Lehrstuhl für Bodenökologie

The effect of plants on the composition and ecological role of the water extractable organic matter (WEOM) in soils

Junko Akagi

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Abbreviations

A	UV ubsorption at 254 nm
Abs.	Absorptivity
AD	Air dried
a.u.	Arbitrary unit
B	Bare plot
BA	"Biological" agriculture
B_{max}	Maximum binding capacity
BDOC	Biodegradability of WEOM
CA	"Conventional" agriculture
[Cu]	Concentration of copper ion
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DOM-B	DOM in the bulk soil
DOM-R	DOM in the rhizosphere
DOM-I	DOM in the micro-pore in soil
DOM-II	DOM in the meso-pore in soil
DOM-III	DOM in the macro-pore in soil
F, Flu., Fluor.	Fluorescence intensity
FE	Fluorescence Efficiency
GSF	GSF-Forschungszentrum für Umwelt und Gesundheit
HIX	Humification Index
HC	High plant coverage
IBÖ	Institut für Bodenökologie, GSF
K_d	Dissociation constant
λ_{em}	Fluorescence emission wavelength
λ_{ex}	Fluorescence excitation wavelength
MC	Medium plant coverage
n.d.	Not determined
RC	Red clover
RSF	Relative summed fluorescence
SB	Sugar beet
SF	Summed fluorescence

SOC	Soil organic carbon
SOM	Soil organic matter
UV	Ultraviolet
WEOC	Water extractable organic carbon from a pre-incubated soil
WEOCa	Water extractable organic carbon from an air-dried soil
WEOM	Water extractable organic matter from a pre-incubated soil
WEOMa	Water extractable organic matter from an air-dried soil
WHC	Water holding capacity
WW	Winter wheat
(+)Gly	Glyphosate applied lysimeter
(-)Gly	Glyphosate non-applied lysimeter
(+)O_3	Ozone fumigated lysimeter
(-)O_3	Ozone non-fumigated lysimeter

Definitions of terminology

Site	Geographical location (Catena or experimental farm)
Location	A position on a site
Plot	A location with a specific treatment

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I. Introduction

Dissolved organic matter (DOM) in soil is organic matter, which is present in soil solution. Although DOM is only a small part of the soil organic matter (SOM), it is important because through its mobility it plays various ecological roles (Zsolnay, 1996; 2003; Kalbitz et al., 2000b). For example, DOM controls the mobility and bioavailability of soil contaminants through directly interacting with them. Furthermore, DOM itself is also the substrate for soil microbes, which directly influences the C-balance between the geosphere and the atmosphere. In which ecological processes DOM participates is dependent on its location, how much of it is available, and also on its quality. Many factors control the quantity and quality of DOM (*cf*. section II). Soil types and conditions, soil hydrology, microbial activity, climate, land use and management, vegetation, *etc*. all seem to have an effect on DOM properties. Since these factors do not function independently but are all intertwined, it is difficult to say which one is the chief controlling factor of DOM. Therefore, in order to understand how DOM functions, we need to know more about its sources and sinks. This dissertation will concern itself with one of the prime sources of DOM in soil: vegetation.

Vegetation is one of the major factors controlling the quantity, quality, and functions of DOM (Zsolnay, 1996; Campbell et al., 1999a; 1999b; Chantigny, 2003), since it is the prime source of both water soluble and insoluble SOM. Vegetation, which has an effect not only through litter, but also through rhizoexudation can contribute directly to the DOM pool (Jones, 1998; Dakora and Phillips, 2002). Despite this, the effects of plants, especially over an entire growing season, are still largely unknown, especially in arable fields (Kalbitz et al., 2000b; Chantigny, 2003).

The main focus in this dissertation is to intensify our knowledge of DOM production and alteration through vegetation and to investigate the ecological implications of this both in regards to DOM's potential interacting ability with a contaminant (*i.e*., copper) and with its substrate availability for microbial processes. The following effects on quantity, quality, and functions of DOM have been investigated with DOM extracted from soil samples, which were taken in different environments such as agricultural sites in Germany, Italy, and Spain; lysimeters in Germany; as well as natural catenae in Spain. This pan-European sampling approach may provide information on the common vegetation effects, which may be observed everywhere in the world. The following effects on quantity, quality, and functions of DOM will be analyzed here.

1. Effect of vegetation:
    a. Overall effect of vegetation,
    b. Seasonal variation of the vegetation effect,
    c. Detailed temporal variation of the vegetation effect,
    d. Effect of different plant types.
2. Effect of agricultural practices:
    a. Monoculture and crop rotation,
    b. Different kinds of fertilizer application (biological agriculture with organic manure vs. conventional agriculture with mineral fertilizer).
3. Effect of stress to plants:
    a. Herbicide application,
    b. Ozone fumigation.

A part of my research was dedicated to finding out the best way of extracting DOM in such a way that it reflects the potential *in situ* state of DOM. Based on the results of this, DOM was extracted with an aqueous solution (10m*M* $CaCl_2$). Therefore, this fraction should be more precisely called water extractable organic matter (WEOM). WEOM was considered as the best estimate for the *in situ* DOM state, because it is composed of all of DOM-III (DOM in macro-pores), most of DOM-II (DOM in meso-pores), and a portion of DOM-I (DOM in micro-pores) (Zsolnay, 1996). In this dissertation DOM and WEOM will usually be used interchangeably. The latter term is more precise, but the former one is by far more ubiquitous. It must also be kept in mind that almost all soil fractions are artificially, that is to say experimentally, defined. For example soil aggregates are defined by their size in the laboratory after they have been removed from the soil matrix. By the same token humic and fulvic acids as well as humin are defined by their ability to be extracted with specific solvents (*e.g.*, NaOH, $Na_4P_2O_7$). These solvents break bounds, which are normally stable *in situ*. Nevertheless research on these humic substances has provided many insights in regards to potential ecological functions of SOM.

The characterization of WEOM was done by measuring its:

- organic carbon content,
- ability to adsorb UV light,
- ability to fluorescence and the nature of its emission spectra.

Furthermore, two well established and important DOM functions were also determined:

- ability to interact with a heavy metal,
- ability to function as a microbial substrate.

UV and fluorescence spectrophotometers have a great advantage in that they can measure small concentrations of WEOM quickly and precisely without any major modification. Also through

WEOM´s optical properties, one can track very well the changes in its quality after it has been microbial modified (Kalbitz et al., 2003a; 2003b; 2003c). The major disadvantage is that only optically active DOM can be determined. For example with the exception of three amino acids, this class of compounds does not fluoresce. Neither do simple carbohydrates or fatty acids. Nevertheless, for a study of such a broad scope as was done here, it was felt that the advantages of optically based characterizations outweighed the disadvantages. The degree of biodegradability and interaction with Cu also gives insight into the quality of WEOM.

The relationships between vegetation and WEOM/DOM can be visualized with a conceptual model (Figure 1). The prime purpose of this research is to validate this model and to clarify the relative importance of fluxes presented by the circled numbers in the figure. This will be discussed in detail in section VII.

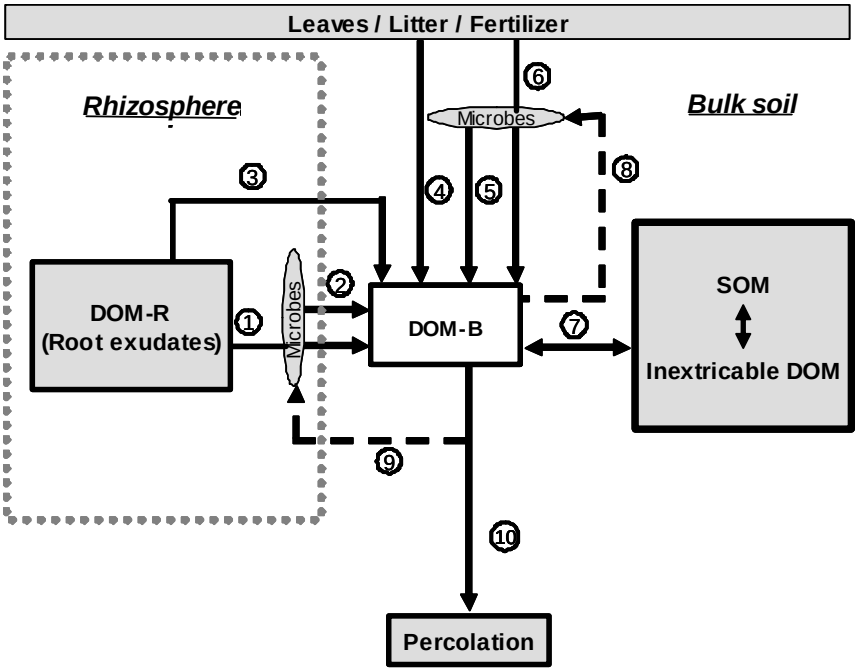

Figure 1 A conceptual model of the impact of vegetation on DOM. DOM-B is bulk soil DOM and is practically equivalent to WEOM. SOM is the soil organic matter imbedded in the soil's matrix. The dashed pathways are not components of this dissertation. DOM losses to the atmosphere are not considered.

II. State of knowledge

1. Dissolved organic matter (DOM)

Dissolved organic matter (DOM) is the organic matter, which is present in soil solution. All organic matter, which is dissolved in water *in situ*, is DOM. Therefore, DOM is a heterogeneous mixture of macromolecules and simple compounds with different structures. These structures reflect the various degradation stages of the soluble organic matter and the different sources of DOM (Stevenson, 1982; Thurman, 1985). It has been postulated that the composition of DOM is highly dependent on its location within the soil matrix (Zsolnay, 1996). This is discussed in more detail in section II 2. Obviously DOM from lysed microbial cells or rhizoexudates will have a different composition (*e.g.*, Zsolnay et al., 1999), especially under drying stress (R. Ruser, A. Embacher, Á. Zsolnay, personal communication), than the DOM originating from the abiotic soil matrix. Therefore DOM obtained from dry soils will consist of material, which differs from its moist soil counterpart.

Furthermore, detailed chemical characterization of DOM in soils is hampered by the fact that DOM amounts in soil are quite low and large quantities of soil are difficult to extract. Therefore, much of our postulated soil DOM composition has been extrapolated from hydrosphere research (Thurman, 1985). The above combined with the lack of standard methods for sampling DOM (Zsolnay, 2003) and with DOM's potential intrinsic temporal and spatial variability (Zeller, 2005) have generated a feeling that DOM remains among the most elusive soil organic matter fractions.

2. Location of DOM in soils

DOM exists wherever there is water. Compared to saturated systems (*e.g.*, lakes, rivers, oceans), it is much more complicated to state where DOM is located in unsaturated systems such as soil. Soil has pore spaces, which have different sizes. These pores have been classified, depending on their size, into macro-, meso-, and micro-pores.

Zsolnay (1996) postulated that DOM has different attributes depending on where it is. He classified DOM into DOM-I, DOM-II, and DOM-III (Table 1). DOM-I exists in the smallest pores (micro-pores). This fraction is well protected against the attack from bacteria and plants. It becomes available only through the diffusion process because high pressure such as over 1500 kPa is needed to remove water out of the micro-pores. Therefore, it must be metabolized abiotically or possibly by exoenzymes (Burns, 1990; Asmar et al., 1994). DOM-III, which exists in the largest pores (macro-pores), is mobile and readily available because the turnover of water in this macro-pore space is smooth and rapid. This fraction can also be called mobile organic

matter (MOM). DOM-II is the material in the meso-pores and has attributes somewhat between DOM-I and DOM-III. It is, presumably, metabolized chiefly by the microheterotrophs. The bacterial metabolism of DOM-II may be different from that of DOM-III, since in the meso-pores the bacteria themselves are fairly well protected from predation, and the water content tends to vary considerably less than in the macropores.

Table 1 Attributes of the different classes of dissolved organic matter (DOM) (After Zsolnay, 1996)

	DOM-I	DOM-II	DOM-III
Pore size (µm)	<0.2	0.2 to 6	>6
Water ´type´	cohesive/adhesive	cohesive	gravitational/cohesive
Water tension (kPa)	<-1500	-1500 to -50	-50
% Water at WHC[a]	~30	~50	~20
Transport mechanism	diffusion	diffusion>convection	convection>diffusion
Metabolism	abiotic, exoenzymes	microbial	biotic
Relative turnover	slow	moderate	rapid
Effect of drought	weak	moderate	strong

[a]Water holding capacity. The values in this row are extremely approximate.

3. Ecological functions of DOM

Even though DOM is only a small organic pool in soils (*ca.* 0.05%, Zsolnay, 1996) compared to SOM (*ca*. 5.0%), it is the most important fraction of soil organic matter, because it can participate in various ecological roles because of its mobility. It is the link between the unsaturated and saturated zones as well as between the non-biosphere and biosphere (Zsolnay, 1996; 2003; Kalbitz et al., 2000b). The known functions of DOM in soil are described here.

3.1 DOM as a substrate for soil microbes

Little is known about the overall composition of DOM, but it is generally assumed that the labile DOM consists mainly of simple carbohydrate monomers, low molecular organic acids, amino acids, amino sugars, and low molecular weight proteins, which are easily water soluble and biodegradable (Lynch, 1982; Qualls and Haines, 1992; Guggenberger et al., 1994; Küsel and Drake, 1999; Kaiser et al., 2001a; Koivula and Hänninen, 2001). Soil microorganisms are basically aquatic and all microbial uptake mechanisms require a water environment (Metting, 1993). Microbes in soil produce atmospheric CO_2, since they mineralize the DOM in soil solution. This means that the production of DOM *in situ* is the key point for understanding the C-balance in terrestrial systems, because the more substrate that is present in soil solution, the more C can be moved from the geosphere into the atmosphere through microbial activity. Also the consumption of DOM can strongly control the redox conditions of soils. Anoxic pockets or micro-sites can be created, which in turn may result in the production and release of

environmentally important gases such as nitrous oxide and methane. DOM, most likely, is also the electron donor when one is required (Zsolnay, 1996).

The amount of DOM, which is biodegradable by soil microbes, is not constant. It varies with soil depth, land use, and soil condition (Boyer and Groffman, 1996; Lundquist et al., 1999; Merckx et al., 2001). DOM quality, and as a result its suitability as a substrate, may also be influenced by environmental stress such as by dry-wet and freeze-thaw cycles. The biodegradability of DOM depends not only on its location in the soil matrix but is also a function of its chemical structure (Marschner and Kalbitz, 2003), which in turn may be a function of its origin. The freshly released DOM from plant roots, leaf litters and from microbes, for example, is known to be easily biodegradable; while DOM released from the soil's abiotic fraction is more refractory (Hongve et al., 2000).

3.2 DOM as a controller of the mobility of metal ions in soils

DOM is known to interact with metal ions in soils (Stevenson, 1982). Metal ions, which are bound to the soil matrix, become mobile not only in consequence of changes in soil solution chemistry, but also through interaction with DOM (Martinez and Motto, 2000). When there is no significant change in solution conditions, DOM must be the chief controller of the fate of metal ions in soils. DOM mobilizes metal ions through interacting with them directly (*e.g.*, Cu) or indirectly by competing with them for binding sites on the soil matrix (*e.g.*, Cs, Staunton et al., 2002). As a result in regards to metal chemistry, the following processes may be caused by DOM.

3.2.1 DOM makes micronutrients more available for plants

The presence of micronutrients (*e.g.*, Fe, Cu, Mg, *etc*.) is essential for plant growth (Dakora and Phillips, 2002). Because the uptake of such elements by the plant roots is restricted to the liquid phase, the content of metals in the solution is of primary importance (Brümmer et al., 1986). If plants can not grow well due to the lack of micronutrients, this limitation results in the lowering of the organic matter, which can be produced and introduced into the soil. Therefore DOM, which can make micronutrients more available for plant roots, becomes less available. However, the DOM, which is involved in this role, may be modified when it is released as the root exudate from the stressed plants. For example, some species excrete root exudates composed of organic acid anions in response to P and Fe deficiency or of phytosiderophores to respond to Fe and Zn deficiency (Haynes, 1990; Jones and Darrah, 1994). Because of this, the released DOM can cause some nutrient elements to be relatively more available for uptake by plants.

3.2.2 DOM controls the level of toxicity of heavy metals in soils

A small amount of metals as micronutrients in soil is essential for the plant growth. However, once specific concentrations are exceeded, they have a negative influence on both microbial activity and plant growth (He et al., 2005). Their bioavailability and toxicity are dependent on their chemical and physical forms (Luoma, 1983), and on the condition and composition of the soil solution (Brümmer et al., 1986). DOM is known to make organo-metal complexes with heavy metals and thus control their mobility, toxicity, and bioavailability (Brümmer et al., 1986; Ma et al., 1999). The degree of interaction and the change in both metal toxicity and availability is dependent on the quality of DOM (Inaba and Takenaka, 2005). Although not "soil studies", much toxicological research in the hydrosphere indicates that Cu-binding of DOM may vary according to the DOM source (Luider et al., 2004; Ryan et al., 2004), and this is presumably also applicable to the soil environment.

3.2.3 DOM leaches metal ions into the hydrosphere and deteriorates water quality

Increase in DOM concentrations in surface and ground waters causes deterioration in water quality by potentially bringing pollutants such as heavy metals into the hydrosphere (Land Ocean Interaction Study (LOIS), 1999). DOM influences water quality not only through co-transporting pollutants, but also *per se*, since it can color water and absorb UV radiation (Engelhaupt et al., 2003; Findlay et al., 2003; Houser et al., 2003). In many regions of Europe, DOM concentrations in surface waters are increasing (Evans and Monteith, 2001; Freeman et al., 2001), and the increased DOM, because of its color, has to be removed at considerable expense to achieve drinking water standards. This increased DOM may cause a problem also in water treatment, because it can react with chlorine and light during chlorination and produce carcinogen chloroform and other halogenated organics (Rook, 1976), which are known to be toxic.

3.2.4 DOM leaches metals out of soil matrix and contributes to pedogenesis

It is generally found that the concentrations of metal ions in a soil solution increases with increasing DOM concentration (excluding acid soils), because the metal ions in these soils are expected to be mainly present as metal-DOM complexes (*e.g.*, Cu, Brümmer et al., 1986). Such interaction plays a critical role in soil weathering (Hongve et al., 2000). DOM is therefore considered to play an important role in podzolization, even though the exact mechanisms governing the mobility of Al, Fe, and organic matter are still not clear (Jansen et al., 2005). Recent reports maintain that low molecular weight organic acids play important role in this (Lundström, 1993; Jansen et al., 2004, 2005).

3.3 DOM as a controller of soil aggregate stability

Soil clay dispersion and aggregation processes in soil are of tremendous importance from an agricultural and environmental point of view. Dispersed clay colloids result in undesirable soil physical properties, such as surface crust formation, pore clogging, and slow water penetration and can also be potential water pollutants. Soil aggregation results from flocculation, cementation, and the rearrangement of particles (Duiker et al., 2003).

Aggregation is mediated by soil organic carbon, biota, ionic bridging, clay minerals, and carbonates. The effect of DOM on the stability of soil aggregates is not well understood. DOM can be considered both as an aggregate stabilizer (Cheshire et al., 1983, 1984; Chaney and Swift, 1984, 1986; Piccolo and Mbagwu, 1989) and as a dispersing agent (Gupta et al., 1984; Visser and Caillier, 1988). It appears that DOM plays a role as a dispersing agent when it co-exists with monovalent cations (*e.g.*, Na, K), while it is a stabilizer when it co-exists with polyvalent cations (*e.g.*, Al, Fe, Ca, and Mg, Gu and Doner, 1993). The quality of DOM appears to be important. DOM, which is composed of humic acids (Chaney and Swift, 1984; Mbagwu and Piccolo, 1989), polysaccharides (Cheshire et al., 1983, 1984), or readily metabolisable chemicals (Tisdall and Oades, 1982), has been reported as an aggregate stabilizer. Since the above processes can control a soil's hydrology, they can be an important factor in desertification and land remediation.

3.4 DOM as a co-transporter of hydrophobic contaminants

DOM controls the toxicity and availability of hydrophobic contaminants such as some pesticides and especially polyaromatic hydrocarbons (PAH) when it interacts with them (Chiou et al., 1986; Steinberg et al., 2000; Akkanen et al., 2001). Association of PAH with DOM considerably increases their solubility and results in their facilitated transport (Chiou et al., 1986; Fang et al., 1998; Sabbah et al., 2004). This association with DOM is probably due to a partition-like interaction of the solute with the microscopic intramolecular DOM environment, which may consist of micelle like structures (Chien et al., 1997; Nanny et al., 1997; Ragle et al., 1997). DOM that has more complicated molecules (*e.g.*, humic acids) can interact better with PAH and can lower the bioavailability of PAH and detoxify them. On the other hand, low molecular DOM seems to enhance the bioavailability of PAH (Muir et al., 1994; Traina et al., 1996), even though this may be a misinterpretation of the experimental results (Haitzer et al., 2001). The degree of interaction seems to be dependent on the quality of DOM, but concentration may be a more important factor in this regards (Persson et al., 2003). Since DOM is an aggregate of both high and low molecular organic matter, the changes caused by DOM on the toxicity and bioavailability of PAH in nature may be hard to observe. Also, the solution conditions such as pH, metal ion concentration, and ionic strength of the medium are known to affect DOM-

pollutant interactions (Carter and Suffet, 1982; Lee and Farmer, 1989; Chien et al., 1997; Döring and Marschner, 1998).

The functions listed above are summarized in Figure 2. In which ecological process DOM is involved, is a function of its quantity and quality, which in turn can be a product of its source. Therefore, it is of considerable importance to understand the influence that DOM sources can have on DOM composition, since this will help improve our understanding of DOM functions as well.

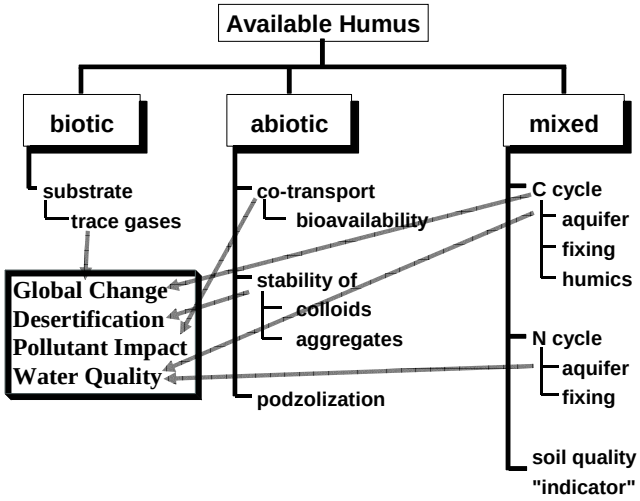

Figure 2 Some ecological roles of DOM (modified from Zsolnay (1996))

4. Sources of DOM

The primary source of DOM must be plants. Vegetation type and the amount of organic matter returned to the soil are major factors in determining the amount and composition of DOM in soils (Meyer and Tate, 1983; Saviozzi et al., 1994; Delprat et al., 1997; Campbell et al., 1999a, 1999b; Qualls et al., 2000). The major sources of DOM are presented and illustrated in Figure 3.

4.1 DOM from living plants

Plants exude DOM from their roots. The root exudates can be significant component of *in situ* DOM (Yano et al., 2000). Plant root exudates consist of a complex mixture, which can contain mucilage, root border cells, extracellular enzymes, simple and complex sugars, phenols, amino acids, vitamins, organic acids, nitrogenous molecules such as purines and nucleosides as well as inorganic or gaseous molecules such HCO_3^-, OH^-, H^+, CO_2, and H_2 (Rovira, 1969; Uren

and Reisenacher, 1988; Marschner, 1995). The exuding pattern of plants is dependent on the age and type of plants (Barber and Martin, 1976; Johansson, 1992; Xu and Juma, 1993), the nutritional status of the plants (Haynes, 1990; Jones and Darrah, 1994), the condition of nutrient availability (Marschner, 1995), and soil conditions such as pH and temperature (Ochs et al., 1993). Also, plants excrete more organic materials when they are under stress (Hale and Moore, 1979). Such low molecular weight organic matter, however, is known to have a significantly short mean residence time (Van Hees et al., 2005), therefore DOM in the non-rhizosphere (bulk) soil, which is commonly researched, may rarely contain original, freshly released exudates but rather microbial processed, relatively refractory organic matter, which nevertheless can still be DOM.

