ARTICLE IN PRESS

Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

Hormone-like activity of the soil organic matter

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A R T I C L E I N F O

Keywords:
Soil organic matter
Humic substances
Hormone-like activity
Forest soils

A B S T R A C T

Over the years, the physicochemical and biological properties of humic substances (HS) continue to attract the interest of researchers. HS which are heterogeneous organic compounds, influence the biogeochemistry of organic carbon in the global ecosystem. Among the properties of HS, their biological activity is of great interest. The reported evidences showed that the effects of HS on plant growth might depend on their source, concentration, molecular size, and on compounds contained into them. It is not known whether this activity is due to the chemical structure of HS or whether it is linked to hormones of microbial origin such as indole acetic acid entrapped into them. In any case, HS exhibit stimulatory effects on plant cell growth and development.

1. Introduction

Soil organic matter (SOM) is a key component of terrestrial ecosystems and thus is an indicator of soil fertility (Seybold et al., 1998). Humic substances (HS), the largest component of SOM, can be considered the top in the stabilization of SOM and an intermediate in the mineralization of SOM to CO_2 (Ertel et al., 1988). The conservation and enhancement of SOM and HS in soils exerts beneficial effects on soil structure, compactability and water-holding capacity. Humus is defined as the total organic fraction in soils exclusive of non-decomposed plant and animal material, their partial decomposition products, and the soil biomass (Stevenson, 1994). Humic substances (e.g. humic acids and fulvic acids) make up the bulk of humus (Tan, 1998). Several researches have shown that HS influence plant growth, the root formation and root hair initiation (Nardi et al., 2009; Canellas and Olivares 2014). Also, HS interact with nutrient assimilation of both macro- and micro-elements, by increasing the nutrient use efficiency (Nardi et al., 2016). Furthermore, enzymes and biochemical route were found to be influenced by HS supplied with different extents of up- and down-regulation (Carletti et al., 2008; Aguirre et al., 2009).

Over the last 30 years Nardi's group have studied the hormone-like activity of HS derived from earthworm faeces and agrarian soils (Muscolo and Nardi, 1997; Muscolo et al., 1999, 2007; Quaggiotti et al., 2004; Nardi et al., 2007; Carletti et al., 2008; Schiavon et al., 2010) and forest soils (Concheri et al., 1996 Nardi et al., 1999; Pizzeghello et al., 2001, 2002; Sitzia et al., 2014 Pizzeghello et al., 2001, 2002, 2015; Carletti et al., 2009). These studies produced relevant results suitable to identify a possible relationship between the structure and the activity of HS. However, it is not yet clear if this activity is mainly linked to the chemical structure of HS or whether it depends on hormones of microbial origin, such as indole acetic acid, entrapped into them.

2. Hormone-like activity of HS

A putative HS hormone-like activity is not surprising as it is known that soils vary in their native auxin amount and fertile soils contain greater amounts of auxin then less fertile ones. Auxin and gibberellin levels are usually higher in the rhizosphere than in the bulk soil, probably as a consequence of increased microbial populations or of an augmented metabolism due to the presence of root exudates. Although several soil and rhizosphere microorganisms, as well as the root of higher plants, have been reported as producing auxin and gibberellins, there is little information about their stability and only indirect conclusions have been made about their presence in amounts high enough to be biologically active. In a first paper Dell'Agnola and Nardi (1987) proven that HS extracted from *Allelobophora (Einsen)* faeces were endowed with hormone like-activity and a year later, the same authors showed, in vitro, that the delivery of the hormone-like activities in HS was influenced by their particular arrangement in the humic aggregate which is influenced by the pH of the humic extract. Acidification of the humic extract elicited a high auxin-like activity, while basic or neutral conditions showed high gibberellin-like one.

Later, Muscolo et al. (1998) revealed the quantity of indoleacetic acid (IAA) in HS with high specificity monoclonal antibodies and with different anti-indoleacetic acid antibodies. The data suggested that IAA

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http://dx.doi.org/10.1016/j.apsoil.2017.04.020
Received 21 November 2016; Received in revised form 20 April 2017; Accepted 24 April 2017

Please cite this article as: Nardi, S., Applied Soil Ecology (2017), http://dx.doi.org/10.1016/j.apsoil.2017.04.020

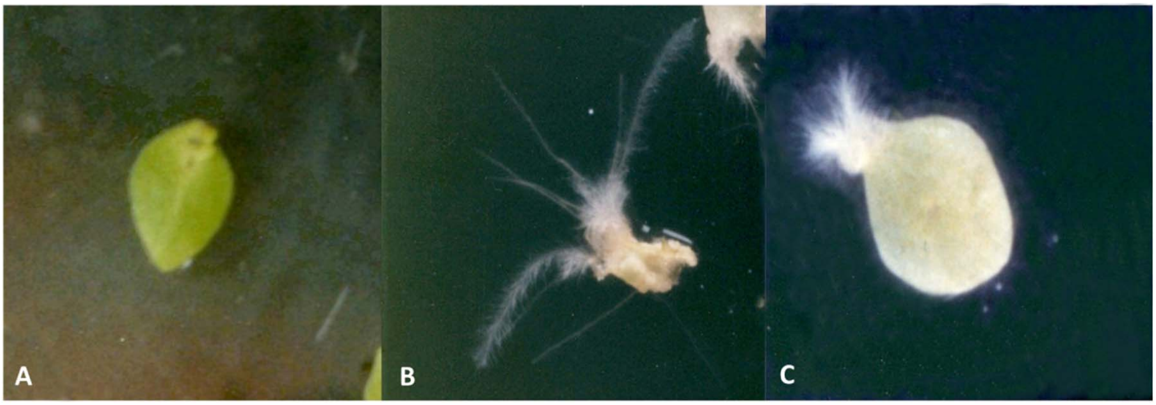

Fig. 1. Photographs of leaf explants of *Nicotiana plumbaginifolia* untreated (A), supplied with IAA (B) and humic substances (C).

was present in humic substances. The results were revealed both with high specific monoclonal antibodies and with different anti-IAA antibodies able to inhibit biological activity of humic substances. The results were confirmed also by on root growth of watercress. Both IAA and humic matter affected root inhibition significantly. The inhibition was partially removed when the antibody was added to the solutions demonstrating that the antibody was linked to IAA- or humus fraction-antigen.

