the prevailing view of bacteria as poor S-mineralizers (Eriksen, 1996).

Santana et al. (2014) explained the metabolic basis for the production of soluble sulfate by soil thermophiles within the phylum Firmicutes (Order Bacillales). The metabolism of proteinaceous compounds, and other sulfur and nitrogen containing organic molecules, by soil thermophiles results in a requirement to process the amino acids methionine and cysteine leading to sulfite synthesis and ultimately to sulfate release; sulfur and nitrogen containing organic molecules, such as humic acids, are common constituents of soils organic matter and an organic source for soil microorganisms (Sutton & Sposito, 2005). The genomes of thermophiles have revealed the presence of genes encoding for the enzymes involved in these metabolic steps leading to sulfite synthesis, while mesophilic bacteria belonging to commonly reported phyla in soils, lack the required genes (Santana *et al*., 2014). Among these genes is the one encoding sulfite oxidase, the enzyme that catalyzes sulfate synthesis in the last step of sulfur-organic dissimilation, thus determinant for sulfate production.

Simultaneously to this sulfate production during organic matter consumption, these thermophiles also release high levels of ammonium as a product of their metabolism of amino acids (Portillo *et al*., 2012; Santana *et al*., 2013; Santana *et al*., 2014). The amount of ammonium produced by these heterotrophic thermophiles is quantitatively equivalent to hyperammonium-producing mesophilic bacteria previously described (Eschenlauer *et al.* 2002; Whitehead & Cotta, 2004). These findings strongly confirm the high potential of soil thermophiles to contribute to global carbon, nitrogen and sulfur cycling under high temperature conditions, thus complementing the ecological role of mesophiles in those environments periodically exposed to high temperature. This inorganic N and S transformed at the upper soil layers can be easily transported down to deeper soil layers (Conant *et al*., 2011) and they represent a nutrient source for plants. Soil thermophilic bacteria could be regarded as adjuvants in soil fertilization; their activity has been correlated with the increment of growth in plant seedlings (Santana *et al*., 2013).

Relevance of soil thermophiles on a global warming context

Warming is known to increase microbial carbon decomposition in soils (Hopkins et al., 2014) leading to a decrease of organic matter residence time and an increase in the release of carbon dioxide from soils into the atmosphere (Conant *et al*., 2011; Davidson & Janssens, 2006; Hopkins *et al*., 2014). As well, Hopkins *et al.* (2014) observed that warming stimulated Gram-positive bacteria to better compete for supplied organic nutrients. Current lack of knowledge on this aspect could have potentially serious effects on our ability to explain or avoid the expected consequences, i.e. typically reduced organic matter content found in soils of warm climatic zones, and potential desertification processes (Conant *et al*., 2011; SoCo, 2009). As an example, almost half of European soils are included in this category (SoCo, 2009). Understanding organic matter and nutrient cycling in soils is a major keystone in soil, environment, and biogeochemical related sciences considering both local and global scales. The generally observed reduced organic matter content in soils in warm climate areas is a major concern mainly during current periods of global warming (Davidson & Janssens, 2006; SoCo, 2009), because it implies the mineralization of that soil organic carbon as carbon dioxide to the atmosphere with significant relevance on global scales (Conant *et al.*, 2011; Davidson & Janssens, 2006; SoCo, 2009). A better understanding of the role of soil thermophiles will greatly contribute to that aim.

In an attempt to comprehend the relevance of soil thermophilic Firmicutes, Gonzalez *et al*. (2014) showed the annual frequency of hot days (those reaching 30ºC or above) from a complete latitude range on Earth. The results confirm that high latitudes offer scarce relevance to the development of thermophilic bacteria, as high temperature events seldom occur and air temperatures above 30ºC are rare. An example is the English town of Cambridge (52ºN) that presents average values of just 1 hot day per year. Interestingly, medium and low latitudes, approximately 40º (both N and S) and below showed over 50 hot days per year (Fig. 1) and this number greatly increases at lower latitudes. This latitude range includes more than half of European soils and it is directly related to the reduced organic matter observed in these soils (Fig. 2). A question raises on the contribution of thermophilic soil bacteria to soil organic matter empoverishment on warm climates, and similarly, on a global warming scenario (Davidson & Janssens, 2006; Stocker *et al*., 2013). Surely, the importance of soil thermophiles gains significance at latitude environments (40º N and S and below) which represent over 60% of terrestrial environments on Earth, where high temperatures are frequently recorded (Gonzalez *et al*., 2014). At these latitudes, the presence of arid or semi-arid soils and desertification events represents a major concern for soil conservation and function. On the other hand, the role of soil thermophilic bacteria in S and N cycles, namely on sulfate and ammonium production, raises questions on their potential use, following soil priming and growing of appropriate crops and cultivars, as a way to avoid desertification. Future research on this topic, focusing on the potential for recovery of unproductive soils, must be envisioned.