4.2 DOM from plant litter

Not only living plants release DOM, but also fallen leaves, branches, and dead roots can be a source of DOM. In forests, the primary source of DOM is considered to be the leaching of substances from fresh litter and the products of plant residue decomposition (Qualls et al., 1991). In this regards, the type of plant appears to be important. For example, water-soluble substances are more easily leached from the leaf litter of deciduous species than from coniferous species (Nykvist, 1963; Harris and Safford, 1996; Hongve, 1999), and coniferous trees accumulate more carbon in the forest floor as compared to deciduous trees (Vesterdal and Raulund-Rasmussen, 1998). Such difference among tree species can affect the microbial community (Bauhus et al., 1998; Priha and Smolander, 1999; Côté et al., 2000; Priha et al., 2001). The difference in tree species, however, does not appear to have an influence on DOM composition and chemical activity in soil solution (Strobel, 2001; Smolander and Kitunen, 2002).

4.3 DOM from soil microbes

Soil microbes play a role as modifiers of DOM. They take simple molecules, such as amino acids and sugars, and then turn them into more complicated molecules, which have a higher aromaticity or concentration of chromophores per organic carbon (Kalbitz et al., 2003c). Ogawa et al. (2001) have also reported that soil microbes modify simple organic compounds (*e.g.*, glucose) to refractory ones, which persisted for up to a year in an aquatic system. Their activity can be influenced by climate conditions. Warmer temperatures result in higher respiration and biological activity in soil, while lower temperatures result in higher standing stock of SOC, because lower temperature makes SOC less available than warm and dry soil (Franzluebbers et al., 2001).

Soil microbes release DOM when they are under the stressed condition. Therefore, they must also be regarded as one of the sources of DOM. It has been shown that exo-polysaccharides

are commonly released by bacteria in soil, especially when under drying stress (Roberson et al., 1993). Their cells can be also a direct DOM source, when their cell wall and/or membranes are torn through dry-rewetting process and freeze-thaw cycles (Zsolnay, 1997; Zsolnay et al., 1999). Soil microbes are more abundant in areas influenced by plants such as the rhizosphere. This means that vegetation through its associated microbial community is an indirect source of DOM in soil. However during periods of stress their associated microbiota can be a DOM source.

4.4 DOM from SOM

The quantity of DOM in soil appears to have a correlation with the quantity of SOM (Saviozzi et al., 1994; Delprat et al., 1997; Gregorich et al., 2000). Therefore, it is reasonable that vegetation, which is a source for SOM, is also the indirect source of DOM. SOM exists in the pore spaces in soil and also is bound to the soil matrix. SOM is composed of various organic substances such as carbohydrates, polysaccharides, phenols, lignin, lipids, and aged humus (Stevenson, 1982). DOM derived from SOM should increase or decrease as the soil environment is changed. This can be caused by a change in water content through wetting and drought, by disturbances such as tillage, removal of plants, or by a freeze/thaw cycle. Agricultural managements such as fertilizer application can also result in changes in pH and in the ionic strength of the soil solution.

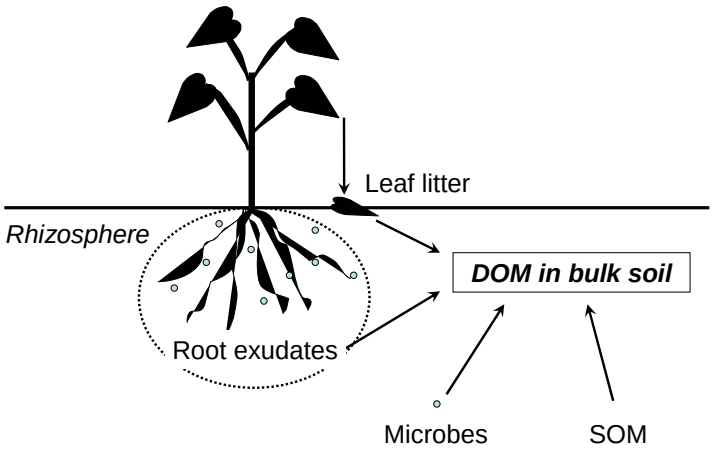

Figure 3 Major sources of DOM in bulk soil

5. Sinks of DOM

The released DOM from the sources listed above is not stable and is exposed to various fates. For example:

1. SOM and also DOM originating from SOM through fertilizer application, living roots, and fresh litter may be quickly metabolized and mineralized (Van Hees et al., 2005), because such DOM can be rich in nutrient elements (*e.g.*, N, P, S). The microbial metabolized DOM can turn into CO_2 and be released into the atmosphere, or it can remain *in situ* as more humified DOM, which is more refractory than the original (Don and Kalbitz, 2005). Such refractory DOM remains *in situ* longer than the original, because it becomes less mobile and can have a stronger affinity to the SOM of the soil matrix (Kalbitz et al., 2003c). As a result it may become adsorbed and immobilized as a part of SOM.
2. Substrate DOM may become part of the biomass. This however can not be considered to be a true sink, because such substrate DOM has a relatively fast turn-over (Jones and Kielland, 2002). Nevertheless, logically more substrate DOM is stored within the biomass, when the biomass increases. Generally speaking, more biomass is present when more vegetation is present, because of an increase in substrate through rhizoexudation.
3. A portion of soil DOM can be transported either horizontally or vertically into the groundwater or surface hydrosphere. The degree of this translocation of DOM from soil into the hydrosphere is dependent on its quality, soil conditions, and of course water availability such as rainfall. Small size DOM should be more mobile than large size DOM. (Corvasce et al., 2006). The soil structure such as its pore size distribution is also important, since DOM in the macropores (DOM-III) can be easily flushed with water. Also, if a vertical “highway” of water flow through a soil exists, DOM in the surface layer can be carried into the deeper layers very rapidly (preferential flow) (Aeby et al., 2001; Bundt et al., 2001). This percolated DOM continues to exist as DOM but once it reaches the saturated zone, it can no longer be considered to be soil DOM.
4. Some of the DOM is composed of material, which is relatively volatile such as the small fatty acids (Jandl et al., 2004) and can escape to the atmosphere.

The sinks of DOM are dependent on the quality of DOM. On the other hand, its quality can also be controlled by the sinks of, for example, microbial modification and adsorption.

6. Factors having an influence on DOM

The presence of plants, types of vegetation (*e.g.*, trees or grasses), and their growing states are highly dependent on environmental conditions. Both sources and sinks of DOM are controlled by the other various factors. The known factors are described here.

6.1. Influence of water - drying/rewetting and freeze/thaw cycles

An obvious effect of water on the dissociation of immobilized SOM is observed in drying/rewetting cycles. Rewetting soils after a dry spell is known to result in more soluble organic matter. Such DOM is from the protective biofilms released by microorganisms during drying (Roberson et al., 1993) and from the material released by cell lysis. Furthermore rewetting (slaking) can release microbial organic matter, which had been sequestered in the soil matrix (Lundquist et al., 1999; Gregorich et al., 2000; John et al., 2003). This is also suggested by the fact that the DOM released by rewetting has a high proportion of hydrophilic bases and neutrals (Christ and David, 1996), is easily biodegradable (Merckx et al., 2001), and has a fluorescence emission spectrum, which is indicative of cell lysis (Zsolnay et al., 1999). It is also enriched in carbohydrates (R. Ruser, Á. Zsolnay, personal communication).

Freeze/thaw cycles also cause a disruption, which can release additional DOM. The amount released is both a function of water content and soil texture (Zsolnay, 1997). This is reasonable in that higher moisture content buffers the speed of freezing, giving microorganisms more time to react. However, higher moisture contents result in a greater expansion of ice and the resulting disruption of pore space structure. This pore space structure is, of course, a function of the soil's texture.

6.2. Influence of land use change

Soil disruption is also caused by the change in land use, which is often associated with clear-cutting of forests and turning grassland into arable soil or *vice versa*. Following clear-cutting, an increase in the amount of DOM has been observed (Hughes et al., 1990; Delprat et al., 1997; Qualls et al., 2000). The increase is caused by soil disturbance, water flux increase, accumulation of decaying wood debris, or stimulation of microbial activity. This state, however, does not seem to last long. Estimates range from less than 2 years (Meyer and Tate, 1983) to at most up to 10 years (Moore, 1989). A progressive decline in the amount of DOM has been reported a few years after clear cutting because of the stabilization of the remaining organic matter (Delprat et al., 1997) and because of the lower organic input to the soil (Meyer and Tate, 1983; Qualls et al., 2000). The soluble material mobilized by clear cutting is mostly composed of medium to large humic acids, colloids, and organo-metal complexes (Hughes et al., 1990; Delprat et al., 1997). However, these materials are gradually replaced by smaller substances when the previous forest is cultivated to maize (Delprat et al., 1997). It has also been published that afforestation stimulated the production of new larger organo-metal complexes (Hughes et al., 1990; Quideau and Bockheim, 1997).

Following grassland to arable cropping, the DOM content decreases, and it appears to become less pronounced as the number of years under arable cropping increases (Gregorich et al., 2000; Haynes, 2000), apparently due to a gradual depletion in the SOM (Saviozzi et al., 1994). Arable soil compared to grassland also contain less water extractable carbohydrates and amino N-compounds (Deluca and Keeney, 1993, 1994). Even though many reports suggest that agriculture decreases DOM, an increase in DOM was observed in crop rotations after a number of years (Campbell et al., 1999a; Haynes, 2000). When an arable soil has turned into a grassland, the size of DOM increases (Von Lützow et al., 2002).

6.3. Influence of agricultural practices

6.3.1. Tillage

Soil disruption caused by tillage changes the quality and quantity of DOM. Soil tillage lower WEOM content (Linn and Doran, 1984), and an increase in tillage intensity can alter soil WEOC composition (Leinweber et al., 2001). Increased tillage intensity furthermore enhanced oxidative microbial activity (Leinweber et al., 2001), made DOM more easily biodegradable (Boyer and Groffman, 1996), and resulted in the rapid recycling of nutrients, crusting, and reduction of water and air availability to roots (Wardle et al., 1999). This in turn can affect DOM. Larger molecules and organo-metal complexes are more abundant in DOM from forest floors (Hughes et al., 1990; Strobel et al., 1999), while agricultural soils exposed to more disruption contain a greater proportion of smaller molecules, such as fulvic and hydrophilic acids, carbohydrates, and amino acids (Delprat et al., 1997; Leinweber et al., 2001). The frequent disturbances in arable soils also prevents the formation of large organo-metal complexes (Delprat et al., 1997).

6.3.2. Inorganic amendments

Inorganic fertilizers may influence DOM/WEOM content. However, this increase does not seem to be long-lasting. Significant increases of DOM concentration have been observed immediately after the application of the urea-based and ammonium-based fertilizers due to an increase in the soil pH (Myers and Thien, 1988; Liu et al., 1995; Hartikainen and Yli-Halla, 1996). Such solubilized DOM, however, is readily biodegradable (Norman et al., 1987, 1988; Yano et al., 2000). Over the long term, reported inorganic nitrogen applications has not been found to significantly influence the amount of DOM in forests (Gundersen et al., 1998; Yano et al., 2000) or in agricultural soils (Zsolnay and Görlitz, 1994).

Liming causes a change in DOM concentration. The increase is caused by an elevation of organic matter solubility (Murayama and Ikono, 1975; Andersson et al., 1994; Erich and Trusty,

1997), enhanced microbial activity and production of soluble molecules (Guggenberger et al., 1994), and by the displacement of previously adsorbed DOM by other mobilized anions (Kalbitz et al., 2000a). On the other hand, a DOM/WEOM decrease can result because of increased microbial consumption (Andersson et al., 1994; Karlik, 1995; Andersson, 1999) and by DOM flocculation or adsorption through cation bridges resulting from higher Ca^{2+} concentrations (Römkens and Dolfing, 1998). All the above mechanisms can work simultaneously after liming. Therefore, the net effect is sometimes not clearly observed (Cronan et al., 1992; Smolander et al., 1995; Ponette et al., 1996). Liming also has been found to affect the composition of DOM/WEOM by increasing the proportion of hydrophobic acids (Andersson et al., 2000), humic acids (Cronan et al., 1992), and carboxylic groups (Karlik, 1995) in DOM, and by precipitating high-molecular-weight DOM with Ca^{2+} (Römkens and Dolfing, 1998), while releasing smaller molecules less complexed with metals (Erich and Trusty, 1997).

6.3.3. Organic amendments

Organic amendments, such as crop residues (McCarty and Bremner, 1992; Jensen et al., 1997; Franchini et al., 2001), animal manure (Kirchmann and Lundvall, 1993; Zsolnay and Görlitz, 1994; Gregorich et al., 1998; Rochette and Gregorich, 1998; Martín-Olmedo and Rees, 1999; Chantigny et al., 2002), and industrial wastes (Gigliotti et al., 1997; Chantigny et al., 2000), always induce an increase in DOM. DOM already present in organic amendments is highly biodegradable and rapidly consumed by microbes, which may result in transient increases in soil DOM content. However, by changing soil properties such as pH, animal manure may result in the improved solubility of indigenous DOM (Bol et al., 1999; Shand et al., 2000). The composition of DOM is also influenced by the type of amendment (Ohno and Crannell, 1996). Even where soil DOM content remains stable for a long period of time after amendment, its composition gradually changes, and plant-derived molecules are mostly replaced by microbial metabolites (Chantigny et al., 2000).

6.4. Influence of stress factors for plants

There are a number of factors, which have an influence on plant growth. If the plant activity is stressed by those factors, the properties of DOM *in situ* can be also influenced. Not only the natural factors (*e.g.*, climate), but also the anthropogenic ones can have an influence on plant growth. For example, the application of herbicides controls the total vegetal input, which decreases DOM quantity. A herbicide can also change DOM quality and functions through directly interacting with it (Ertunç et al., 2002). In many urban area and in the industrialized regions, the increase in ozone in the troposphere has resulted in the wide spread occurrence of visible plant damage throughout Europe (Benton et al., 2000). The direct negative effects of

ozone on photosynthetic C fixation, which leads to productivity losses, have also been reported (*e.g.*, Lehnherr et al., 1997). Due to this negative influence on plants, the quantity as well as the quality of DOM *in situ* may also be affected through the fact that the plant activity has been changed.

Many factors listed above have an effect not only on the vegetal and DOM properties, but also on the function and composition of the soil biota (Filser et al., 2002) and on the stability of soil humus and aggregates (Bronick and Lal, 2005). Since the latter effects can also alter the activity and the growth of plants; it is difficult to observe pure vegetation effects on DOM. Furthermore, these factors lead to the spatial and temporal variation of DOM quantity and quality. This all makes DOM the least understood and most difficult to investigate organic matter pool in soils.

7. Methods for characterization of DOM

7.1 DOM extraction from soils

DOM does not have a representative structure, because it is composed of a mixture of organic substances, which are under different stages of degradation and vary over time. Also, there is no way to extract DOM, which is identical to the DOM *in situ*, because no method can quantitatively extract DOM from all the different pore spaces in soil. A fractionation is bound to occur. All of DOM-III (*cf.* Table 1) will be obtained by almost all methods but the extracted amounts of DOM-II and DOM-I will vary. It can be assumed that DOM-I in the soil's microspores is especially elusive to quantitative extraction. Therefore this fraction will be underrepresented in any given sample. Also DOM can be physically modified by using inadequate extraction medium. Only one definition coming from the aquatic sciences and which has a general consensus is: The organic matter, which can go through a filter with a pore size of 0.45 µm, is regarded as DOM (Zsolnay, 1996). However, from a practical point of view, there are no significant differences between DOM, which has gone through a 0.4 or 0.6 µm pore size filter (Á. Zsolnay, personal communication).

Commonly utilized extraction methods are batch extraction, extraction with centrifugation, extrusion with pressure, and suction cups. Each method has advantages and disadvantages. This has been reviewed in detail by Zsolnay (2003). Extraction methods based on suction are only capable of obtaining water and its associated DOM, which is retained by the soil at over -100 kPa, that is to say essentially DOM-III. Centrifugation is attractive in that one can theoretically control, from which pore spaces water and its associated DOM is removed. The same is true for extrusion with pressure. However, in both cases realistically only very small volumes can be

obtained and none at all in studies, which concern themselves with potential DOM in dry soils. Furthermore, the small extraction volumes resulting from these methods can result in significant "wall effects", consisting of the adsorption and subsequent desorption of extracted DOM (memory effect) and of the release of extraneous DOM from the container and filter walls. One additional difficulty with centrifugation is that the physical nature of the soil sample is destroyed, and it can not be used for further studies such as those dealing with DOM replenishment. Attempts to use pF pots to extrude DOM under pressure have been unsuccessful because of the "wall effects" (S. Stein, personal communication).

A study of the literature shows that, with the exception of research dealing with DOM percolation to ground and surface waters, the batch extraction method is by far the most commonly used (Zsolnay, 1996). It has the advantage in that it is very simple to perform, and therefore a great number of samples can be handled simultaneously. The disadvantage is that it is unknown to what extent from the different pore spaces DOM is extracted. This is especially true in regards to DOM-I. Nevertheless, it is probably the best estimate of DOM *in situ*, which can be obtained in a practical manner. Realistically, all methods are faulty. One can only choose one, which is consistent and does not produce too many artifacts. Therefore the batch approach was used to attain the goals of this dissertation (section III).

However caution has to be paid to the following factors, which can influence the extracted DOM properties.

1. Soil to solution ratio
2. Type of extractant
3. Solution condition of extractant (*e.g.*, pH, ionic strength)

In order to perform the DOM extraction under optimal conditions, the effects of these factors have to be considered before the DOM extraction is carried out. There have been several workshops, which dealt, among other things, with extraction methodology. One was sponsored by the European Science Foundation and was convened in 2002 in Beilngries by Á. Zsolnay. The others were convened in Thurnau by K. Kalbitz and K. Keiser in 2002 and 2004. However, no definitive extraction method was recommended.

7.2 Soil sample handing before the DOM extraction

Not only the extraction method, but the water content of soil is known to influence the DOM quantity and quality. Rewetting of dried soil increases the concentration of DOM (Lundquist et al., 1999; Gregorich et al., 2000; Kaiser et al., 2001a; John et al., 2003). The composition of DOM is also significantly influenced by the water content of soils (Christ and David, 1996; et al., 1999; Merckx et al., 2001; R. Ruser, Á. Zsolnay, personal communication). Obviously the water content of soil is strongly dependent on weather and climate. The soil,

which is freshly taken from a field on one day, is not necessarily in the same condition as soil that is taken at the same sampling location on another day. The water content of soils must be brought to the same level for comparison of DOM from various places at various times. Air-drying before extraction could provide relatively standard conditions in this regard. However, it was confirmed that both quantity and quality of water extractable organic matter (WEOM) kept changing over time (Á. Zsolnay, A. Embacher, personal communication). They showed that changes as the result of air-drying over a three year period did not appear to have an end point, even though the rate of the change decreased over time. An alternative is to incubate air-dried soils at constant water content before they are used for DOM extraction. The validity of such a pre-incubation is also investigated in this work.

7.3 Characterization of DOM

There are a number of analytical tools available (*e.g.*, Pyrolysis, IR, NMR, UV, Fluorescence) for characterizing DOM. UV and fluorescence spectrometry are recently commonly used for detecting the quality of DOM (*e.g.*, Coble, 1996). Even though they do not characterize the structure of DOM, their advantages are offset:

- Quick
- Sensitive
- Highly reproducible
- Need only small sample volume
- Can measure DOM without modification
- Nature of fluorescence emission peak can tell the source of DOM
- Can show the degree of humification of DOM
- Fluorescence data can estimate roughly the molecular size of DOM.

Since many simple organic compounds such as fatty acids, carbohydrates, and amino acids (with the exception of tyrosine, tryptophane, and phenylalanine (Wolfbeis, 1985)), are not optically active, DOM can not be fully characterized with UV and fluorescence. Even some large molecules such as cellulose and starch are also not optically active. Nevertheless this disadvantage is offset for the reasons given above and by the fact that optical measurements allow a large number of measurements to be made within a short time period. Also, it is an important point that they do not require any modification of extracted DOM, because the DOM analyses should be performed to understand DOM as it is, not to characterise strongly modified DOM, which may not exist in the soil solution in nature. This is essential when one deals with such a complicated environment as soil.

The main interest of the DOM research is to understand DOM's functions *in situ*, and how they influence and are influenced by various factors. Since the functions of DOM are dependent

on the quality of DOM, characterization of DOM can also be done through measuring its functions. Two major functions of DOM are investigated in this dissertation: the ability of DOM to interact with copper and to act as a substrate for microbiological processes. Since the *in situ* conditions are almost impossible to mimic, the experimental conditions used to monitor these functions are done solely in the liquid phase without a solid matrix with complicated pore structures. Therefore, the results must be considered to indicate the potential ability of DOM to control processes of environmental interest. The fluorescence quenching has been found to be a valuable method for determining the ability of DOM to interact with heavy metals (*e.g.*, Cu) in solution. A significant breakthrough in standardizing DOM substrate potential has been published by McDowell et al. (2006).

III. Purpose of work

DOM plays important ecological roles in soil functioning and atmospheric and water quality. We have to manage soils to maximize their ability to serve as a sink for carbon, buffer for ground water quality, basis for food production and biodiversity, *etc*., This requires that we investigate DOM's relevance and improve our understanding of this relatively little researched organic matter pool. Significant deficits are especially present in our understanding of the sources of DOM and in how they influence its functions.

Although it is believed that the prime source of DOM is vegetation, how it influences DOM quantity, quality, and its ecological functions is essentially unknown, especially in non-forest fields. The purpose of this work is to significantly increase our understanding of the effect of vegetation on DOM quantity, quality, and its functions in regards to its biodegradability as well as to its ability to interact with Cu within various ecosystems.

To accomplish this, a large number of samples had to be extracted from numerous, differing sites (e.*g*., catenae, long term agricultural plots, lysimeters, all with control plot/locations) in southern and central Europe. Seasonal or even more frequent soil sampling in various ecosystems was needed to reveal common vegetation effects, which may be present everywhere in the world. The results are to be utilized for validating the conceptual model, which shows the possible pathways of plant influence on DOM *in situ* as was presented in the Introduction (Figure 1). The following are to be elucidated:

1. Effect of vegetation on quantity, quality, and functions of DOM
    a. Overall effect of vegetation
    b. Seasonal variation of the vegetation effect
    c. Detail temporal variation of the vegetation effect
    d. Effect of plant types
2. Effect of agricultural practices on quantity, quality, and functions of DOM
    a. Monoculture versus crop rotation
    b. Different types of fertilizer application (biological agriculture with organic manure versus conventional agriculture with mineral fertilizer)
3. Effect of stress to plants on quantity, quality, and functions of DOM
    a. Glyphosate application
    b. Ozone fumigation

IV. Site selections and descriptions

1. The sites for investigating the effect of vegetation

The following requirements were needed to investigate this aspect.

1. Sites with and without vegetation
2. Sites with different types of plants

The sites for requirement 1 are located in Puch, Bavaria, Germany and in Abanilla, Santomera, and Los Cuadros, Murcia, Spain. The site in Puch is located about 40 km northwest of Munich, Germany. The experimental station belongs to the Bayerische Landesanstalt für Landwirtschaft (Bavarian State Research Center for Agriculture). The field does not have a slope. Each agricultural plot has about 450 m^2 with a silt-loam soil. The other three sites are all natural catenae in Murcia region, southeast Spain. The vegetation condition of each site is listed in Table 2. In Table 3, typical plant species in the Murcia region are listed, since native shrubs at the sampling locations are mixture of them.

These four sites were selected, because they all have the locations with and without or little vegetation both in artificial and natural system. The bare plot in Puch is artificial, because the plot has been kept without vegetation since 1953 by plowing. The bare plots on the other Spanish sites are occurred as a result of mismanagement and due to the semi-arid climate.

It is obvious that the climate conditions in Germany and Spain are totally different (Table 4). Soil textures between sites are also different (Figure 4). Therefore, the comparison of WEOM quantity and quality between different vegetation conditions was done basically only within each site.

Soil samples (A horizons) were taken every second week at Puch site from June 2004 until June 2005: while three times at Spanish sites (Ah horizons) in 2004 by the colleagues in the Centro de Edafología y Biología Aplicada del Segura of the Consejo Superior de Investigaciones Científicas (CEBAS-CSIC).

Table 2 Properties of sampling locations in Puch, Abanilla, Santomera and Los Cuadros.