In another experiment (Nardi et al., 1994), leaf explants of *Nicotiana plumbaginifolia* were compared in cultures supplemented with IAA, inhibitors of IAA (TIBA = 2,3,5-Triiodobenzoic acid and PCIB = 4-chlorophenoxy-isobutyric acid) and a humic substance (HEf) a low molecular size fraction, obtained from the faeces of *Allolobophora caliginosa* and *A. rosea*. The results show that the HEf at a concentration of 1 mg CL^{-1} causes root development from leaf explants that appears to be similar to IAA-induced activity, while the control did not develop roots (Fig. 1). Furthermore, the HEf induced longer roots than those grown in IAA with fewer hair roots. In the presence of the IAA inhibitors, the leaf explants were without roots.

Morphological changes induced by HS were also observed in carrot cells (Fig. 2) culture (Muscolo et al., 1999). The HEf was able to induce growth and shape changes similar to those induced by auxins (2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthylacetic acid (NAA), 2,4-D induced the best cell growth stimulation, while HEf mimicked 2,4-D action. The presence of IAA, NAA, or 6-benzylaminopurine (6-BAP) stimulated growth, although only to a minor extent compared to HEf. Optical microscopy revealed that the cells grown in the presence of HEf displayed an extended shape similar to those grown in IAA medium. Cells supplemented with 2,4-D and 6BAP, or with 6BAP alone, formed round clumps of cells, while cells cultured only in the presence of 2,4-D formed small round cells. Cells cultured in the presence of NAA exhibited both round clumps and a few extended cells. Cells grown in nutrient medium without hormones showed cellular detritus and a few round clumps.

Humic substances have also demonstrated gibberellin- (Nardi et al., 2000a,b; Pizzeghello et al., 2002) and cytokinin-like activities (Nardi et al., 1988; Piccolo et al., 1992), even if no significant amounts of these plant growth regulators have been found. As regard cytokinins, a recent study (Pizzeghello et al., 2013) showed in several HS (i.e., lignosulphonate-humates, leonardite humic acid, and HS from earthworm faeces) the presence of physiologically active concentrations of isopentenyladenosine.

3. Hormone-like activity of HS in forest soils

The biological activity of humic matter is known to depend on factors such as the type of the originals remains, the pathways of their decomposition, and the conditions of the synthesis of new molecular species. In forest ecosystems, these factors are magnified by the type of vegetation, mineral substrate, climate, the activity of soil organisms, and management practices.

In order to investigate the interaction between forest productivity and biological humus activities, three undisturbed forests, located in an unique climatic area but under different vegetal covers (*Abies alba*, *Fagus sylvatica* and *Abies* plus *Fagus*) were assessed (Concheri et al., 1996). The presence of two species combined induced the best basal annual increments, litter production, and humus content compared to the average overall data obtained from the monospecific cover. Besides, the hormone-like activity varied according to the sample site and the collection horizon. Within each profile, auxin- ad cytochinin-like activities were found mainly in the overlying g horizons (Ah), while gibberellin-like activity was present in the underling ones (Bwk) (Concheri et al., 1996).

In Nardi et al. (2000) studying the biochemical activities of forest humic fractions in *Pinus mugo* and *Pinus sylvestris* seedling, the authors revealed that the roots treated with humic fractions were highly autofluorescent, thus showing a high rate of differentiation compared with that of the control (Fig. 3).

In Pizzeghello et al. (2002), 32 soil horizons from a large area of northern Italy typical for silver fir (*Abies alba* Mill.) were evaluated. These soils had developed under the following five silver fir forests: montane *Fagion sylvaticae* with *Cardamino pentaphylli–Abietetum albae* (MO); high montane *Fagion sylvaticae* with *Adenostylo glabrae–Abietetum albae* (HMA), high montane *Fagion sylvaticae* with *Adenostylo glabrae–Abietetum caricetosum albae* (HMC), acidophilous *Luzulo–Fagion* with *Luzulo niveae–Abietetum* with *Calamagrostis* (AC), and *Luzulo–Fagion* with *Pyrolo–Abietetum albae* (A). The development of organic and humic matter in the five silver fir forest types was assessed by quantifying chemical and biochemical parameters. Soil pH differed in almost all silver fir types, while the soil C:N ratio and the humic carbon: organic carbon (HC:OC) ratio distinguished the montane from the acidophilus with *Calamagrostis* and from the acidophilus types. Phytohormone-like activity of HS was evaluated by measuring auxin-like (IAA-like) and gibberellin-like (GA-like) activities as well as invertase and peroxidase activities. The results showed that IAA-like, GA-like and peroxidase activities distinguished the montane from the high montane with *Adenostylo*, and from the high montane with *A. caricetosum*, while GA-like activity varied significantly between the high montane with *Adenostylo* and high montane with *A. caricetosum*. Acid conditions were essential for the release of the IAA-like activity, whereas neutral conditions promoted GA-like activity, thus confirming that a different collocation of the hormone-like activity along the profile is well in accordance with seeds germination and the first stage of seedlings growth.

4. Conclusions

The complexity of humic substances and their remarkable proper-