Conclusion

In summary, recent findings suggest that during high temperature conditions soil thermophilic bacteria can develop and contribute to soil ecosystem functioning. In soils of medium and low latitudes, this process would occur within a temporal basis necessary to the proliferation of soil thermophilic bacterial communities. This ecological shift of responses by complex and diverse soil microbial communities contributes to complement the functional role of the microbiota in processing soil organic matter and ultimately in the cycling and mineralization of essential nutrients (C, S and N) for plant growth. The importance of these processes must be considered to understand the potential soil use, its contribution as carbon sink or link and the actual role of soil microorganisms within different environmental conditions, so that sustainable agricultural practices can be incorporated, and current estimates of the balance of elements in a global warming scenario on Earth can be adjusted.

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Figure legends

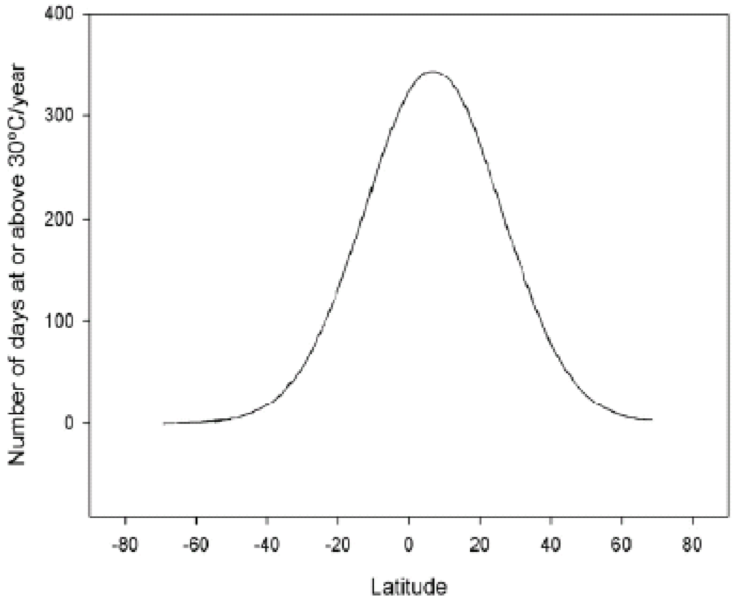

Fig. 1. Curve-fitting, Gaussian-shaped, curve of number of hot days (30ºC or above) per year along a complete set of latitudes. Negative latitudes are at the S hemisphere and positive latitudes at the N hemisphere. Extracted and modified from Gonzalez *et al.* (2014).

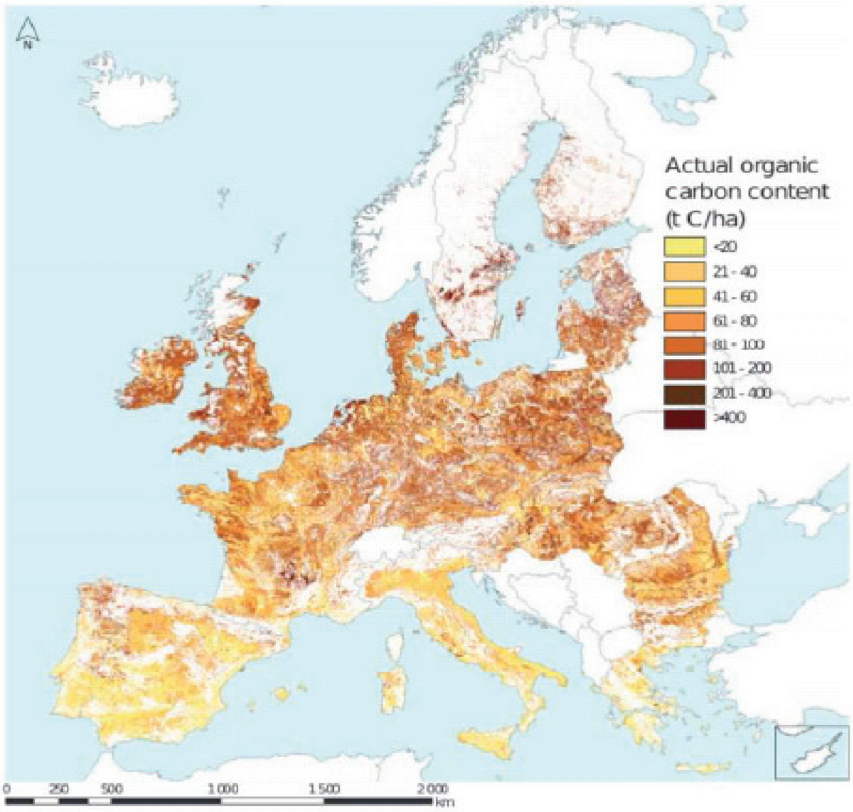

Fig. 2. Map showing the actual organic carbon content in agricultural soils for the States members of the European Union. Source: Sustainable agriculture and soil conservation (2009).