Sampling location		Puch (Germany)	Abanilla (Spain)	Santomera (Spain)	Los Cuadros (Spain)
Bare or Low plant coverage (1)	Plant coverage (%)	0	0	5-10	0
	Litter production[1] (t ha^{-1})	0	0	~0	0
	Type of vegetation	-	-	Native shrubs	-
Medium plant coverage (2)	Plant coverage (%)	0-100%	20-40 %	20-25 %	20-40 %
	Litter production[1] (t ha^{-1})	1.4	1.2	1.2	1.2
	Type of vegetation	Winter wheat	Native shrubs	Native shrubs	Native shrubs
High plant coverage (3)	Plant coverage (%)	100%	60-70 %	60-80 %	60-80 %
	Litter production[1] (t ha^{-1})	unknown	3.14	3.14	3.14
	Type of vegetation	Grass	Native shrubs	Pine trees	Pine trees

[1]Estimated by M. Kuderna (wpa Consulting Engineers).

Table 3 Typical plant species in the Murcia region

Location	Plant species
Low and mid areas	*Stipa tenacissima* communities and dwarf-shrubs *Rosmarinus officinalis, Cistus clusii, Thymus membranaceu*
Upper areas with a relatively steep slope	*Rhamno lycioidis-Quercetum cocciferae*
Shrublands	*Juniperus oxycedrus* and *Pistacia lentiscus*
Woodlands	*Pinus halepensis*
Bare gypsum containing soils on slopes	*Ononis tridentata, Salsola genistoides, Teucrium carolipaui, Helianthemum squamatum, Senecio auricular, Thymus zygis subsp. gracilis, etc*

Table 4 Climate and soil conditions in Puch, Abanilla, Santomera and Los Cuadros.

Sampling site	UTM Geographical coordinates	Mean temperature (°C)	Annual preciptation (mm yr^{-1})	Soil type	Sampling location (*cf.* Tab. 2)	SOC[3] (%)	SIC[4] (%)	pH[5]	WHC[6] (%)
Puch	X: 658697, Y: 5330958	7.9	900	Orthic Luvisol[1]	(1)	0.59	2.5	5.75	55.1
					(2)	1.20	4.3	7.13	40.2
					(3)	2.60	4.2	6.16	75.6
Abanilla	X: 667900, Y: 4231400	19-20	200	Xeric Torriortents[2]	(1)	0.45	53.9	7.49	55.6
					(2)	0.44	54.9	7.68	47.0
					(3)	0.77	53.5	7.58	56.2
Santomera	X: 672660, Y: 4219651	17	300	Lithic calcixeroll[2]	(1)	1.00	46.8	7.75	45.0
					(2)	1.70	54.6	7.81	55.9
					(3)	2.8	52.0	7.75	53.0
Los Cuadros	X: 667230, Y: 4216561	18	300	Calcic xerosol[1]	(1)	0.76	36.3	7.67	33.1
					(2)	1.2	45.5	7.73	45.8
					(3)	2.3	32.4	7.88	60.1

[1]FAO, 1974.
[2]Soil Survey Staff, 1975.
[3]Total organic carbon in soils determined with the Dicromate oxidation. Data were provided by Garcia et al. (CEBAS-CSIC).
[4]Total inorganic carbon (carbonate) in soils determined with the Calcimeter. Data were provided by Garcia et al. (CEBAS-CSIC).
[5]WEOM filtrate (Soil: 10mM $CaCl_2$ = 1: 2 (w/v)).
[6]Maximum water holding capacity.
[3, 4, 5, 6]Data for A-horizons.

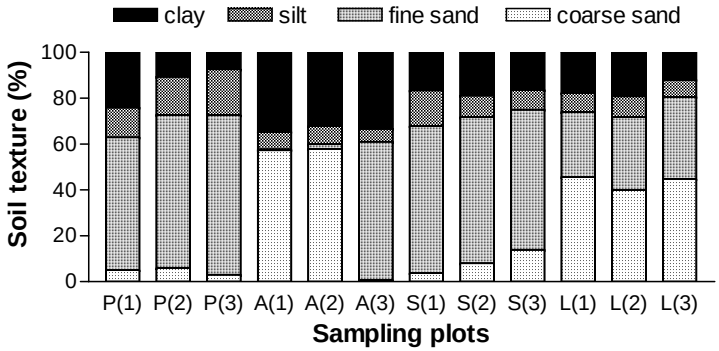

Figure 4 Soil textures of the investigated soils (A horizons) in four different sites. P: Puch, A: Abanilla, S: Santomera, L: Los Cuadros. (1) Bare or low plant coverage, (2) medium plant coverage, (3) high plant coverage (*cf.* Tab.2). Clay: < 0.002 mm, silt: 0.05-0.002 mm, fine sand: 0.25-0.05 mm, coarse sand: 2- 0.25 mm.

The site for requirement 2 is again Puch and sampling was performed three times in 2004. Detail conditions of sampling plots are given in Table 5 and Figure 5.

Table 5 Management of sampling plots with different kinds of crops in Puch and the soil conditions.

Sampling plot	Plant		Fertilizer application in 2004	SOC[1] (%)	SIC[2] (%)	pH[3]	WHC[4] (%)
Monoculture 1	Winter wheat (*Triticum aestivum L.*)		70 N (10.03.), 50 N (29.04.)	1.20	4.3	7.13	40.2
Monoculture 2	Potato (*Solanum tuberosum L.*)		150 N, 150 P, 215 K (14.04.)	0.98	2.3	6.45	37.2
Monoculture 3	Sugar beet (*Beta vulgaris L.*)		100 N, 100 P, 142 K (31.03.) 90 N, 90 P, 127 K (28.05.)	1.20	1.2	6.16	58.2
	Plant in 2003	Plant in 2004					
Rotation 1	Red Clover	Winter wheat	50 N (10.03.), 50 N (29.04.)	1.40	2.5	6.66	34.4
Rotation 2	Barley	Red Clover (*Trifolium pratense L.*)	60 P, 95 K (29.03.)	1.50	2.3	7.06	43.6
Rotation 3	Winter wheat	Barley (*Hordeum vulgare L.*)	50 N, 50 P, 70 K (18.03.) 20 N (05.04.)	1.40	2.2	6.92	39.0
Rotation 4	Potato	Winter wheat	50 N (10.03.), 30 N (29.04.)	1.60	2.3	6.77	52.7
Rotation 5	Oats	Potato	80 N, 113 K, 80 P (14.04.) 30 N (15.06.)	1.50	2.3	7.10	41.1
Rotation 6	Winter wheat	Oats (*Avena L.*)	50 N, 50 P, 70 K (18.03) 20 N (04.05.)	1.50	2.9	7.04	43.4

[1]Total organic carbon in soils determined with the Dicromate oxidation. Data were provided by Garcia et al. (CEBAS-CSIC).
[2]Total inorganic carbon (carbonate) in soils determined with the Calcimeter. Data were provided by Garcia et al. (CEBAS-CSIC).
[3]WEOM filtrate (Soil: 10mM $CaCl_2$ = 1: 2 (w/v)).
[4]Maximum water holding capacity.
[1, 2, 3, 4]Data for Ap-horizons.

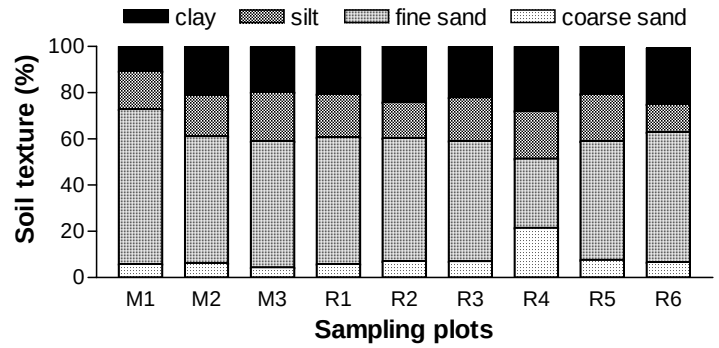

Figure 5 Soil texture and water holding capacity (WHC) of the investigated soils (Ap horizons) in Puch, Bavaria, Germany. M: monoculture, R: rotation (*cf.* Tab. 5). Clay: < 0.002 mm, silt: 0.05-0.002 mm, fine sand: 0.25-0.05 mm, coarse sand: 2-0.25 mm.

2. The sites for investigating the effect of agricultural practices

In addition to the above, the effects of agricultural practices on DOM properties have also been investigated. The observed practices are:

1. Monoculture vs. Crop rotation
2. "Biological" agriculture with organic fertilizer vs. "Conventional" agriculture with inorganic fertilizer.

The site for practice 1 is Puch. There are two sets of monoculture and rotation plots where the same crop species was growing in 2004 (Table 5, monoculture 1 and rotation 1 (winter wheat) and monoculture 2 and rotation 5 (potato)). The winter wheat plot at rotation 4 was not used for this comparison, since the soil texture was slightly different (Figure 5).

The sites for practice 2 are in Manejo, Murcia, Spain, and in Alberese, Tuscany and Pantanello, Basilicata, Italy. Manejo had broccoli (*Brassica Oleracea*) and the latter two sites had durum wheat (*Triticum durum*). The mixed organic fertilizer (grape residues and goat manure) was applied to Manejo "biological" agricultural plot, while the green manure was applied to Italian biological agricultural plots. The chemical fertilizers were applied to the "conventional" agricultural plots in all sites. Detail information of each sampling plot is in Table 6, 7, and Figure 6.

Table 6 Management of sampling plots in Manejo, Tuscany, and Basilicata.

Sampling site	Agricultural practice	Crops in 2004	Applied fertilizer
Manejo (Murcia, Spain)	Biological (BA)	Broccoli	Sheep and goat manure (2-2.5 % N content) Organic fertilizer from grape (2.5-3% N content)
	Conventional (CA)	Broccoli	Ammonium nitrate (33.5% N content) Calcium nitrate (15.5% of N content)
Alberese (Tuscany, Italy)	Biological (BA)	Durum wheat	Green manure: Egyptian clover (*trifolium alexandrinum*) and faba beans (*vicia faba minor*)
	Conventional (CA)	Durum wheat	Ammonium nitrate
Pantanello (Basilicata, Italy)	Biological (BA)	Durum wheat	Green manure: Egyptian clover (*trifolium alexandrinum*) and faba beans (*vicia faba minor*)
	Conventional (CA)	Durum wheat	Ammonium nitrate (25% N content) Ammonium phosphate (15% P content)

Table 7 Climate and soil conditions in Manejo, Tuscany, and Basilicata.

Sampling site	UTM Geographical coordinates	Mean temperature (°C)	Annual preciptation (mm yr^{-1})	Soil type[1]	Sampling location (*cf.* Tab. 6)	SOC[2] (%)	SIC[3] (%)	pH[4]	WHC[5] (%)
Tuscany, Italy	X: 687898, Y: 4748636	15	680	Eutric Cambisol	BA	0.77	19.2	7.63	26.3
					CA	0.95	14.4	7.68	48.8
Basilicata, Italy	X: 664442, Y: 4496502	16	450	Eutric Vertisol	BA	0.69	18.6	7.67	52.0
					CA	0.81	16.2	7.79	52.3
Murcia, Spain	X: 613350, Y: 4168771	18	300	Calcaric fluvisol	BA	0.94	4.95	7.89	38.2
					CA	0.68	36.3	7.89	70.1

[1]FAO, 1990
[2]Total organic carbon in soils determined with the Dicromate oxidation. Data were provided by Garcia et al. (CEBAS-CSIC).
[3]Total inorganic carbon (carbonate) in soils determined with the Calcimeter. Data were provided by Garcia et al. (CEBAS-CSIC).
[4]WEOM filtrate (Soil: 10mM $CaCl_2$ = 1: 2 (w/v)).
[5]Maximum water holding capacity.
[2, 3, 4, 5]Data for Ap-horizons.

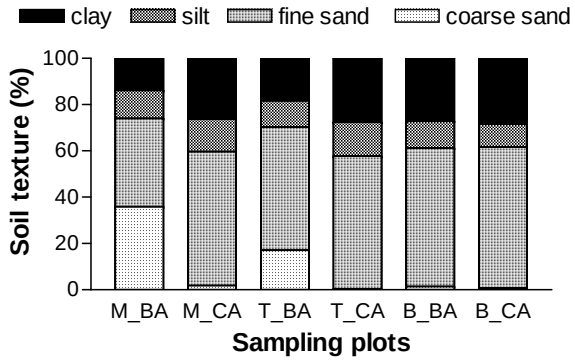

Figure 6 Soil texture and water holding capacity (WHC) of the investigated soils (Ap horizons) in three different sites. M: Manejo, T: Tuscany, B: Basilicata. BA: biological agriculture, CA: conventional agriculture (*cf.* Tab. 6). Clay: < 0.002 mm, silt: 0.05-0.002 mm, fine sand: 0.25-0.05 mm, coarse sand: 2-0.25 mm.

3. The site for investigating the effect of glyphosate application on DOM properties through plants

Since herbicide application can affect plants, it conceivable that such an effect can then in turn influence DOM through the vegetation. In order to determine this, the effect of herbicide application on DOM was observed in 2004 and 2005 with the lysimeters of the GSF, Neuherberg, Bavaria, Germany. These lysimeters were set up for the project: "Effects of transgenic, glyphosate tolerant soybeans in combination with the corresponding herbicide glyphosate on the soil ecosystem - A risk assessment study using lysimeters".

The lysimeters used were stainless steel cylinder (V4A) with a surface area of 1 m^2 and a depth of 2 m. The lysimeters filled with Neumarkt soil (Bavaria, Germany) were intact monoliths. Both lysimeters had 40 glyphosate-tolerant soy plants (*Bradyrhizobium japonicum*), which were seeded once in May 2004 and three times in 2005. Due to the cool weather in 2005,

the germination finally occurred at the 3rd seeding in July. Plant growth was also poor; the number of nodules observed in 2005 was less than half compared to the ones in 2004. There were neither flowers nor beans in 2005. Glyphosate was applied to one lysimeter once in 2004 and twice in 2005. The other lysimeter served as a control.

Table 8 Management of sampling plots (lysimeters) inside of the GSF in 2004**.**

Sampling plot (lysimeter)	Vegetation	Date of seeding	Date of glyphosate application and amount	Sampling dates	Sampling depth
Control ((-) Gly)	40 glyphosate tolerant soy per lysimeter	28.05		29.07 13.08	5-20cm 5-20cm
Glyphosate applied ((+) Gly)			17.05 (110 mg glyphosate/ m^2)	13.09	5-20cm

Table 9 Management of sampling plots (lysimeters) inside of the GSF in 2005.

Sampling plot (lysimeter)	Vegetation	Date of seeding	Date of glyphosate application and amount	Sampling dates	Sampling depth
Control ((-) Gly)	40 glyphosate tolerant soy per lysimeter	06.06 23.06 19.07		17.05 01.06 09.06	0-5cm/ 5-20cm 5-20cm 5-20cm
Glyphosate applied ((+) Gly)			24.05 (110 mg glyphosate / m^2) 06.09 (110 mg glyphosate / m^2)	20.07 29.08 14.10	0-5cm/ 5-20cm 0-5cm/ 5-20cm 0-5cm/ 5-20cm

Table 10 Climate and soil conditions at the sampling plots (lysimeters) inside of the GSF**.**

Sampling plot (lysimeter)	UTM Geographical coordinates	Mean temperature (°C)	Annual preciptation (mm yr^{-1})	Soil type[1]	Sampling location	SOC[2] (%)	SIC[3] (%)	pH[4]	WHC[5] (%)
With Neumarkt soil	X: 675445, Y: 5334250	8	1000	Haplic Arenosol	Neumarkt	1.20	2.2	5.81	66.7

[1]FAO, 1990
[2]Total organic carbon in soils determined with the Dicromate oxidation. Data were provided by Garcia et al. (CEBAS-CSIC).
[3]Total inorganic carbon (carbonate) in soils determined with the Calcimeter. Data were provided by Garcia et al. (CEBAS-CSIC).
[4]WEOM filtrate (Soil: 10mM $CaCl_2$ = 1: 2 (w/v)).
[5]Maximum water holding capacity.
[2, 3, 4, 5]Data for Ap-horizons.

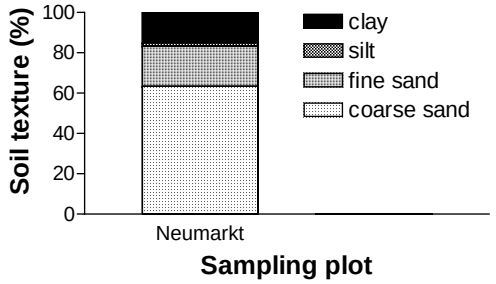

Figure 7 Soil texture and water holding capacity (WHC) of the investigated soil (A horizon) at the lysimeters inside of the GSF. The lysimeters are filled with the soil from Neumarkt, Bavaria, Germany. The soil was analyzed before the treatments had started. Clay: < 0.002 mm, silt: 0.05-0.002 mm, fine sand: 0.25-0.05 mm, coarse sand: 2-0.25 mm.

4. The site for investigating the effect of ozone fumigation on DOM properties through plants

The effect of atmospheric concentration of ozone to DOM properties through plant activity was investigated with the lysimeters of the GSF. These lysimeters were set up for the project: "Influence of biotic and abiotic stressors on the soil-plant-system, on the example of young beeches".

Eight lysimeters were located inside of the GSF, Neuherberg, Bavaria, Germany. The size of these lysimeters was the same as written above. Soil inside and outside of the lysimeters was filled with natural forest soil characterized as a dystric Cambisol derived from Pleistocene Loess above Tertiary sediments originating from the Hoegwald near Augsburg (Bavaria, Germany). All eight lysimeters and their surroundings had 5-year-old beech trees (*Fagus sylvatica*) in 2004. Four lysimeters were treated with ambient air. The other four lysimeters were exposed to air, which contained two times higher ozone concentration than the ambient air. The ozone concentration in the ambient air was continuously measured and the ozone concentration of the air, over the experimental lysimeter was adjusted accordingly. The ozone fumigation experiment was once performed in 2003 and again in 2004. However, soil samples were taken only in 2004. The ozone fumigation in 2004 was started in April and stopped in December of the same year.

Table 11 Conditions of sampling plots (lysimeters) inside of the GSF in 2004.

Sampling plot (lysimeter)	Vegetation	Date of planting	Period of fumigation	Sampling dates	Sampling depth
Control ((-) O_3)	Beech trees	November 2002		28.06	0-15cm
				29.07	0-15cm
				31.08	0-15cm
Fumigated with ozone ((+) O_3)	Beech trees	November 2002	April to December	01.10	0-15cm
				02.11	0-15cm
				06.12	0-15cm

Table 12 Climate and soil conditions at the sampling plots (lysimeters) inside of the GSF.

Sampling plot (lysimeter)	UTM Geographical coordinates	Mean temperature (^oC)	Annual preciptation (mm yr^{-1})	Soil type[1]	Sampling location (*cf.* Tab. 11)	SOC[2] (%)	SIC[3] (%)	pH[4]	WHC[5] (%)
With Hoegwald soil	X: 675445, Y: 5334250	8	1000	Dystric Cambisol	(-) O_3	2.70	2.2	3.96	84.0
					(+) O_3	3.00	2.4	3.92	84.0

[1]FAO, 1990
[2]Total organic carbon in soils determined with the Dicromate oxidation. Data were provided by Garcia et al. (CEBAS-CSIC).
[3]Total inorganic carbon (carbonate) in soils determined with the Calcimeter. Data were provided by Garcia et al. (CEBAS-CSIC).
[4]WEOM filtrate (Soil: 10mM $CaCl_2$ = 1: 2 (w/v)).
[5]Maximum water holding capacity.
[2, 3, 4, 5]Data for A-horizons.

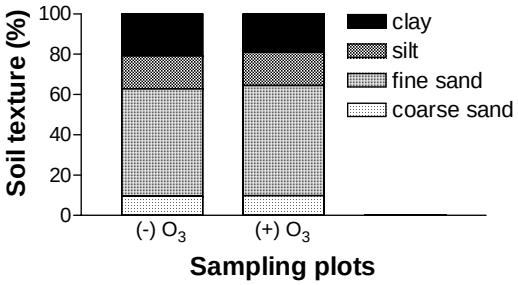

Figure 8 Soil texture and water holding capacity (WHC) of the investigated soils (A horizons) at the lysimeters inside of the GSF. The lysimeters are filled with the soil from Hoegwald, Bavaria, Germany. (-) O_3: Lysimter exposed to the ambient air, (+) O_3: Lysimeter exposed to the air, which contained two times higher ozone concentration to the ambient. Clay: < 0.002 mm, silt: 0.05-0.002 mm, fine sand: 0.25-0.05 mm, coarse sand: 2-0.25 mm.

V. Experimental

1. Soil sampling

Soil samples were taken between plants (mainly non-rhizosphere soils) with a spade from the A horizons. Since the sampling strategies were different at each sampling site, information is given here in greater detail.

1.1 Sampling at the experimental station in Puch, Germany

Soil samples were taken every second week from June 2004 to June 2005 at three plots: meadow, agricultural (winter wheat, monoculture), and bare. Sampling was not performed in February 2005, since the field was covered with snow during the entire month. From each plot four random replicates were taken at each sampling date.

In addition, sampling was performed three times in 2004 (May, July, and October) at the other agricultural plots with different crops grown. From each plot three random replicates were obtained at each sampling.

These samples above were taken from 0-20 cm. They were not composite samples, because the sampling plots were rather homogeneous (ploughed with the exception of the meadow, no slope).

1.2 Sampling at three catenae and one agricultural site in Murcia region, Spain

Sampling was performed at three locations with different degrees of vegetation coverage on three catenae (Abanilla, Santomera, and Los Cuadros) three times in 2004 (March, July and December) and also at the agricultural site (Manejo) twice in March and October 2004. Three replicates were taken each time, and each replicate was composed of the soils taken from 0-15 cm at eight different random locations or plots (composite sampling approach). These sampling was performed by the colleagues of the CEBAS-SCIC Murcia, Spain.

1.3 Sampling at two agricultural sites in Tuscany and Basilicata, Italy

Sampling was performed at two agricultural sites (Tuscany and Basilicata). Each site had two agricultural plots, where different types of fertilizers have been applied. The sampling was three times in 2004 (March, August and December). Three random replicates (0-15 cm) were taken at each plot. These samples were also not composite, since the expected variability at agricultural sites is expected to be considerably less than that found in locations composed of forests and bush dominated vegetation. These samples were taken by the colleagues of the Studio degli Ecosistemi (ISE), Pisa, Italy.

1.4 Sampling at lysimeters of the GSF, Neuherberg, Bavaria, Germany

1.4.1 Sampling at the lysimeters with and without glyphosate application

Sampling was performed in 2004 and 2005. Soil samples were taken with a 20 cm long metal cylinder in order to obtain samples from two different depths: 0-5 cm and 5-20 cm. Three samples were taken at each sampling time at each lysimeter: one with glyphosate application, the other as control. The samples were provided by S. Grundmann (IBÖ, GSF).

1.4.2 Sampling at the lysimeters with and without ozone fumigation

Sampling was performed every month six times in 2004 between June and December 2004. The soil samples were not taken directly from the lysimeters, but around them, because the outside of the lysimeters had the same experimental conditions (*i.e*., same trees, density of plants, soil type) as the inside of the lysimeters. Around each lysimeter, four random samples were taken from 0-20 cm and then combined (composite sampling approach). Since there were four lysimeters for each treatment, four replicates were obtained both for the ozone fumigated lysimeters and for the controls.

2. Handling of soil samples

The soil samples were brought back directly from Puch and the GSF lysimeters and then a small portion of each soil sample was used for the determination of the water content of soils. The rest were allowed to air-dry in the dark at room temperature. The soil samples from Spain and Italy were transported after having been air-dried in the dark at the local laboratory.

The air-dried soils were sieved with a 2-mm sieve in order to remove roots and stones. A small portion of each air dried soil sample was also used for the determination of the water holding capacity of soils (see next section V 3). This information was needed for the DOM, more precisely, WEOM extraction in order to bring these air-dried soils to the "standard" moisture content (pre-incubation). The bulk of the sieved soils were stored in a dark place at room temperature until used for the pre-incubation.

3. Determination of the water holding capacity of soil

The water holding capacity (WHC) of each soil had to be determined for the pre-incubation. The WHC was determined by placing *ca*. 10 g of air-dried soil in a pre-weighed funnel with filter paper, whose wet and dry weight had also been determined, and then over saturating the soil with distilled water. The top of the funnel was covered lightly with aluminium foil in order to prevent drying of the soil surface. After water flow had stopped, the wet soil with funnel and

filter paper was weighed before and after oven drying at 105°C for a day. The mass difference was considered to be the WHC.

4. Pre-incubation and WEOM extraction

The sieved soil was weighed in a centrifuge bottle (250 cm^3) and incubated aerobically with distilled water at 50% of the water holding capacity (WHC) in a cool room (4°C) in the dark for 1 week. The following extraction method was modified from Zsolnay (1996). After incubation, DOM was extracted with 10 m*M* $CaCl_2$ solution ($CaCl_2 \cdot 2H_2O$, p.A., Merck) at a soil (air-dried state) to solution ratio of 1 to 2 by shaking for 10 min. with an over-head shaker (Heidolph). The soil suspension was centrifuged (Sigma) at 4000 rpm for 10 min. in order to facilitate the filtration step. The supernatant was filtrated with polycarbonate membrane (Whatman), which had a 0.4 μm pore size. The filtrate was considered to be WEOM and was used for further analyses. The WEOM filtrates were preserved in the freezer (-20° C), if the characterization could not perform immediately after the extraction.

5. Characterization of WEOM

5.1. Measurements

The characterization was done optically with UV and fluorescence spectrometry (both from Varian) with 1cm quartz cuvettes. UV absorption of WEOM was measured at 254 nm. The fluorescence emission spectrum was scanned with the scan rate of 4800 nm min^{-1} though λ_{em} 300 ~ 480 nm (λ_{ex} 254 nm). The slits were 10 nm for the excitation and 20 nm for the emission light. The selection of the excitation wavelength is somewhat arbitrary, but 254 nm is most often used (Zsolnay, 1996). This is partially for historical reasons, since 254 nm is one of the chief wavelengths of the light produced by mercury lamps. However, this wavelength also has the advantage that it provides a relatively large amount of energy. This improves the sensitivity of the analyses. Much lower (higher energy) excitation wavelengths are not advisable, since the presence of nitrite can result in difficulties. The emission wavelengths were chosen to provide a maximum range not influenced by the scatter peaks around 254 nm and 508 nm (1^{st} harmonic). The optical density was adjusted to a UV absorption of 0.08 cm^{-1}; otherwise the fluorescence peak would be influenced by inner filter and molecular condensation effects (Zsolnay, 1996). WEOM filtrate, which had a higher optical density, was accordingly diluted with distilled water. Since fluorescence is sensitive to pH (Laane, 1982; Chen and Bada, 1994), all measurements were carried out at the standard pH of 2. One reason for this also was that WEOM needed to be acidified in order to determine the WEOC. The acidification was done with HCl (p.A., Merck).

WEOM solution pH was measured with the pH electrode and meter (WTW).

The WEOC concentration was measured with a total carbon analyzer (TOC- 5050A, Shimadzu) with different concentrations of potassium phthalate (p.A., Merck) solutions as standards. In order to remove carbonate the WEOM at pH 2 was purged with oxygen for 2 min. and the injected on a platinum catalyzer at 680 °C. The resulting CO_2 was quantified with an infrared detector. This method unfortunately results in the loss of volatile organics. The volatile organic carbon, however, is only expected to make up a small portion of the total (Fukushima et al., 1996).

5.2. Analyses

Although the dilution to an optical density of less than 0.08 cm^{-1} minimizes inner filter effects, an additional correction for inner-filter effects on the fluorescence emission was made (Zsolnay et al., 1999).

$$F = F_o \, e^{A} \qquad (1)$$

where F is the fluorescence peak after the correction, F_o is the measured fluorescence peak, and A is the UV absorption at 254 nm. This corrects only the excitation light intensity. The attenuation of the emitted light was not determined, since the absorption of light energy at higher wavelengths (lower energy) is relatively small (Zsolnay, 2002).

The UV and summed fluorescence (SF, integrated fluorescence emission intensity) were divide by WEOC in order to normalize them for comparison purposes. The normalized UV absorption is called “Absorptivity”, “Aromaticity” or “Standardized UV Absorption, SUVA”. It provides an estimate of the chromophore concentration per carbon mass and is usually given in $dm^3\ mg^{-1}\ C\ m^{-1}$. The normalized SF is generally called “Relative Summed Fluorescence (RSF)” and provides an estimate of the fluorophore concentration per carbon mass. It usually has the unit a.u. $dm^3\ mg^{-1}$ C, where a.u. stands for arbitrary units. The unit, a.u. can be standardized, but this is not readily done, since standards are needed for the entire emission range (*cf.* Ewald et al., 1983). Furthermore, this is not necessary in an internal study such as this one.

By taking the ratio of the higher quartile (λ_{em} 435 - 480 nm) divided by the lower quartile (λ_{em} 300 - 345 nm) of the fluorescence emission spectrum, a Humification Index (HIX) was calculated (Figure 9). It is dimensionless and is based on the fact that the fluorescence emission shifts to lower (redder) energy regions as the structure of an organic molecule becomes internally more condensed. This is reflected in a higher C/H ratio (Figure 10) (Zsolnay et al., 1999). This is presumably caused by the fact that more condensed molecules tend to dissipate absorbed energy as heat, and as a result the fluorescence photons possess less energy (Turro, 1978). Most models of humified material (Stevenson, 1982) indicate a structure, which is more condensed than

possible humic substance precursors such as carbohydrates, fatty acids, amino acids, and aromatic phenols (Zsolnay et al., 1999; Zsolnay, 2003).

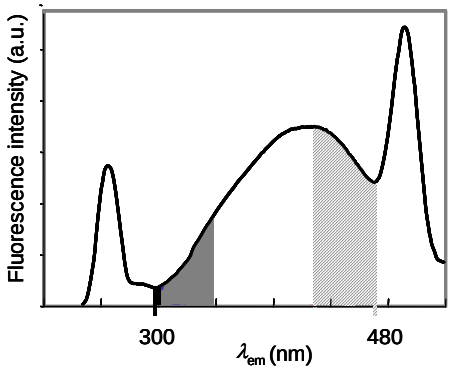

Figure 9 Typical fluorescence peak of WEOM. HIX is higher quartile (λ_{em} 435-480nm) divided by lower quartile (λ_{em} 300-345nm).

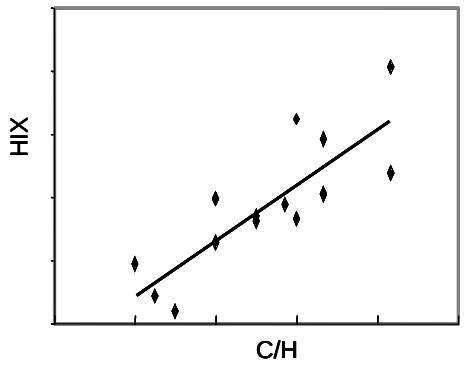

Figure 10 Relationships between HIX and condensation of an organic molecule. This was compiled by Å. Zsolnay from literature data.

By taking the ratio between SF and UV absorption, Fluorescence Efficiency (FE) was obtained. It is proportional to the quantum yield of DOM. FE can be a measure of molecular weight of DOM, since it is negatively correlated with molecular weight of humic samples due to the internal quenching in higher weight molecules (Ewald et al., 1988). It usually has the units of a.u. cm.

In summary, the following parameters were obtained with the optical measurements:

- Absorptivity (UV absorption per unit WEOC)
- RSF (SF per unit WEOC)
- HIX (Higher quartile / Lower quartile)
- FE (SF / UV absorption)

5.3. Interaction with copper

Five test tubes, containing the same WEOM solution (*ca*. 4 mg C dm^{-3}) and adjusted with acetate buffer (CH_3COOH and CH_3COONa, both p.A., Merck) to pH 5 were prepared. Dilution, if necessary, was done with 10 m*M* $CaCl_2$ solution ($CaCl_2 \cdot 2H_2O$, p.A., Merck), to maintain the same ionic strength. Different concentrations of $CuSO_4$ solutions ($CuSO_4 \cdot 5H_2O$, p.A., Merck) were added to each test tube. The Cu^{2+} concentrations were 0, 0.25, 0.5, 0.75, and 1.0 x 10^{-4} mol dm^{-3}.

A fluorescence emission spectrum for each copper concentration was measured (λ_{ex} 254 nm, λ_{em} 300 - 480 nm). The degree of quenching caused by the addition of Cu was calculated based on the decrease in SF of WEOM after the correction of the inner-filter effect.

The potential quencher such as dissolved oxygen (Huang et al., 2003) was not eliminated. The oxygen concentration in each solution was thought to be the same, because all were aqueous solutions with the same ionic strength and pH. Furthermore other heavy metals can also act as quenchers (Chang et al., 1999). However, it was confirmed in advance that the available heavy metal concentrations, which were measured by colleagues in CEBAS-CSIC, did not differ much. Also, the heavy metal ion concentrations, which were found in WEOM, were significantly lower than the Cu concentrations used in the experiment. Therefore, it was considered that such co-existing quenchers did not play a significant role in the Cu quenching measurements.

The typical figure of fluorescence quenching is shown in Figure 11 (a). This procedure is partially based on Lombardi and Jardim (1997). The curve, which was obtained by calculating the degree of quenching, was fitted with the one-site binding equation (Eq. 2) and the maximum binding capacity of WEOM (B_{max}) and the dissociation constant (K_d) were obtained. A WEOM, which has higher B_{max} value, has more capacity to interact with Cu. Since the main WEOM-Cu interaction must be through covalent bonding, B_{max} can give the information on the number of functional groups that are involved in this interaction with WEOM. K_d is the kinetic parameter, which indicates the efficiency of WEOM-Cu interaction, because K_d is the Cu concentration, which is needed to occupy half of the interacting sites (functional groups such as $-COO^-$, $-OH^-$) on WEOM. A low K_d means that the interaction is efficient, since it indicates that the affinity of WEOM to Cu is strong. Through this measurement, the difference of WEOM quality between different samples can be investigated, because the values of the copper interaction parameters obtained from Eq.2 are based on WEOM quality.

$$y = B_{max}\, x / (K_d + x) \qquad \textbf{(Eq. 2)}$$

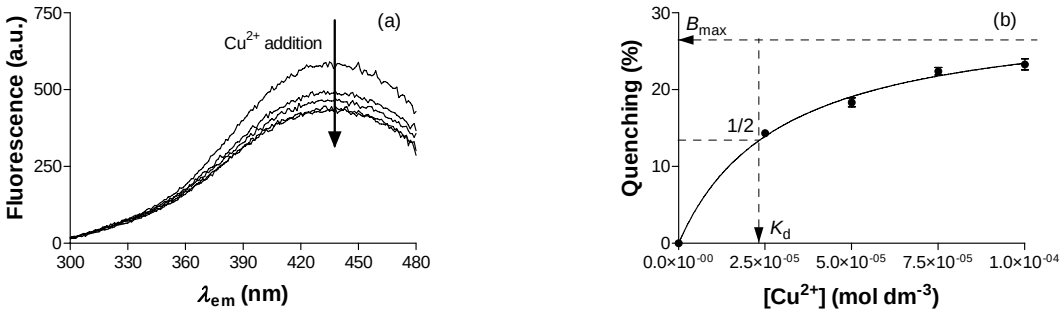

Figure 11 Typical fluorescence quenching due to copper. (a) Original fluorescence peaks with and without different degrees of quenching. (b) Curve fitting of the degree of quenching with the one-site binding equation (Eq. 2). The degree of quenching was calculated by using the entire emission spectrum.

5.4. Biodegradability measurement of DOM

WEOM for this study was extracted from air-dried soils, because pre-incubation diminishes the biodegradable DOM. This aspect will be discussed in the Results section. In order to avoid the confusion with WEOM from pre-incubated soils, this WEOM from air-dried soils is written as "WEOMa". The WEOMa extraction was done in the same way as written in V.4 but without the pre-incubation step.

The biodegradability of 5 cm^3 of WEOMa was measured with a 7-day incubation in 20 cm^3 Teflon ® vials. 2 cm^3 of nutrient solution (concentration in the vials: 3.6 m*M* NH_4NO_3 and 2.1 m*M* KH_2PO_4, both p.A., Merck)) and an inoculum (0.05 cm^3) were added to the original WEOMa filtrates to make sure that the lack of essential nutrient elements and soil microbes in the solution do not affect the results. The inoculum was the supernatant of a manually shook soil-water mixture (*ca*. 1: 2 (w/v)). The air-dried soil used for the inoculum preparation was a mixture of all soil samples analyzed in this dissertation. The rationale behind this was to assure that the microbial populations from all sites could participate in the metabolism of the WEOMa. Control was a 25 mg dm^{-3} glucose solution (10 mg dm^{-3} glucose carbon, p.A., Merck). This control had the same inorganic nutrients and inoculum as the experimental vials. The glucose control had the function of assuring that the incubation conditions were suitable for metabolism. After 7 days usually 80% of the glucose C was metabolized.

Since the incubations were performed in a liquid phase under optimal conditions, the results reflected potential rather than *in situ* substrate values. The biodegradability (BDOC, %) and the substrate value (mg C dm^{-3}) were calculated simply by taking the difference of WEOCa on day 0 and day 7 of the incubation. More details and justification can be found in McDowell et al. (2006).

It should be mentioned here that this method can not distinguish between WEOCa, which has been mineralized, from WEOCa, which has been incorporated into the microbial biomass. It also does not distinguish between the original WEOC or between DOC, which is released from the microbial biomass during the incubation. This measurement simply shows how much WEOCa can be eliminated from the system through microbial activity.

6. Data processing

Data of each sampling location or plot had either three or four replicates. A Q test was used to eliminate outliers (Dean and Dixon, 1951). Data in this dissertation was presented as mean values with the standard error. The fitting of data to a one-site bonding model was done with "Prism" from Graph Pad Software, Inc.

7. Statistics

The software "Winstat" (Kalmia, Co.) was used to evaluate significant differences ($p < 0.05$) within parameters. Either independent or paired t-test was used for comparing two data sets. Analysis of variance was used for comparing more than two data sets.

VI. Results

1. Evaluation of suitable DOM extraction conditions and the conditions for Cu quenching measurements

The quantity and quality of water extractable organic matter (WEOM), which is extracted with the batch extraction method and is the best estimate of *in situ* DOM, are strongly influenced by the experimental conditions (*e.g.*, extractant composition, ratio between soil and extractant) and the condition of soil samples (*e.g.*, soil water content) at the time of extraction (Zsolnay, 1996; 2003; Kaiser et al., 2001b). Before starting the investigation on the effect of vegetation, an adequate "standard" condition for WEOM extraction had to be determined. It has to be kept in mind that the goal here is not have a high yield of organic matter; it is to obtain an organic matter fraction, which as much as possible, reflects the *in situ* state. Therefore one can almost say, "Less is more". Also, the experimental condition for determining the interaction of WEOM and Cu needed to be assessed.

This section shows the background information why the conditions, which were described in the Experimental section, were chosen.

1.1. Selection of a suitable condition for DOM extraction

Effects of following conditions on WEOM quantity and quality were investigated.

1.1.1. Extractant and its ion concentration and composition: distilled water, 10 m*M* $CaCl_2$, 100 m*M* $CaCl_2$, 500 m*M* K_2SO_4 (soil:solution = 1:2). The use of $CaCl_2$ is commonly found in the literature and K_2SO_4 is used in fumigation-extraction studies to determine microbial biomass.

1.1.2. Ratio between soil and solution: 1:1, 1:2, and 1:10 (extractant 10 m*M* $CaCl_2$)

1.1.1. Extractant and its concentration and composition

The results indicate that the presence of cations and their valence are of great importance (Figure 12 (a)). Cation free water and K_2SO_4 solution with its monovalent cation can extract more material than $CaCl_2$ solution with its divalent cation, and at the same time they extract more humified materials (Figure 12 (b)). As one can see, the salt concentration did not play so much an important role compared to the cation type, since $CaCl_2$ solutions (10 and 100 m*M*) could not extract WEOC as much as K_2SO_4 solution did. K_2SO_4 solution was the second most efficient extracting solution after the distilled water, even though it had a higher concentration (500 m*M*) than $CaCl_2$ solutions. This result confirms that polyvalent cations, which make a bridge between organic matter and soil matrix (Bronick and Lal, 2005), disrupt soil aggregates to

a lesser degree than pure water or a solution with the monovalent potassium ion. Thus $CaCl_2$ solution makes DOM less extractable and controls both quantity and quality of WEOM. The results imply that distilled water and K_2SO_4 solution are relatively strong extractants. They probably extract more organic matter, which is not only truly dissolved, but also that, which is bound to clay particles under natural conditions. Clays without polyvalent cations form suspension, which do settle with centrifugation at only 4000 rpm and can pass through the filter pores (0.45 μm), which are used to differentiate DOM from particulate organic matter. Even though rainwater in nature is ion poor, many kinds of chemicals, including polyvalent cations, in the soil can dissolve in freshly precipitated rainwater, and the *in situ* water is no longer pure water. In order not to obtain such additional WEOM, $CaCl_2$ solution was selected as the extractant. Also, Ca^{2+} is an abundant cation in non-acidic soils.

Even though the salt concentration did not have as strong an influence as the type of cation, there were differences both in quantity and quality of WEOM between 10 and 100 m*M* $CaCl_2$. 10 m*M* $CaCl_2$ can extract more WEOC, however its quality was significantly different from the one extracted with 100 m*M* $CaCl_2$. WEOM in the 100 m*M* $CaCl_2$ solution might be of the same chemical composition as that in the 10 m*M* extracts, but the organic molecules are most likely overly condensed because of the high ionic strength. For further experiment, 10 m*M* was chosen, because the ionic strength of soil solution has been reported as being *ca*. 30 m*M* (Houba et al., 1997).

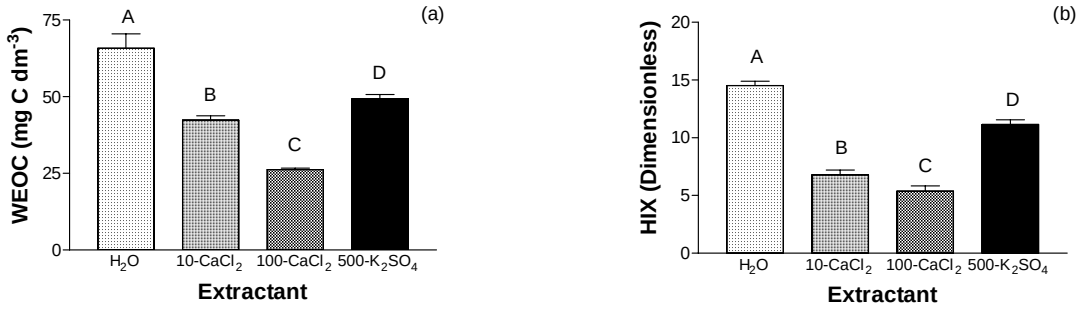

Figure 12 (a) Water extractable organic carbon (WEOC) and (b) Humification Index (HIX) of WEOM extracted from the same soil with different extractants. Extraction was performed with an air-dried soil: extractant ratio of 1: 2. Numbers in the X-axis are concentrations in m*M*. The bars are standard errors.

1.1.2. Ratio between soil and extractant

The ratio between soil and extractant makes a large difference in the absolute amount of WEOC (Figure 13 (a)). It shows that the WEOC concentration, based on extracted soil mass, is more when a relatively large extractant volume is used and the extracted DOM tends to be composed of more humified organic molecules (Figure 13 (b)). The reasons for this can only be

theorized. One possibility for this is that larger volumes of extractant can disrupt soil aggregates and extract more DOM sequestered in smaller pores. Obviously, the soil:extractant ratio must be fixed, otherwise the data are no longer comparable. The ratio 1:2 was used, because a smaller ratio than 1:2 results in a very difficult to filter extract; while higher ratios dilute the WEOC unnecessarily.

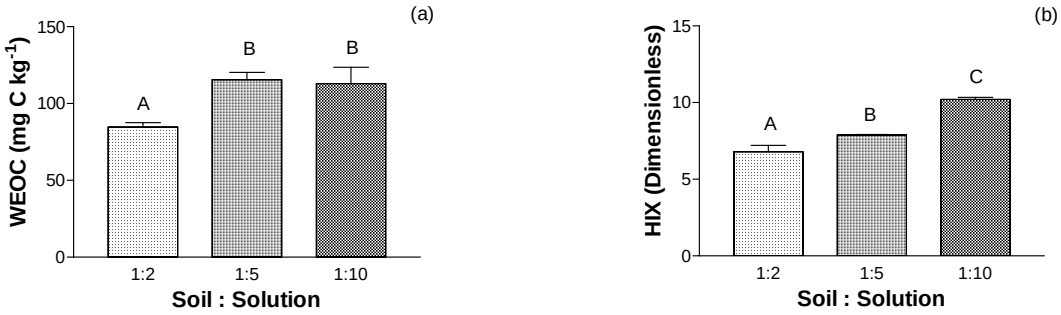

Figure 13 (a) Water extractable organic carbon (WEOC) per air-dried soil mass and (b) Humification Index (HIX) of WEOM extracted from the same soil with different ratios between soil and extractant. Extractant: 10 m*M* $CaCl_2$ solution. The presented data are mean values of three replicates with the standard errors.

The results showed that the extraction conditions significantly influence the WEOM quantity and quality. The final choice was based on the results obtained here and on the scientific literature. For example 10 m*M* CaCl was selected to prevent the extraction of clays, which are immobile *in situ,* and because of its relatively common use in other research projects. A soil to extractant ratio of 1:2 is also common, but it was also selected here from a practical point of view. Again, even though the selection of extraction condition to be used here is somewhat arbitrary and may possibly not be the best, there is actually no "best" way of extracting DOM (Zsolnay, 2003). However, as long as the conditions are fixed, the extracted WEOM will be comparable.

1.2. Selection of suitable soil conditions for DOM extraction

1.2.1. Influence of soil water content to the quantity and quality of DOM

The most significant controlling factor of the DOM concentration *in situ* is the water content. Considering the fact that DOM is the "dissolved organic matter in soil solution", this certainly is logical. However, a soil's moisture content also affects the yield of DOM extracted with the batch method. On the other hand, moist soils such as can exist under field fresh condition and pre-incubated samples do not release as much DOM even though the extraction is performed under the same condition and procedure.

The results from the study dealing with the same air-dried soil samples, two-third of which were re-wetted and then half of which were allowed to dry again, show that more WEOM is extractable as the water content decreases (Figure 14 (a) and (b)). The increased DOM fraction is mainly easily biodegradable organic matter (Figure 14 (d)), which has lower HIX (Figure 14 (c)), suggesting that it derives from biomass in soil. On the other hand, the WEOC content of soil did not change as long as the moisture content was kept at the same level. The obvious effect of water on WEOC was seen even immediately after the soils were re-wetted (Figure 15). This is supported by the results of Zsolnay et al. (1999). The degree of rewetting appeared to have also an influence.

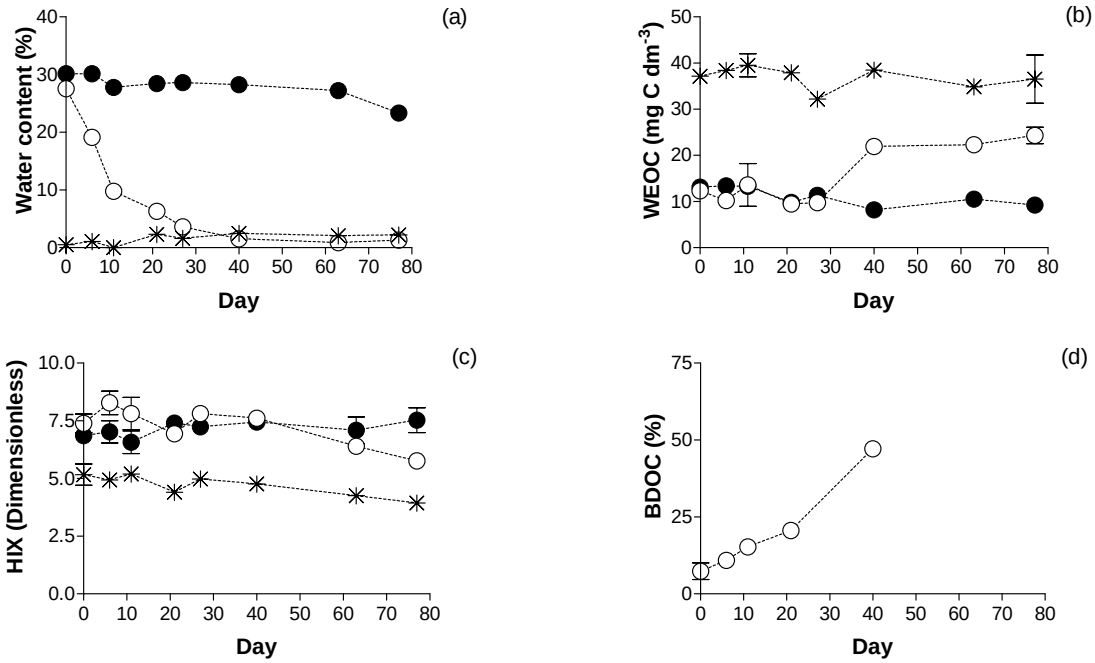

Figure 14 (a) Change in soil water content, (b) water extractable organic carbon (WEOC), (c) Humification Index (HIX) and (d) biodegradability of WEOM (BDOC) over time. One-third of soil was kept air-dried (control) (*); the other two soils were re-wetted. One out of two was kept moist at the constant water content level (●), while the other was air-dried again immediately after the re-wetting at room temperature (○). The soil used for this experiment was all same. Extractant: 10 m*M* $CaCl_2$ solution. Soil: Extractant = 1: 2.

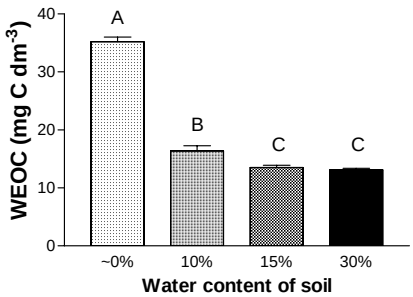

Figure 15 Water extractable organic carbon (WEOC) immediately after re-wetting air-dried soils with different amounts of water (*ca*. 1 hour later). The water content level was adjusted to ~0%, 10%, 15%, and 30%. The soil used for this experiment was all same. Extractant: 10 m*M* $CaCl_2$ solution. Soil: Extractant = 1: 2.

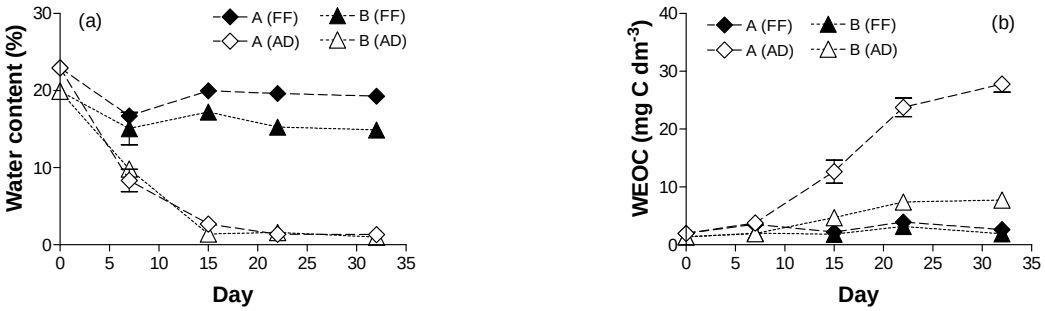

Figure 16 (a) Change in water content of soils and (b) water extractable organic carbon (WEOC) over time. Soil samples were taken at an agricultural plot with winter wheat (A (♦)) and at a bare plot (B (▲)) in Puch. Half of these soils was kept field fresh condition (same water content level as day 0, FF (solid symbols)), and the other half was let to air-dry (AD (open symbols)). Extractant: 10 m*M* $CaCl_2$ solution. Soil: Extractant = 1: 2.

Another preliminary study for investigating the effect of water content of soils on WEOM quantity and quality was performed dealing with "field fresh" soils; one was from an agricultural plot while the other one was from a bare plot in Puch. Half of the samples were kept at the same water content level, and the other half was air-dried. The results show that the reaction of soil to drought is different between the two soil samples. For example the increase of WEOC in a function of change in water content was more significant for the agricultural WEOC than for bare one (Figure 16). This is probably due to differences in the total biomass between these two soils. As was presented in section II 4, the biomass is a significant contributor to DOM during desiccation stress. On the other hand, as long as the soil moisture content was kept at the same level, WEOC did not change at all.

As also mentioned in section II 6, WEOC from air-dried soil continues to increase and its quality also tends to change even after a three year period (A. Embacher, Á. Zsolnay, personal communication). Although the magnitude of these changes decrease strongly over time, it can be seen that air drying is not optimal for standard conditions either. Soil in nature contains usually some water, even though the soil water contents may be significantly low in summer in arid lands or in strongly vegetated agricultural fields. Therefore it appears better to investigate DOM with moist soils. However, WEOC properties are different depending on the degree of soil moisture content. Also, "field fresh" soils are difficult to transport and to store, and the moisture level of the soil can be different at each sampling time. It is also impossible to adjust the water content level constantly during transport and storage. For that reason, soil samples were allowed to air dry first and then were pre-incubated for 1 week at 4 ^{o}C at 50% of their water holding capacity before WEOM extraction. Such a pre-incubation step is commonly used for the soil respiration measurements (*e.g.*, Prokop et al., 2003). WEOC did not have any major change over a month as long as the water content was kept constant after re-wetting an air-dried soil (Figure 14 (b)). The quality data did not show significant change over a month either (Figure 14 (c)). If

there was no major change in WEOM quantity and quality, the incubation time and temperature used here was considered to be suitable, and allowed samples to be processed within a reasonable period of time.

1.2.2. The effect of pre-incubation on DOM properties

The idea of pre-incubation is attractive, but how does it influence the quantity and quality of WEOM? This aspect was evaluated with soils from the agricultural and bare plots in Puch.

The use of field fresh samples was initially rejected, because each soil sample could have different moisture content level at each sampling location/plot and also at each sampling time. In addition, it is almost impossible to keep soil moisture content the same for long time. Although this may not be a problem if the WEOM extraction could be carried out immediately after the sampling, this is not always possible, especially when a large number of samples are taken or provided at once. It is also likely that the field fresh soils become no longer field fresh during transport and storage. Transport consideration were of considerable importance here, since many of the samples had to be shipped from Spain and Italy.

Pre-incubation of once air-dried and then rewetted soils brings WEOC relatively close back to the state of field fresh conditions (Figure 17), even though this is obviously not the case when the field fresh soil is from a dry field. Nevertheless, such a step brings soils to a standard moisture conditions. The pre-incubation may result in the stabilization of aggregates, but more importantly it results in the reactivation of the microbiota. This reactivation removes much of the DOM, which resulted through desiccation stress on the microbiota. Although such DOM production is perfectly "natural", it makes it difficult to have standardized conditions to investigate the prime purpose of this dissertation. Based on these results, a pre-incubation step was introduced before WEOM extractions were as performed.

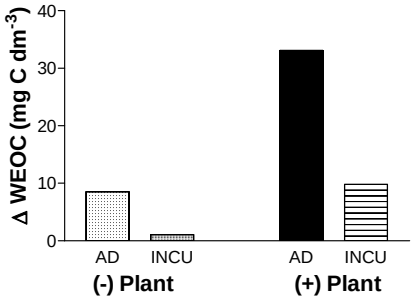

Figure 17 Additional water extractable organic carbon (ΔWEOC) content obtained after different post-sampling treatments of field fresh soils. 0 of y-axis is WEOC extracted from field fresh soils. AD: WEOM extracted from air-dried soil, INCU: WEOM extracted from once air-dried and then pre-incubated soil. (-) Plant: soils from bare plot, (+) Plant: soils from agricultural plot. Soil incubation was for 1 week.

For the study of the biodegradability of WEOM, however, WEOM from a pre-incubated soil was not usable, because the microbial efficiency resulted in a steady-state of WEOM, which was essentially refractory (Figure 18). This decrease in biodegradability was observed for all

WEOM extracted from various soil samples from various ecosystems, suggesting that the efficient microbial re-activation occurs irrespective of soil types. Therefore the WEOM extracted from air-dried soils was used only for this biodegradability measurement. The WEOM extraction of air-dried soil is absolutely same as the procedure described in section V 4 without the pre-incubation.

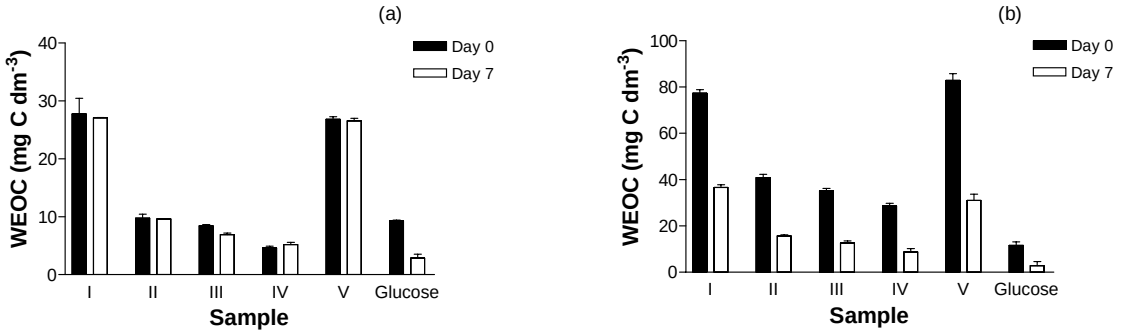

Figure 18 Water extractable organic carbon (WEOC) before and after the 7-day incubation of WEOM in Teflon® vials. (a) WEOM extracted from pre-incubated soils, (b) WEOM extracted from air-dried soils. Black and white columns: WEOC before and after the incubation, respectively. Glucose was measured as a control. WEOC I to V are extracted from the followings: I: soil amended with 0.5% sewage sludge from Abanilla, Spain, II: bare soil from Tres Caminos, Spain, III: agricultural soil from Gödöllö, Hungary, IV: agricultural soil from Basilicata, Italy, and V: forest soil from Tuscany, Italy.

1.3. Selection of an adequate condition for Cu quenching measurement

The maximum binding capacity (B_{max}) and the dissociation constant (K_d) of WEOM were determined by measuring the degree of quenching of the fluorescence emission intensity of WEOM caused by the addition of Cu containing solutions. The interaction of DOM/WEOM with Cu is known to be influenced strongly by the solution conditions (Gamble et al., 1980; Cabaniss and Shuman, 1988). As a preliminary experiment, influence of pH on the fluorescence peak of Cu dissolved in distilled water was investigated. Dissolved Cu does not fluoresce. However, Figure 19 clearly shows that there was a significant influence of solution pH on the 3D fluorescence measurement (λ_{ex} = 250-500 nm, λ_{em} = 300-550 nm, 10 nm increments). Since the unknown fluorescence peaks were detected at pH 6 almost over the entire area of the graph (Figure 19 (b)), these peaks might have been emitted from the amorphous Cu hydroxides in the solution. The scatter peaks (two diagonal lines in figures) at pH 6 and 7 were also widened compared to at pH 5, suggesting an increase of colloids in the system. The peaks disappeared again at pH 7 (Figure 19 (c)), since Cu at this pH is in its hydrolyzed form, which may no longer be dissolved. This effect at the Cu quenching measurement region ((λ_{ex} = 250 nm, λ_{em} = 300-480 nm) was also detected (Figure 19, bottom). Therefore, in order to avoid such interference, the quenching measurement was done at pH 5, adjusted with acetate buffer.

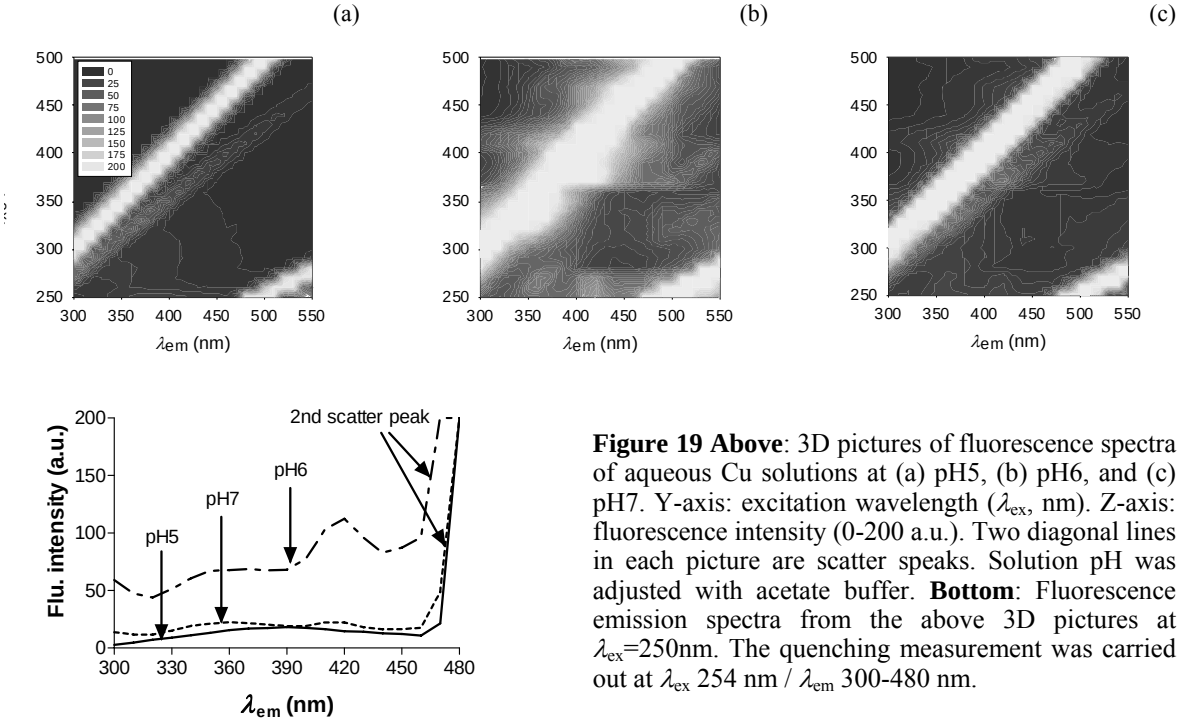

Figure 19 Above: 3D pictures of fluorescence spectra of aqueous Cu solutions at (a) pH5, (b) pH6, and (c) pH7. Y-axis: excitation wavelength (λ_{ex}, nm). Z-axis: fluorescence intensity (0-200 a.u.). Two diagonal lines in each picture are scatter speaks. Solution pH was adjusted with acetate buffer. **Bottom**: Fluorescence emission spectra from the above 3D pictures at λ_{ex}=250nm. The quenching measurement was carried out at λ_{ex} 254 nm / λ_{em} 300-480 nm.

The degree of the quenching of a WEOM fluorescence emission peak caused by the addition of Cu solutions was different in different regions of the fluorescence emission spectra (Figure 20). The fluorescence peak in the higher emission region was more quenched compared to the lower emission region. The nature of fluorescence emission spectrum is different depending on the type of DOM/WEOM. For example, simple DOM such as root exudates and DOM, which is microbial derived through fumigation, fluoresces better in the lower (blue, higher energy) fluorescence emission region (Figure 21 (a)), while a humified DOM such as fulvic acid fluoresces better in the higher (red, lower energy) emission region (Figure 21 (b)). Since the typical WEOM fluorescence peak was quenched mainly in the high emission region (Figure 20 (b)), these data suggests that more humified WEOM is involved in this interaction. Nevertheless, the degree of quenching was determined by using the entire emission spectrum after Cu addition, because the other analyses (*e.g.*, SF, HIX, FE) were done not with a particular fraction of WEOM but with the entire WEOM. This made a better comparison between the parameters possible.

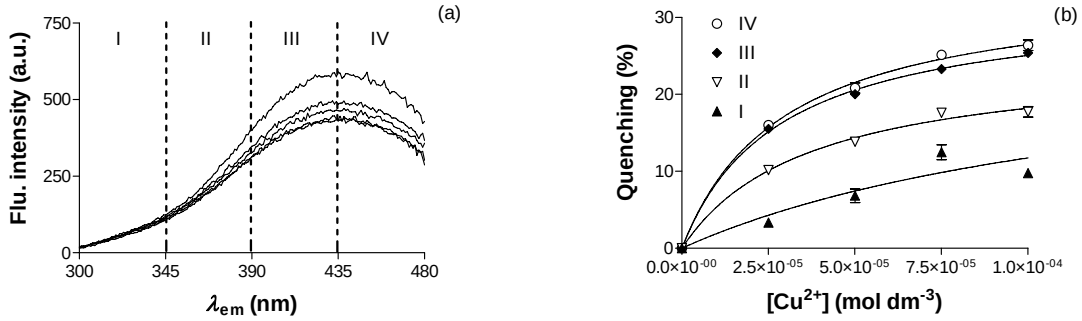

Figure 20 Typical fluorescence quenching of a WEOM peak caused by the addition of Cu containing solutions (a) and the degree of quenching in the 4 different emission ranges (I – IV) (b).

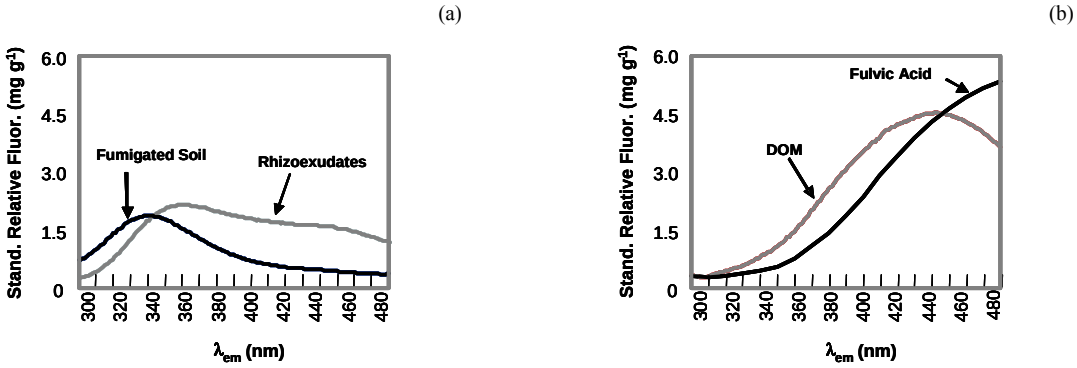

Figure 21 Typical fluorescence emission peak of DOM/WEOM, which is composed of (a) microbial material (fumigated soil) and rhizoexudates, and (b) DOM (from a bulk soil) and fulvic acid. After Zsolnay et al., 1999.

General summary of the evaluation of suitable DOM extraction conditions and conditions for Cu quenching measurements

The background information for the experimental conditions written in section V is given here. It has been confirmed that the type of extractant has an effect on both the efficiency of WEOM extraction (WEOC) and the quality of WEOM. Especially the cation, and whether it is polyvalent or not, plays an important role through influencing the stability of soil aggregates and/or suspending clay particles, which organic matter may be bound to. The salt concentration of the extractant does not influence the efficiency of the WEOM extraction and the quality of WEOM as much as the kind of cation does. Since the ionic strength of the soil solution was about 30 m*M* (Houba et al., 1997), 10 m*M* $CaCl_2$ solution was selected as the extractant. The ratio between soil and extractant influenced the efficiency of the WEOM extraction as well. Even though more WEOC is available when a larger extractant volume was used, the ratio 1: 2

(soil: extractant) was chosen, because it was found the most comfortable to work with and it had less volume than 1: 5 or 1: 10, which dilute WEOM unnecessarily.

The other aspect evaluated here was the condition of soil samples. It was found here again that more WEOM can be extracted from dry soils; while moist soils do not release WEOM as much as dry soils do. Air-dried soil was decided not to be used for the WEOM extraction, because the quantity and quality of WEOM keep changing even after the water content reached a constant level, even though the change rates decreased exponentially over time (Zsolnay, Embacher, personal communication). Field fresh soil was also decided not to be used for the WEOM extraction, because the moisture level of soils might be always different at each sampling time, even though the soil is taken at the same sampling location. Also, the field fresh soils may be changed during transport and storage. To overcome these problems, a pre-incubation step was introduced. The pre-incubation was found to bring the WEOC and WEOM quality close back to the "field fresh" condition.

WEOMa was extracted from an air-dried soil in order to perform the biodegradability measurement, which determines the potential substrate amount for microbes. WEOM extracted from a pre-incubated soil contained little microbially available organic matter. It suggests that microbially available DOM is efficiently taken by microbes during the pre-incubation. It suggests also that the accumulation of substrate in soil occurs only during the dry spell, when many microbes are desiccated and are also less active due to the water shortage. It means that the substrate for soil microbes at the location, where is no vegetation input, can be released from the soil microbial biomass and/or native SOM because of weather fluctuations. By analyzing the biodegradability of WEOMa fraction, the potential substrate amount in a soil could be determined, because this fraction is potentially available, when the soil becomes dry in nature. Before extracting WEOMa the soil was allowed to air-dry for at least a month, since as mentioned above the rate of WEOM change decreases drastically over time.

Even though the evaluated and selected conditions for WEOM extraction might not be the best, the most important point in the WEOM extraction was to fix the extraction condition for the entire experiment.

The solution conditions for the Cu quenching measurement were also evaluated. The solution pH has to be adjusted, otherwise the hydrolysis of Cu may interfere with the fluorescence peak of WEOM. The fluorescence quenching was not uniform over the entire fluorescence emission region, indicating that more humified WEOM participates in this interaction, because more quenching was observed in the higher (lower energy, red region) emission region, where more humified DOM fluoresces such as fulvic acid.

2. Effect of vegetation on the quantity, quality, and functions of DOM

2.1. Overall effect of vegetation on the quantity, quality, and functions of DOM

The mean values of all data obtained from June 2004 until June 2005 for Puch and from March until December 2004 for the Spanish sites are presented below in order to show the general effect of vegetation on the quantity, quality, and functions of DOM as determined through the analysis of WEOM. This results in an evaluation of the overall effect of vegetation, disregarding seasonal and other influential factors, which will be considered in the following sections.

The site in Puch had three investigated plots, which were a bare plot, an agricultural plot (winter wheat monoculture), and a meadow. The meadow had been covered with grasses over the whole year, even though the grasses were regularly mowed and taken away. Unfortunately, no records were kept of this. The agricultural monoculture plot had plants only during a limited period of the year. The bare plot has been kept absolutely without plants by plowing and scratching the surface frequently since 1953. These differences in plot conditions resulted in the difference in SOC (Table 13). More SOC ($p < 0.005$) and WEOC ($p < 10^{-5}$) was present in meadow than the other two plots, namely more WEOC was available at the more SOC containing plots. Since WEOC/SOC at meadow was also higher ($p < 0.007$), the type of SOC appeared to be also different from the other two plots. Since litter input at meadow was limited because of mowing, WEOM at meadow must be chiefly from the root deposits, which can be more readily extractable with aqueous solution (10 m*M* $CaCl_2$), because they are known to be composed of simple and water soluble organic matter (*e.g.*, fatty acids, carbohydrates, most amino acids). This result suggests that root input can be significant source of WEOC.

The catena in Abanilla had three investigated locations: bare, covered with shrubs (medium coverage, MC, 20-40%), and covered also with shrubs but with more plant coverage (HC, 60-70%). SOC was singificantly higher at the HC location than the others ($p < 0.05$, Table 13). Despite of the similar SOC values at the bare and the MC, significantly more WEOC was available at the MC, and HC contained more SOC than the MC ($p < 0.0005$). WEOC/SOC was higher at the MC and the HC than the bare ($p < 0.05$). Therefore, the WEOC availability was not controlled by the SOC, but more by the plant frequency at each plot. Fallen plant litter might have become an additional source of WEOC. The extraction efficiency of WEOC with respect to SOC was significantly higher at HC compared to the others (Table 13). This also supports that this additional source of WEOC was present more at the HC location.

Table 13 Soil organic carbon (SOC), water extractable organic carbon (WEOC), and their ratio at all plot at four different sites (A-horizons). Sampling location, see Fig. 22. Letters next to figures show the significant difference within each site ($p < 0.05$).

Sampling site	Sampling location	SOC (g 100 g^{-1})	WEOC (µg g-1)	WEOC/SOC (%)
Puch	Bare	0.59 (a)	6.6 (a)	0.11 (a)
	Agricultural	1.20 (a)	13.8 (a)	0.12 (a)
	Meadow	2.60 (b)	58.5 (b)	0.22 (b)
Abanilla	Bare	0.45 (a)	5.6 (a)	0.13 (a)
	Shrub (MC)	0.44 (a)	11.7 (b)	0.27 (b)
	Shrub (HC)	0.77 (b)	18.3 (c)	0.24 (b)
Santomera	Shrub (LC)	1.00 (a)	12.4 (a)	0.12 (a)
	Shrub (MC)	1.70 (b)	27.0 (a, b)	0.16 (a)
	Pine (HC)	2.80 (c)	35.4 (b)	0.13 (a)
Los Cuadros	Bare	0.76 (a)	9.2 (a)	0.12 (a)
	Shrub (MC)	1.20 (a)	16.9 (a)	0.14 (a)
	Pine (HC)	2.30 (b)	66.3 (b)	0.28 (b)

Note: SOC was measured only once in spring 2004 (3 replicates per plot). WEOC is the mean value of seasonal sampling, namely data obtained in spring, summer, and fall 2004 (9 replicates per plot). Therefore the ratio was calculated through dividing individual WEOC by the mean SOC value. The presented ratio values are the mean of 9 replicates.

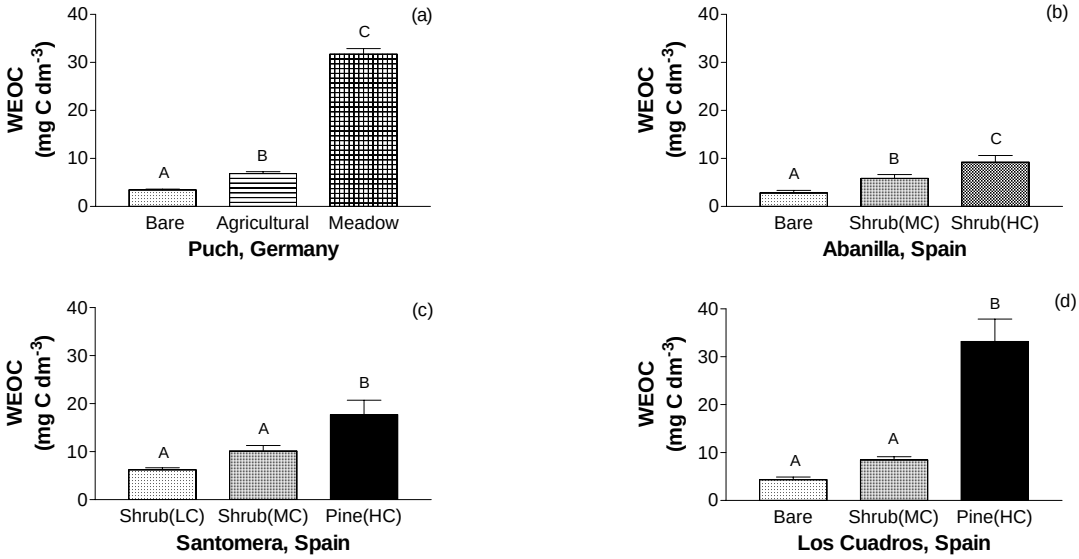

Figure 22 Water extractable organic carbon (WEOC) in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

Both catenae in Santomera and Los Cuadros had three investigated locations: low plant coverage plot (LC, Santomera) or bare plot (Los Cuadros), shrubs (MC, 20-25% (Santomera),

20-40% (Los Cuadros)), and pine trees and with more plant coverage (HC, 60-80% for both catenae). The type of vegetation was different between MC and HC in these catenae.

These differences in plot conditions resulted in the difference in SOC (Table 13). The location with more vegetation coverage contained more SOC in Santomera ($p < 0.0001$); while only the pine forest contained signficantly more SOC than the other two locations in Los Cuadros ($p < 0.01$). WEOC content was also higher at the HC in both catenae ($p < 0.05$ for Santomera, $p < 10^{-6}$ for Los Cuadros). However, WEOC/SOC was not significantly different between locations in Santomera; while HC had higher value than the others in Los Cuadros ($p < 0.0005$). The result for Santomera suggests that the quality of SOC was similar irrespective of the vegetation coverage and type. However, Los Cuadros HC had high WEOC/SOC value, even though both of HC at these catenae had same type of vegetation, vegetation coverage, and located in the same Murcia region. It might be due to the difference in soil conditions such as the subtle difference in clay content in Los Cuadros HC (Figure 4).

All in all, vegetation increased SOC and consequently WEOC as well, even though some exceptions were present. WEOC/SOC was also higher at the plots with more vegetal input. This ratio appeared to be affected not only by the amount of litter input, but also by the type of vegetation and soil conditions.

The quality of WEOM in regards to its Absorptivity shows slight, inconsistent differences between the plots/locations at each site (Figure 23). The differences were also not as pronounces as those for WEOC.

The agricultural plot had the highest Absorptivity in Puch (Figure 23 (a)). There are several possible reasons for this difference: 1) difference in microbial acitivity, 2) difference in litter input, and 3) difference in solution pH. Microbially processed DOM has more aromatic structure than the original (Kalbitz et al., 2003a; 2003b; 2003c). Their activity must be high at both agricultural plot and meadow in Puch, because obviously more WEOM (WEOC), which is the substrate for microbes, is available. Even though ATP at meadow plot was not determined, ATP at the agricultural plot was significantly higher than the bare counterpart (agricultural plot: 1000 ng g^{-1}; bare: 300 ng g^{-1}, mean values of 9 replicates in 2004, measured at the CEBAS-CSIC). Therefore, WEOM at the agricultural plot could be more aromatic. However, there was no difference between bare and meadow, where the latter is supposed to have significantly high microbial activity. Therefore, the difference in microbial activity was not the chief reason for this. The agricultural plot was the only plot, which had substantial litter input in Puch. Meadow had little litter production, since grasses have been mowed and taken away. Litter in general has aromatic organic matter such as lignins, phenols, *etc*. Therefore, this could be the reason for this difference.

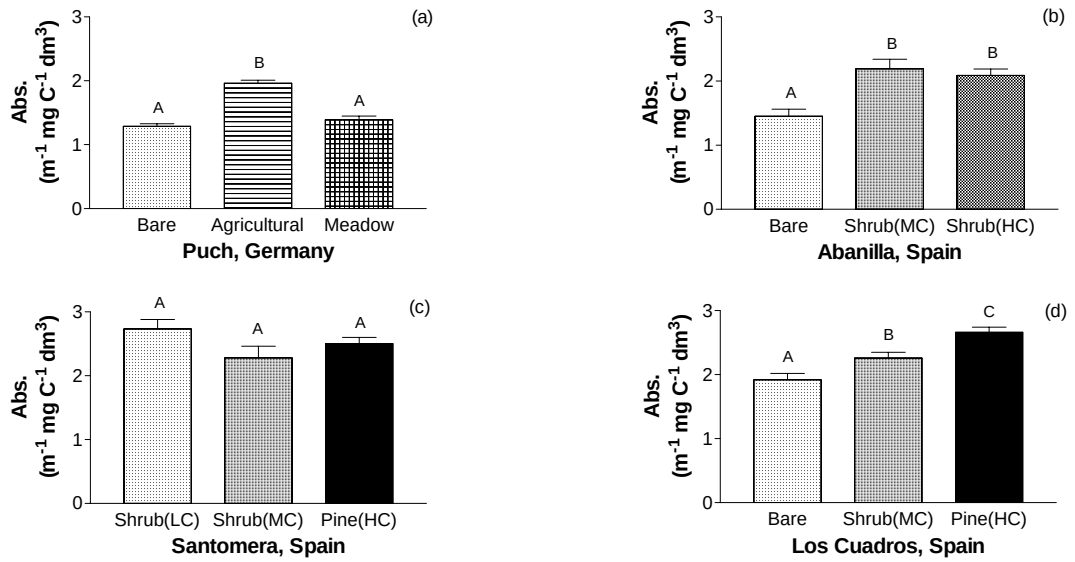

Figure 23 Absorptivity (Abs.) of WEOM in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

In addition, difference in solution pH could be also a significant factor, since high pH solution can mobilize more organic matter (Stevenson, 1982). Even though the utilized extracting solution was the same 10 m*M* $CaCl_2$ solution, the extracted WEOM solutions had different pH (Figure 24), suggesting the *in situ* pH is also different. The presence of litter and the high pH condition at the agricultural plot resulted in extracting WEOM, which is composed of more aromatic compounds.

The catena in Abanilla shows differences between the bare location and the locations with shrubs (MC and HC, Figure 23 (b)). Solution pH (Figure 24 (b)) might be the cause, but the reason for this difference was probably more because of the difference in litter input.

The data from the catena in Los Cuadros show significant differences between locations: the more vegetation, the more Absorptivity (Figure 23 (d)). Since there was a difference between MC (shrub) and HC (pine), the vegetation type, which is in conjunction with the amount of litter input and the nature of the litter, may also be important. Solution pH at the HC location was slightly higher (Figure 24 (d)), but it probably did not produce so much differences in Absorptivity.

However, such differences were not observed at the catena in Santomera (Figure 23 (c)). The reason is basically unknown. Nevertheless, the data from the other sites suggest that the

Absorptivity of WEOM is influenced by vegetation. The Puch data suggested that the agricultural management may also be of importance for this parameter.

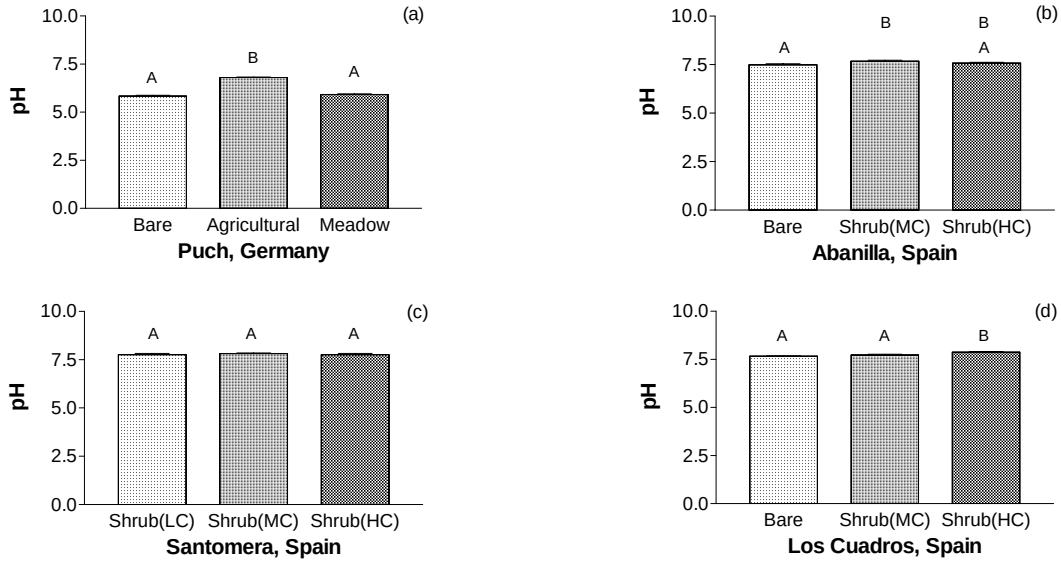

Figure 24 Solution pH of WEOM in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

Even though the Absorptivity showed inconsistent results, fluorescence intensity of WEOM per unit WEOC (RSF) shows a relatively consistent influence of vegetation. With the exception of the agricultural plot in Puch, the more vegetation, the lower the RSF (Figure 25). This could reflect again the input of the plant litter, which has more aromatic DOM that does not fluoresce well. Or the WEOM at the vegetated plots/locations was enriched in non-fluorophores. Such non- or weak-fluorophores are thought to come from vegetation (*e.g.*, carbohydrates, phenols). Components of root exudates such as amino acids, carbohydrates, proteins, do not fluoresce until they contain tryptophane, tyrocine, and/or phenylaranine (Wolfbeis, 1985). Lignins and phenols are components of plant litter. However the former is not water soluble and the latter do not fluoresce well. Therefore RSF may be able to indicate up to certain degree that DOM is composed of either more fresh plant derivatives or not.

The WEOM from the agricultural plot in Puch had the highest RSF at that site (Figure 25 (a)). This plot, however, was the only cultivated plot; therefore this difference might be caused

not only by vegetation, but also by the management, which had resulted in an increased soil solution pH. Higher solution pH might have made fluorophores more available at this plot. RSF at the meadow was low. It was probably because of the significant root input.

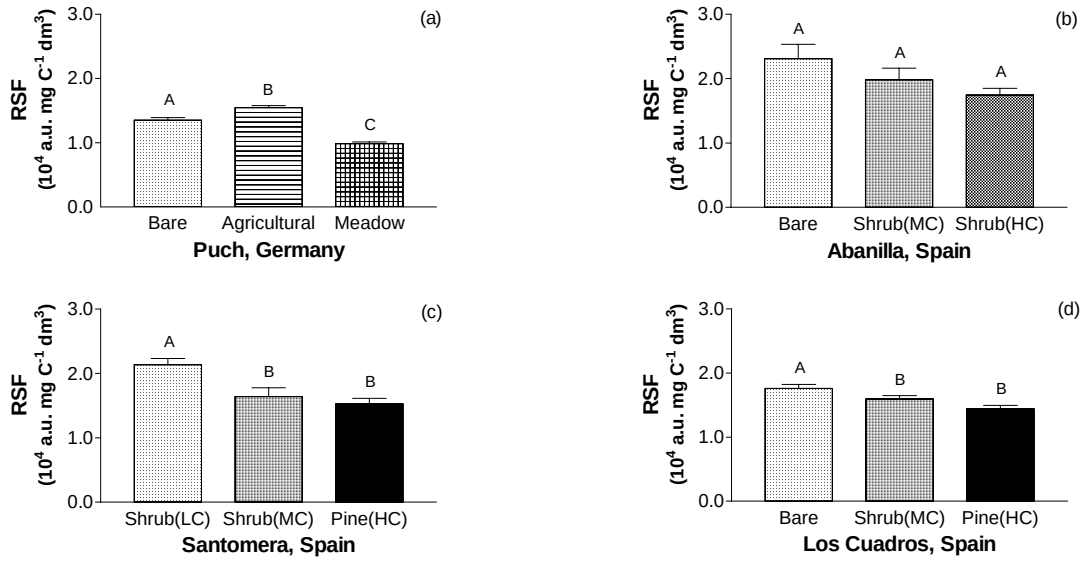

Figure 25 Relative summed fluorescence (RSF) of WEOM in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

Largely because of the differences in RSF, Fluorescence Efficiency (FE) showed significant difference between bare and vegetated plots at all investigated sites: $FE_{bare} > FE_{vegetated}$ (Figure 26). Based on the result of Absorptivity and RSF, even though it was not always found, the WEOM from the locations with more vegetation had slightly higher UV absorption and/or lower fluorescence intensity. Fluorescence emission occurs only when there are chromophores. The vegetated plots had WEOM composed of more chromophores, which for some reason did not fluoresce well. This low FE of WEOM was not due to the concentration of WEOM (concentration effect), since the optical measurements were performed at the same optical density (UV *ca*. 0.08 cm^{-1}). It can be said that this difference might be caused by the presence and the degree of plant input.

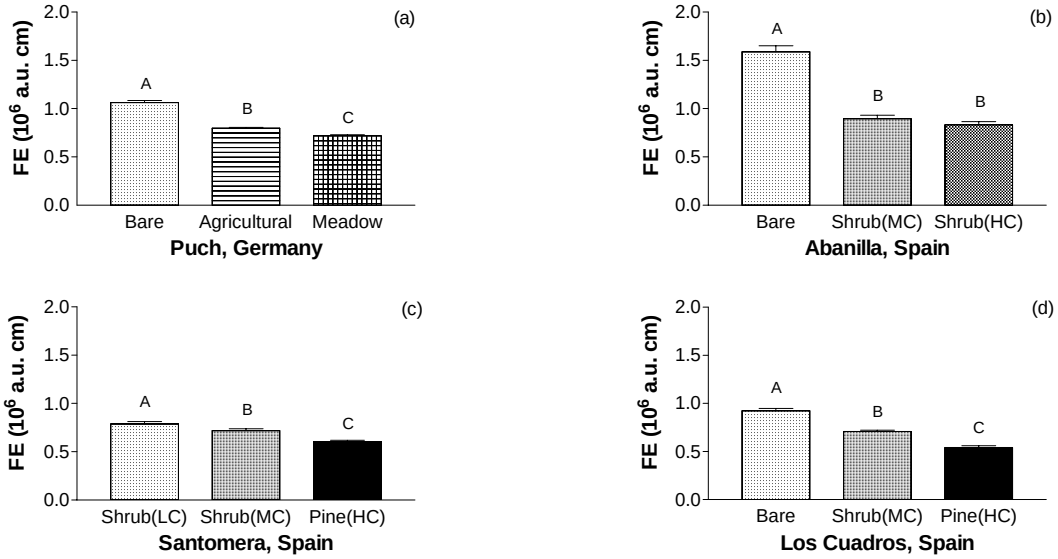

Figure 26 Fluorescence Efficiency (FE) of WEOM in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

The possible reasons why the WEOM does not fluoresce well can be due to:

1) co-existence of quenchers
   (*e.g.*, dissolved oxygen, heavy metal ions),
2) internal quenching within WEOM
   (inner- and/or inter-molecular condensation of the organic molecules),
3) composition of WEOM
   (simply more organic matter, which does not fluoresce well).

The heavy metal ions in WEOM solutions should not be the reason for this, because the measurement was carried out at pH 2, at which all the binding sites of WEOM are supposed to be occupied with H^+, therefore quenching should not occur any more. Furthermore, the data of available heavy metals in the soils at each location, which were measured by the colleagues at the CEBAS-CSIC, showed that the concentrations of such metal ions did not significantly differ between plots/locations. Even though the dissolved oxygen was not determined, its concentration should be the same for all WEOM solutions, because they all had similar solution conditions. Therefore, the possibility 1 is regarded as not the reason for the differences in FE. However, both possibilities 2 and 3 could be the reasons. The condensation of organic molecules was presented

in the study dealing with humic acid. Humic aggregates held together by weak bonding mechanisms such as H-bonding and hydrophobic interactions (Wershaw, 1993; Conte and Piccolo, 1999; Piccolo and Conte., 2000). Even though the WEOM measured here is supposed not to be as much aromatic as humic acids, this weak inter-molecular interaction might have been occurred. Since vegetated plots/locations in nature such as meadow in Puch and pine forests in catenae should have larger molecular size of DOM as opposed to arable soils (Von Lützow et al., 2002), it can be imagined that such larger DOM restrict each other more than small size DOM does and has more three-dimensional condensation. Therefore, the energy of absorbed light is lost as heat and/or the fluoresced light is re-absorbed within a molecule before it reaches to the detector. In addition, the DOM of freshly released from plants is composed of low molecular weight organic matter, most of which does not fluoresce well. The component of litter such as phenols do not fluoresce well either. The WEOM at vegetated locations must have had such non-fluorescing, but UV absorbing organic matter input. For these reasons, the WEOM from vegetated locations had lower FE.

Humification Index (HIX, Figure 27) did not show such a consistent trend as FE, confirming that this parameter indicates different properties of WEOM. HIX shows the degree of intra-molecular condensation (*i.e.*, C/H ratio) in a molecule. Since WEOM is an aggregate of various organic substances, HIX is a measure of the condensation of relatively humified organic molecules. While FE shows the three-dimensional, inter- molecular condensation, which appeared to be influenced by vegetation.

HIX of meadow WEOM had the lowest value in Puch. It was presumably because the plot had more root input, which is usually composed of simple DOM and it fluoresces in the region of short emission wavelengths (Zsolnay et al., 1999). The other Puch plots contained more humified WEOM, which was probably induced by the microbial activity because of plowing, which exposes SOM to the air. The increase in HIX through the microbial degradation has been verified (Marx et al., 2004). HIX values for the other Spanish sites showed inconsistent differences and were relatively similar between plots/locations within each site.

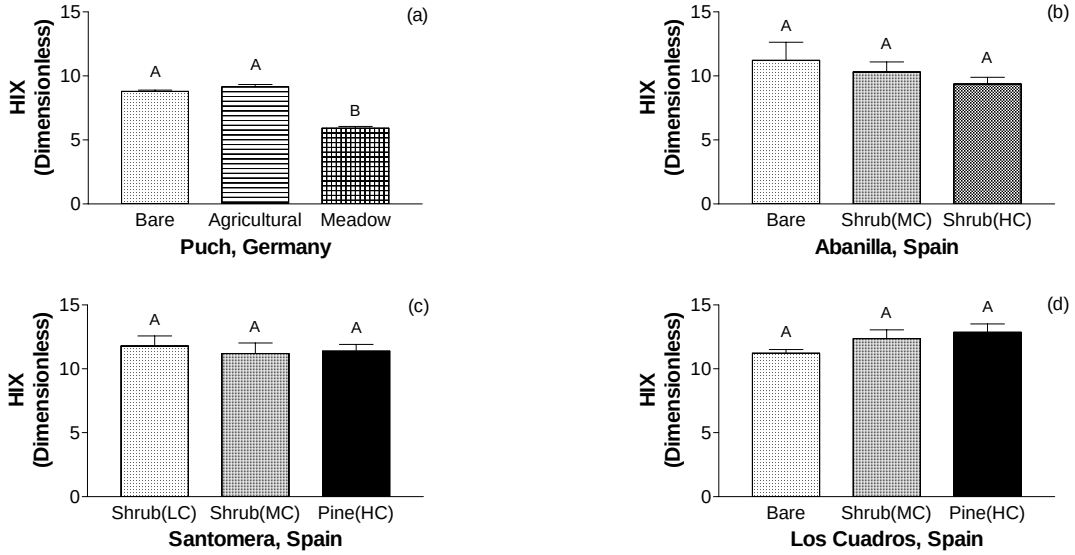

Figure 27 Humification Index (HIX) of WEOM in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

WEOM, which was extracted from the pre-incubated soils and characterized above, was also used for the analysis of the interaction between WEOM and Cu. The maximum Cu binding capacity (B_{max}) of WEOM was hardly affected by vegetation (Figure 28). However, the cultivation and/or solution pH at Puch site (agricultural > bare = meadow) did appear to increase B_{max}. The typical fluorescence quenching of WEOM, which is caused by Cu addition to the WEOM solution, is shown in Figure 20 (a). The degree of quenching was plotted against Cu concentration in Figure 20 (b) and the B_{max} and K_d was obtained by the curve fitting with the one-site binding equation. The quenching data in the four regions of fluorescence emission spectra (Figure 20 (b)) showed that the most quenching occurred in the region of longer emission wavelength, indicating that the more humified WEOM is more involved in this interaction. The results shown in Figure 29 ($p < 10^{-12}$) support this hypothesis; more humified WEOM (higher HIX) tend to have more ability to interact with Cu (higher B_{max}). Such humified DOM is presumably mainly dissociated from plant litter and/or SOM, which was bound to the soil matrix and plays a preferential role. Solutions, which have higher pH, can leach more humified organic matter. The quality of such humified DOM may be affected by the properties of soils, which can be influenced by management such as cultivation, since WEOM from the agricultural plot had the highest B_{max} at the Puch site.

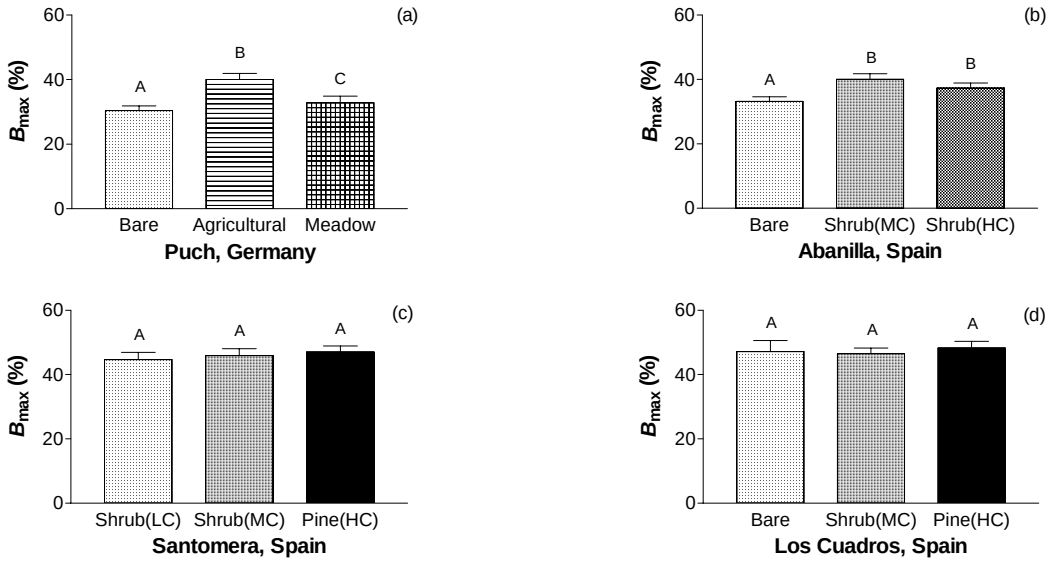

Figure 28 Maximum binding capacity (B_{max}) of WEOM in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

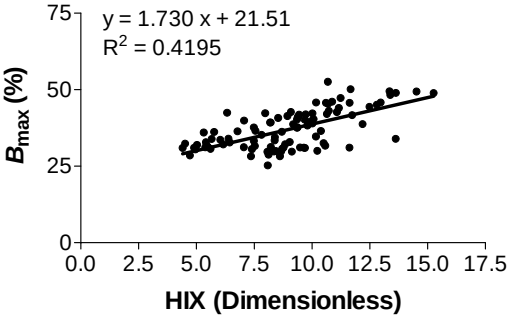

Figure 29 Relationships between the maximum binding capacity (B_{max}) and the Humification Index (HIX). Presented data point is the mean value at each sampling plot/location at each sampling time. Sampling in Puch was every 2 weeks; while it was only 3-times (spring, summer, and fall) on the Spanish catenae (Abanilla, Santomera, and Los Cuadros).

The kinetics of the interaction between Cu and WEOM was reflected by the dissociation constant (K_d), which appeared to be affected by vegetation: the more vegetation, the higher K_d (Figure 30). B_{max} indicates the maximum Cu binding capacity of WEOM; while K_d indicates inversely how readily WEOM and Cu can interact. A lower K_d value means that Cu occupies half the number of binding sites of WEOM at lower concentrations, that is to say more readily. K_d was higher for the WEOM from vegetated plots/locations, indicating that the WEOM in the more vegetated plots/locations had slower interactions with Cu. This result suggests that the WEOM at the vegetated locations was composed of more organic matter, which was rather

simple non-humified organic matter. The negative relationships between K_d and HIX (Figure 31, $p < 10^{-5}$) suggests also more humified WEOM (higher HIX) can interact with Cu more efficiently (less K_d). The possibility of quenching by other quenchers such as heavy metal ions was not likely, because the concentrations of possible quenchers were low (*e.g.*, [Cu] < 1 x 10^{-5} mol dm^{-3}) and they were not so significantly different between the sampling locations at a given site.

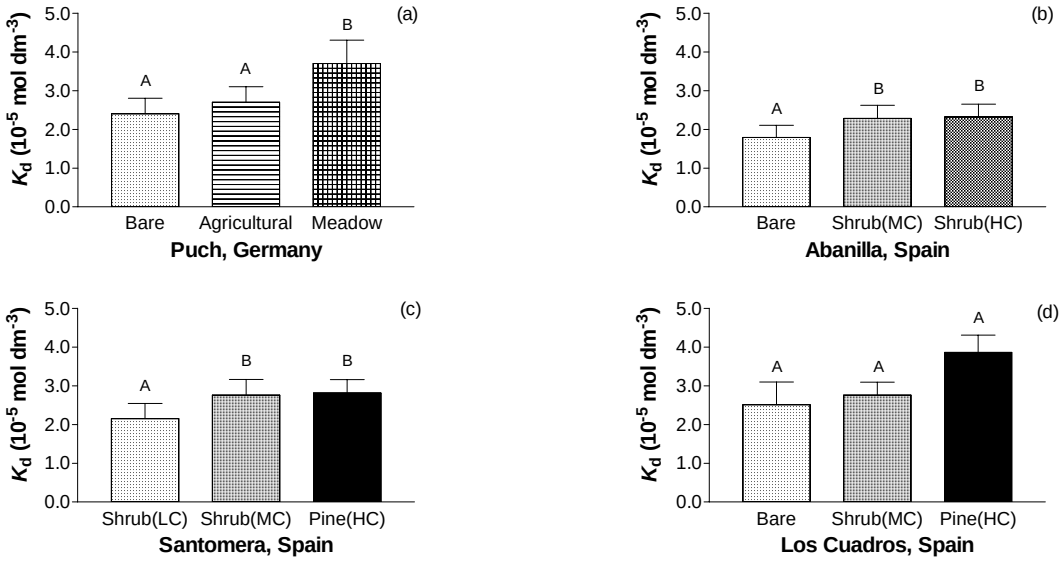

Figure 30 Dissociation constant (K_d) of WEOM in four different sites (A-horizons). WEOM was extracted from pre-incubated soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

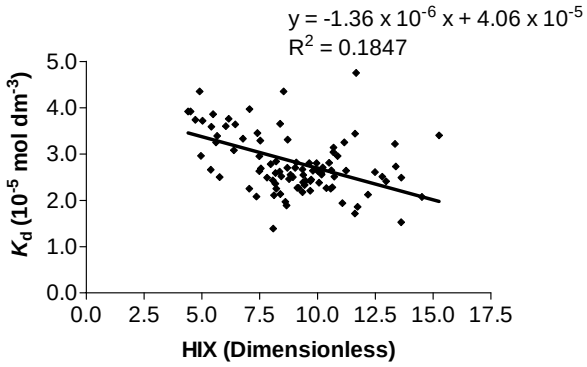

Figure 31 Relationships between the dissociation constant (K_d) and the Humification Index (HIX). Presented data point is the mean value at each sampling plot/location at each sampling time. Sampling in Puch was every 2 weeks; while it was only 3-times (spring, summer, and fall) on the Spanish catenae (Abanilla, Santomera, and Los Cuadros).

For the biodegradability studies, WEOMa, which was extracted from air-dried soils, was used. The incubation was carried out in a liquid phase in the Teflon® vials. Since it did not contain any complicated system such as soil matrix, the values were more potential rather than *in situ*.

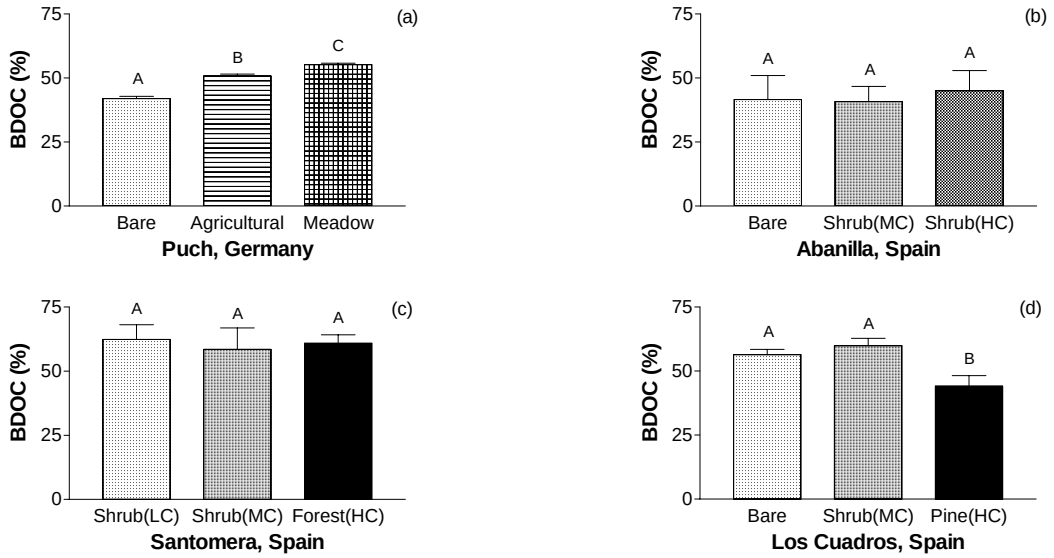

Figure 32 Biodegradability (BDOC) of WEOMa in four different sites (A-horizons). WEOM was extracted from air-dried soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

The biodegradability of WEOMa obtained from the bare plot in Puch was surprisingly high, even though there has been essentially no organic matter input for more than 50 years, indicating that soil is capable of sequestering labile material for long periods of time. The overall effect of vegetation was most pronounced in Puch and Los Cuadros (Figure 32 (a) and (d)). Not surprisingly, meadow WEOMa was more microbially degradable than agricultural WEOMa, which was more easily degradable than bare WEOMa. The difference was presumably caused by the difference of root exudates quantity. Pine WEOMa in Los Cuadros had lower biodegradability than the others. Since the difference was not observed in Santomera (Figure 32 (c)), where same type of vegetation was presented; the reason is not known. This again shows the difficulties in attempting inter-site comparisons, even when the sites are located in the same general area.

Even though the BDOC was not so much different between plots/locations, the absolute amount of substrate was significantly different. When more vegetation was present, more substrate was available at all sites (Figure 33).

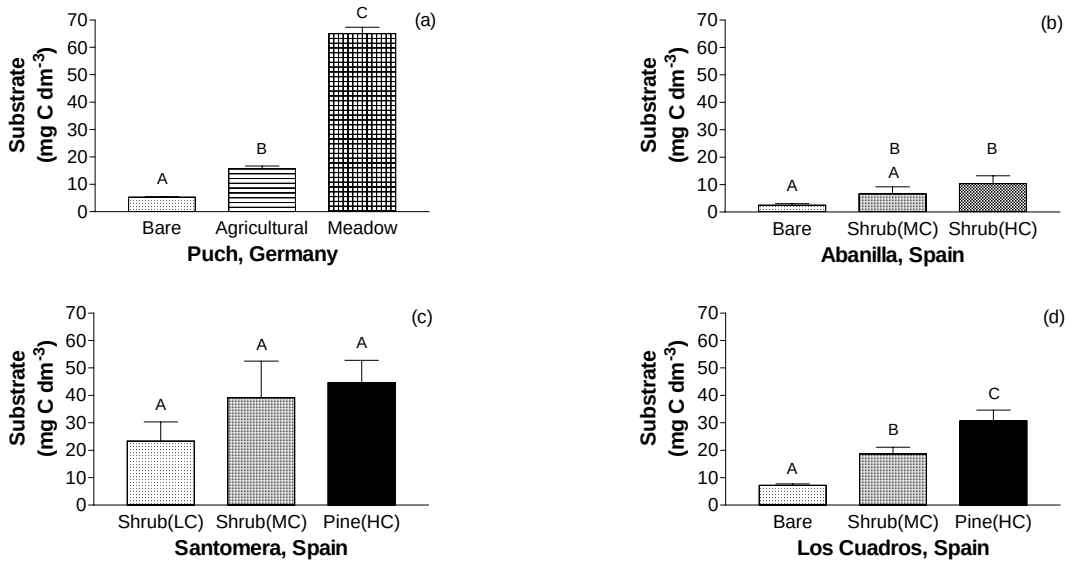

Figure 33 Substrate amount of WEOMa in four different sites (A-horizons). WEOM was extracted from air-dried soils taken at plots or locations with different vegetation conditions. (a) Site in Puch, Bavaria, Germany. Agricultural plot was winter wheat monoculture. (b) Catena in Abanilla, Murcia, Spain. (c) Catena in Santomera, Murcia, Spain. (d) Catena in Los Cuadros, Murcia, Spain. Sampling locations in Spanish catenae had different degrees of plant coverage. LC: low coverage, MC: medium plant coverage, and HC: high plant coverage. Presented data are the mean values of entire sampling period with the standard error of mean. Data points in Puch are over 90, which were taken June 2004 through June 2005; while 9 for Spanish sites, which were taken in March, July and December 2004. Letters stand for the difference is significant ($p < 0.05$).

In summary,

- Plants had a significant influence on WEOC: the more vegetation, the more WEOC. Not only litters but also root exudates appeared to be a significant WEOC component.
- Both UV absorption (Absorptivity) and fluorescence intensity (RSF) of WEOM appeared in general to be influenced by vegetation. Vegetation increased Absorptivity but decreased RSF. These differences might be caused by the difference in amounts of the litter input, nature of the litter, and microbial activity. Therefore FE showed clear differences: $FE_{bare} > FE_{vegetated}$, and this difference was seen at all investigated sites.
- HIX showed hardly any differences except for the meadow in Puch. The lower HIX at meadow was presumably caused by more plant input through roots, which were very dense at the surface layer of soil.
- In regards to the ability of WEOM interacting with Cu, K_d indicated that more plant input caused a less efficient interaction with Cu. However, the maximum Cu binding capacity

of WEOM (B_{max}) was not affected by vegetation. If anything, it might have been affected through agricultural management. Both parameters had correlations with HIX. If a WEOM was composed of more humified organic matter, it should have higher B_{max} (more ability to interact with Cu) and low K_d (more efficiency for interacting with Cu).

- The relative biodegradability of WEOMa was surprisingly high for the samples obtained from soils barren of vegetation. Vegetation had little impact on this with the exception of the Puch meadow, which was enriched in DOM that is easily decomposable. However, the absolute substrate amount was significantly affected by vegetation because it was related to the absolute amount of WEOC.

2.2 Seasonal effect of vegetation on quantity, quality, and functions of DOM

The seasonal effect of vegetation was assessed with the same site-sets and presented here. Soil samples were taken in May (spring), July (summer), and October (fall) at Puch site, while in March (spring), July (summer), and December (fall) at the other Spanish sites in 2004.

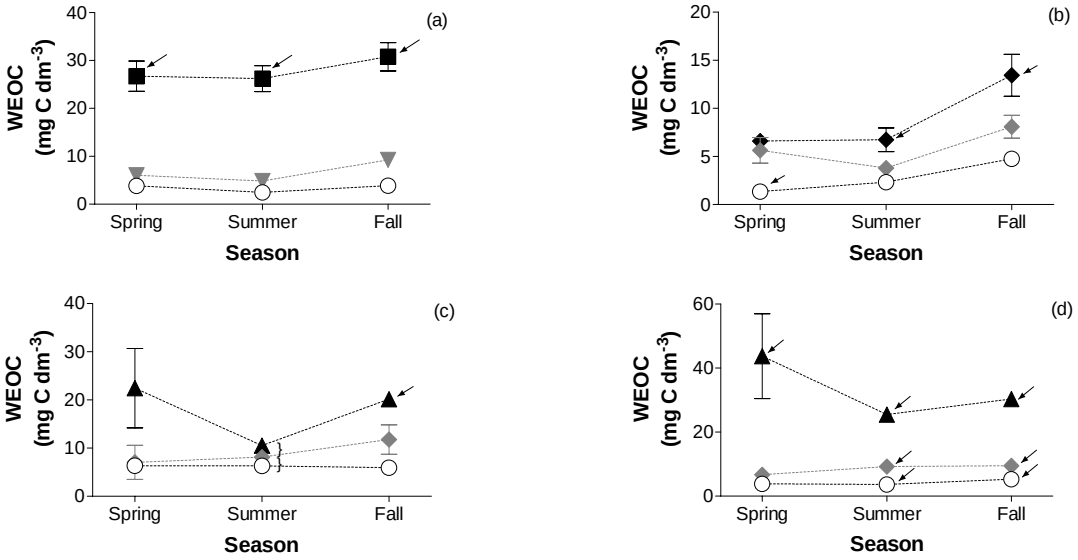

Figure 34 Seasonal development of water extractable organic carbon (WEOC) in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

WEOC in Puch had only minor seasonal variation (Figure 34 (a)). On the other hand, WEOC in the Spanish sites varied over season (Figure 34 (b), (c), (d)). Not unreasonably, the

change was especially significant at the locations with vegetation and relatively small at the bare sites. Furthermore the seasonal effect was greater at the high vegetation coverage locations than at the medium coverage locations. WEOC at the vegetated locations tended to be lowest in summer. WEOC in summer might be consumed by microbes, which are active under warmer temperatures (Marschner and Bredow, 2002) and/or water stress may have restricted rhizoexudation. WEOC had the following tendency at any season: meadow > agricultural plot > bare in Puch, and high plant coverage (HC) > medium plant coverage (MC) > bare in the Spanish sites. This trend was also shown previously in Figure 22.

Both Absorptivity and RSF of WEOM changed a little over the seasons (Figure 35 and 36). For example, both agricultural and meadow WEOM (Puch) decreased their Absorptivity as the year progressed. Meadow WEOM decreased its RSF as well. Also both Absorptivity and RSF of the bare WEOM in Abanilla decreased over the year. The decrease reflected the change in WEOC. It suggests that there was more non-optically active WEOM input in fall at this location. Otherwise no major seasonal change was observed.

FE was also surprisingly stable over the seasons (Figure 37). HIX changed over the year at the three Spanish sites (Figure 38 (b), (c), (d)). However, the change was not consistent at all sites.

B_{max} (Figure 39) and K_d (Figure 40) were constant over seasons, except Los Cuadros. K_d of WEOM from Los Cuadros showed the significant differences between locations in summer: HC > MC > bare. This difference might have been caused by plant activity. However, it was not seen at the other sites, which also had vegetation.

BDOC decreased in fall at Santomera and Abanilla (Figure 41 (b), (c)). The value was, however, constant at the other sites. Since this decrease was also the case for the bare site, it was not a function of vegetation, including litter input. The substrate amount varied over time, even though the variation was not the same at all sites (Figure 42). The value of Puch was relatively constant, reflecting its constant BDOC values. The amount at the vegetated plots decreased strongly in Santomera from spring to summer. The decrease was especially significant at medium plant coverage. On the other hand, the substrate value increased in Los Cuadros and Abanilla, even though this value decreased in fall in Abanilla.

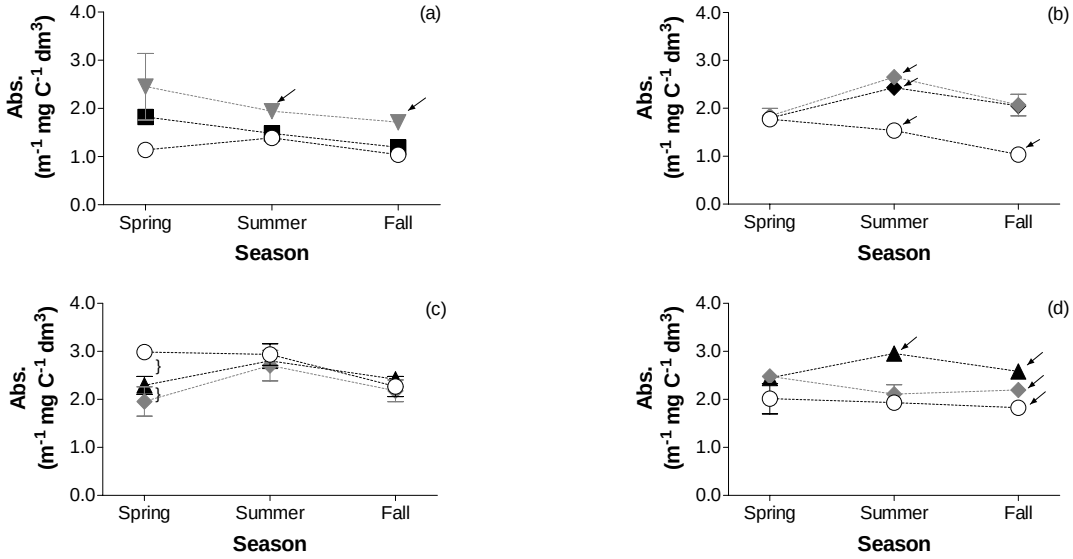

Figure 35 Seasonal development of Absorptivity of WEOC (Abs.) of WEOM in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

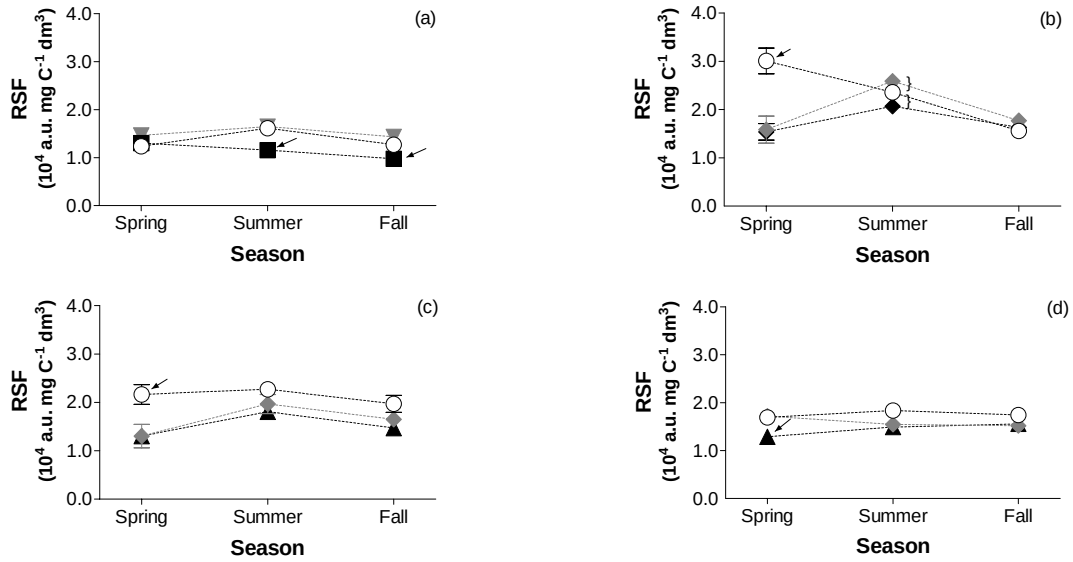

Figure 36 Seasonal development of relative summed fluorescence of WEOM in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

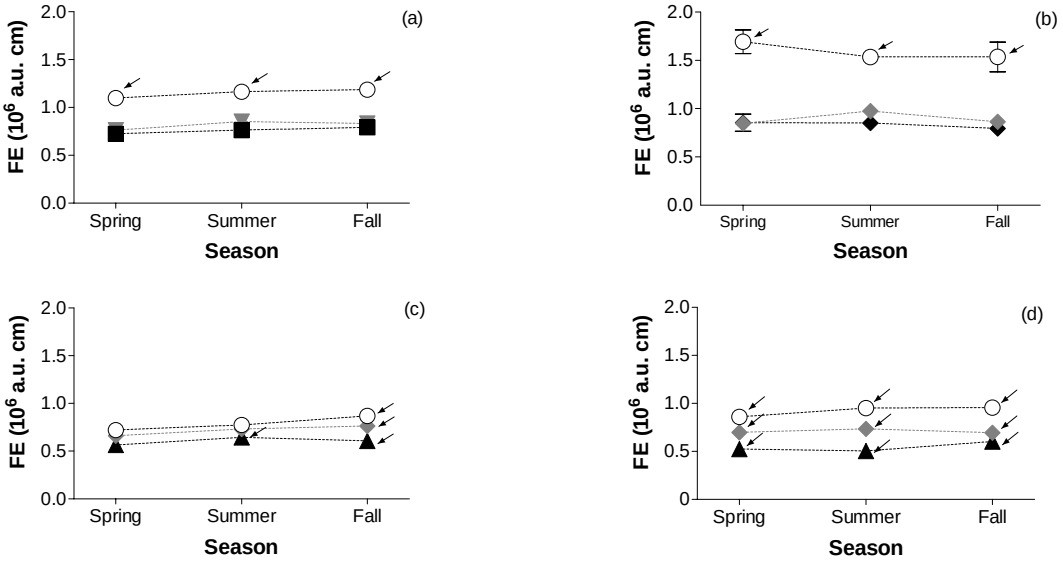

Figure 37 Seasonal development of Fluorescence Efficiency (FE) of WEOM in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

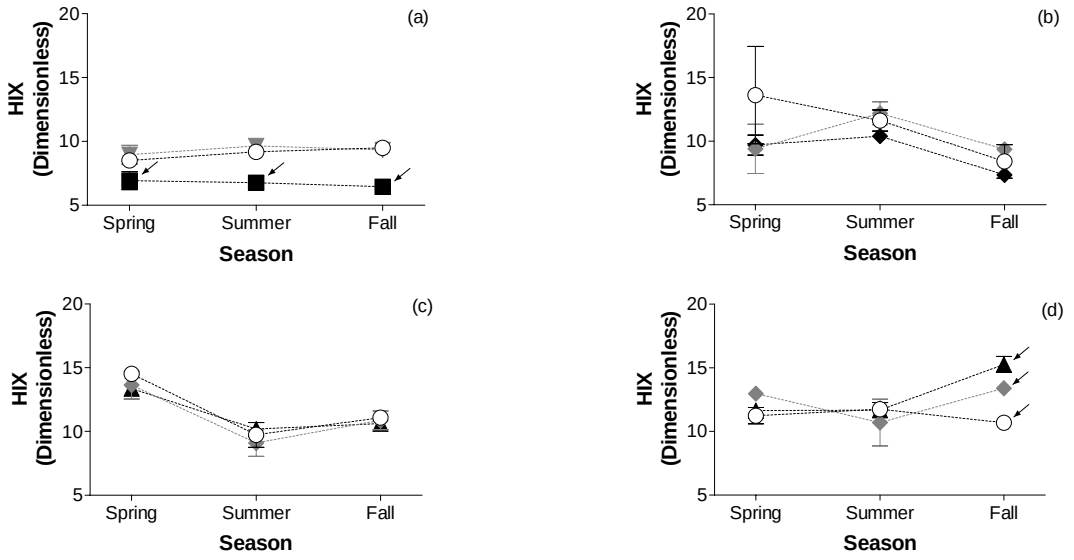

Figure 38 Seasonal development of Humification Index (HIX) of WEOM in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

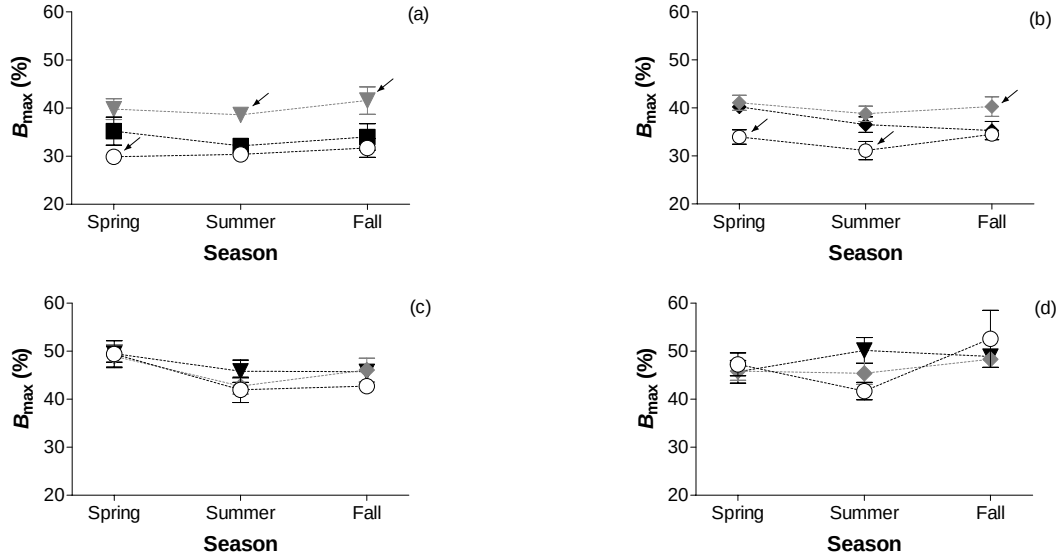

Figure 39 Seasonal development of maximum binding capacity of WEOM (B_{max}) in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

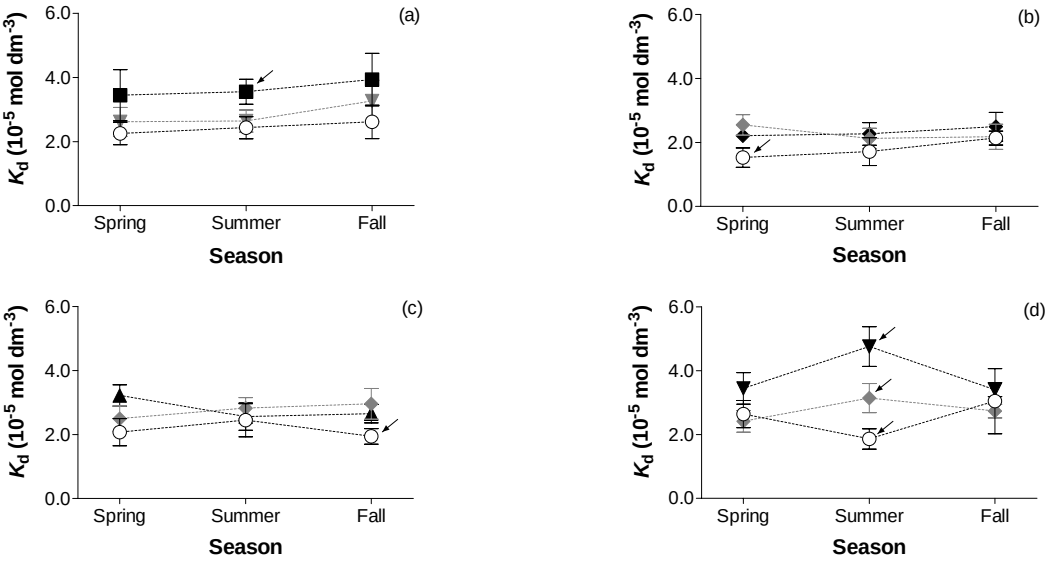

Figure 40 Seasonal development of dissociation constant of WEOM (K_d) in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

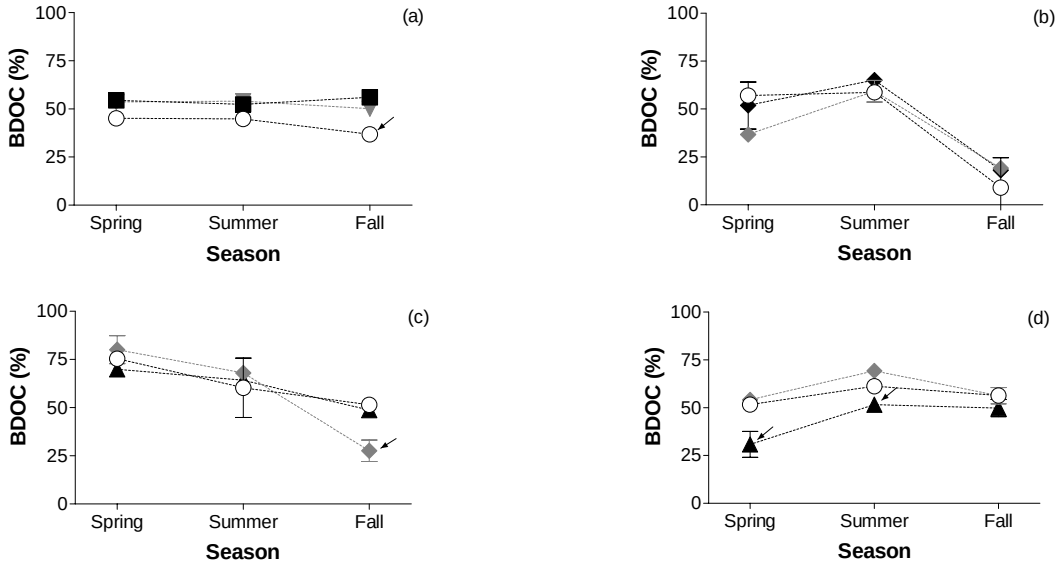

Figure 41 Seasonal development of biodegradability of WEOMa (BDOC) in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

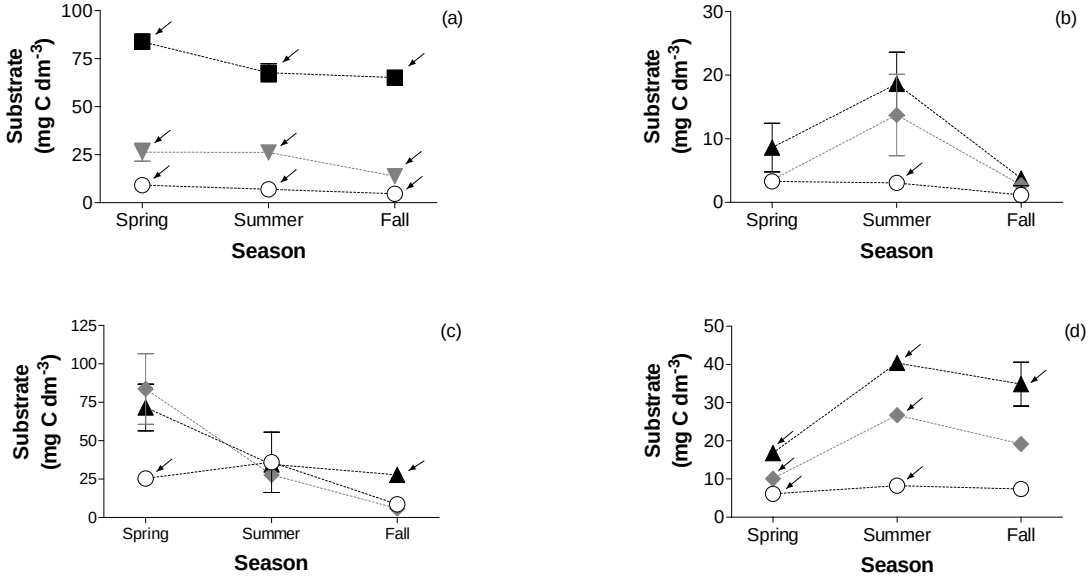

Figure 42 Seasonal development of substrate amount of WEOMa in four different sites (A-horizons). (a) Puch, Bavaria, Germany: ■ meadow, ▼ agricultural plot with winter wheat, ○ bare, (b) Abanilla, Murcia, Spain: ♦ shrub, high coverage (HC), ♦ shrub, medium coverage (MC), ○ bare, (c) Santomera, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare, (d) Los Cuadros, Murcia, Spain: ▲ pine forest, HC, ♦ shrub, MC, ○ bare. Each datum is the mean value of four (Puch) or three (Spanish sites) replicates with the standard error of mean. Arrows show the significant difference ($p < 0.05$).

In summary,

- Little quantitative seasonal variation was observed at the site in Puch, while the chief seasonal impact was observed at the locations with vegetation in the semi-arid Spanish region.
- The quality of WEOM changed surprisingly little in the course of a year.
- The degree of interacting with Cu changed over season only at one site.
- The substrate availability changed over time, but how it changes over time appeared to be site dependent.
- There appeared to be seasonal variations at most of parameters. However, more frequent sampling was obviously needed to determine more precisely the temporal variations.

2.3 Detailed temporal change of vegetation effects on quantity, quality, and functions of DOM

The significant temporal variation, which has been reported by others, was most likely due the fact that most DOM research has been done with extracts obtained from field fresh or air dried soils, while the results here, based on extraction after pre-incubation, reflect more *in situ* steady-state conditions. To expand on this, the temporal change of vegetation effect on WEOM was observed in Puch in greater detail. To do this, soil samples were frequently taken (every second week, except in February 2005 when the ground was frozen) from June 2004 until June 2005.

The results confirm that vegetated plots have more WEOC throughout an entire year (Figure 43). A paired t-test indicates that the values for both the agricultural and meadow were significantly larger than for the bare plot ($p << 0.001$ in both cases). Absolutely, meadow WEOC changed more strongly than both agricultural and bare plot WEOC. However on a relative basis, the changes were of the same size. This is difficult to see in Figure 43, because of the large differences in the WEOC values and also of the unevenness of the results. Therefore the figure was redrawn with some elementary curve smoothing. First the results for WEOC for a given plot were divided by the average value for the whole year. This placed everything on the same scale. Secondly a running average composed of the average of a value and its 2 nearest neighbors were calculated and plotted against time.

$$X = (X_{i-1} + X_i + X_{i+1}) / 3 \qquad \text{(Eq. 3)}$$

where *X* is the running average value, and i the position of the original values in the time sequence (Figure 44). One can now see that a temporal cycle appears to be present. Values increased during the late summer and then declined in the fall. There was a substantial increase, especially in the agricultural plot during the late fall and early winter. This may have been due to decreased microbial activity and possible cell death during the colder months. This increase may have been more pronounced on the agricultural plot, perhaps due to litter decomposition. It can also be postulated that this WEOC increase may contribute to the well known release of N_2O during free-thaw periods (Chen, 1995). Also of interest is the fact that the bare soil had a similar pattern to the plots with vegetation, even though vegetation itself, especially in the case of an agricultural plot, has a very strong temporal cycle (growing season).

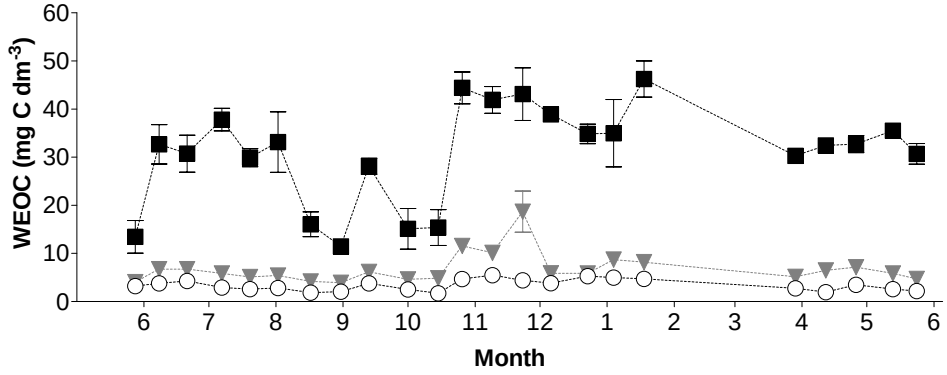

Figure 43 Temporal variation of water extractable organic carbon (WEOC) at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

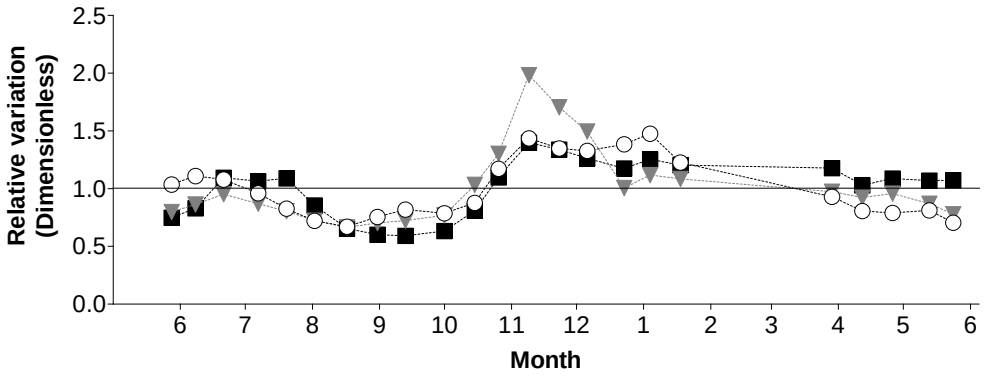

Figure 44 Standardized and smoothed illustration of the results shown in Figure 43. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare. *Cf.* text for details.

The quality of WEOM was fairly constant over time. The Absorptivity of WEOM, which was extracted from the soils that came from significantly different vegetation condition such as bare (plant coverage: 0%) and meadow (plant coverage: 100%), was amazingly similar over the whole year. The Absorptivity of the agricultural WEOM was constantly higher than meadow and bare WEOM (Figure 45). It suggests that the Absorptivity is not affected by the vegetation, but probably more by the land management such as cultivation and litter input. The Absorptivity of the agricultural WEOM increased at late fall, when the litter input was significant. A paired t-test shows no difference between the meadow and the bare plot but very significant differences between the meadow and the agricultural plot ($p < 0.001$). Since the values from the different plots were almost of the same size, it is not necessary to normalize the results as was one for WEOC.

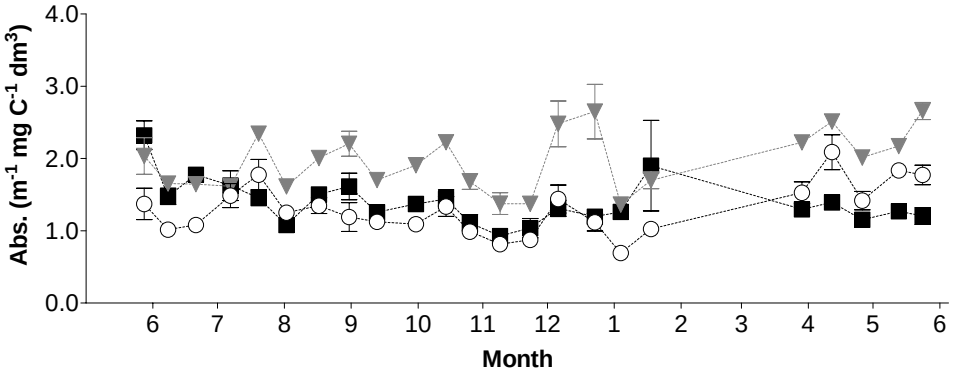

Figure 45 Temporal variation of Absorptivity of WEOM at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

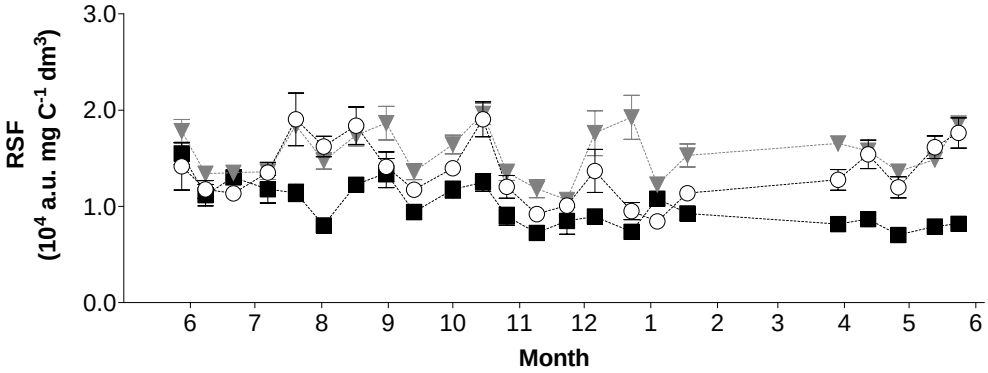

Figure 46 Temporal variation of relative summed fluorescence of WEOM (RSF) at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

As opposed to Absorptivity, RSF had a constant difference (paired t-test: $p < 0.001$) between meadow and bare WEOM, even though the difference was small in the early summer and winter (Figure 46). A paired t-test also indicated that the differences over the year between the agricultural plot and both the meadow and bare plots were significant (in both cases $p < 0.001$). This may also be a function of management. No seasonal cycles were detectable.

WEOM from the vegetated plots (meadow and agricultural plot) had significantly ($p < 0.001$) lower FE than that of the bare plot. This trend existed all though the year. Again to better illustrate the temporal stability of FE, the curves of Figure 47 were standardized and smoothed in the same way as for WEOC above and are given in Figure 48. It can be seen that there is almost little temporal variation. This means that FE shows some promise as a stable soil quality indicator (Zsolnay, 2005).

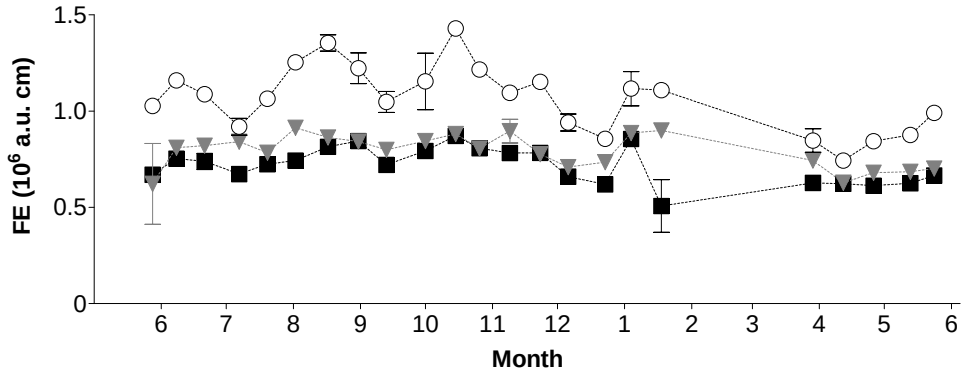

Figure 47 Temporal variation of the Fluorescence Efficiency of Puch WEOM (FE) at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

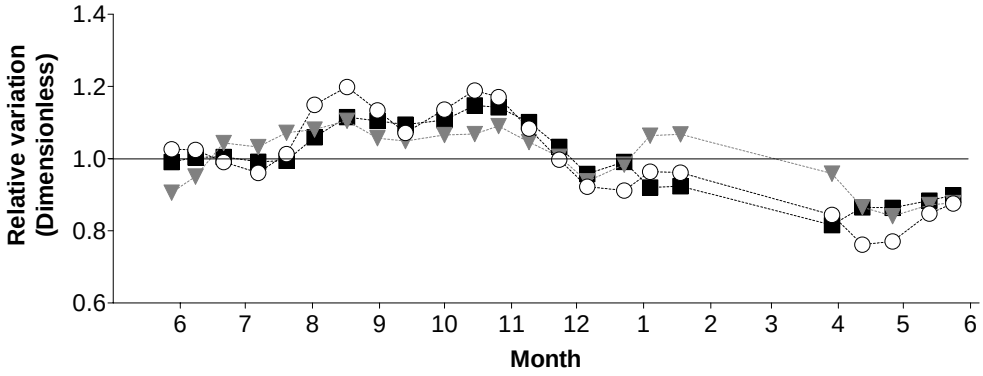

Figure 48 Standardized and smoothed illustration of the results shown in Figure 47 (Relative variation, dimensionless). Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare. *Cf*. text for details. To improve comparison, the figure is drawn on the same scale.

HIX showed also difference constantly between plots: bare = agricultural (p = 0.14) > meadow (p << 0.001) (Figure 49). As was presented in section VI 2.1, more organic matter input from plant roots occurred under meadow. However, the temporal variations seem to be too small and not corresponding to season. For example, the root activity should be high in summer and the roots excrete more exudates. Therefore, logically speaking, HIX of meadow WEOM in summer should be lower than winter time. This trend was, however, not observed. On the other hand plant activity can decrease the amount of water available for the diffusion of low HIX, rhizoexudation WEOM into bulk soil. This should result in a higher HIX.

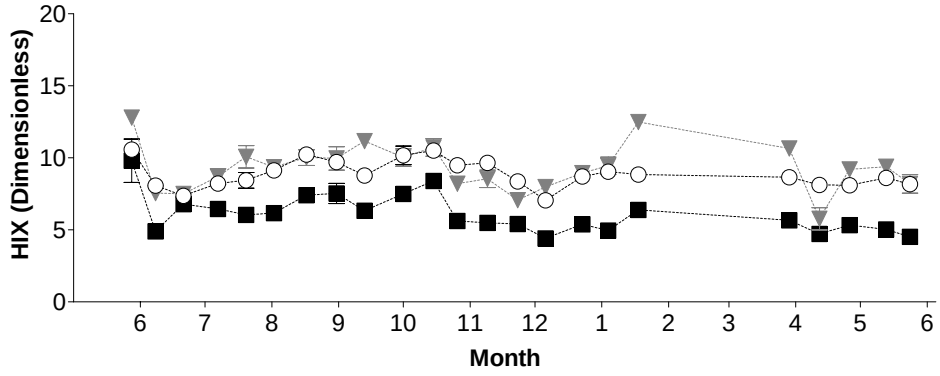

Figure 49 Temporal variation of Humification Index of WEOM (HIX) at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

B_{max} of agricultural WEOM was significantly (p << 0.001) higher than the others at any time (Figure 50). Bare and meadow WEOM had very similar binding capacity to Cu. Again, it appears that land management has more influence than the vegetation.

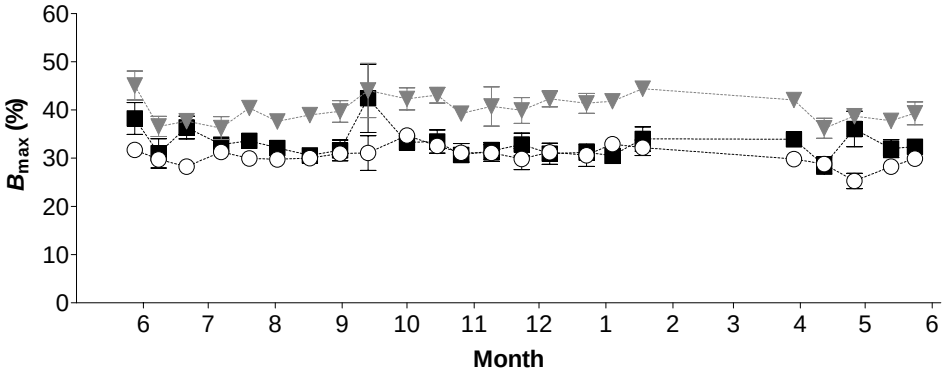

Figure 50 Temporal variation of the maximum binding capacity of WEOM (B_{max}) at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

The overall K_d results (Figure 51) indicated that the plots with vegetation had higher K_d values than the bare one ($p < 0.01$ in both cases). This result supports the hypothesis: more plant input, more K_d (*i.e.*, less efficiency for interacting with Cu). No seasonal trend was apparent.

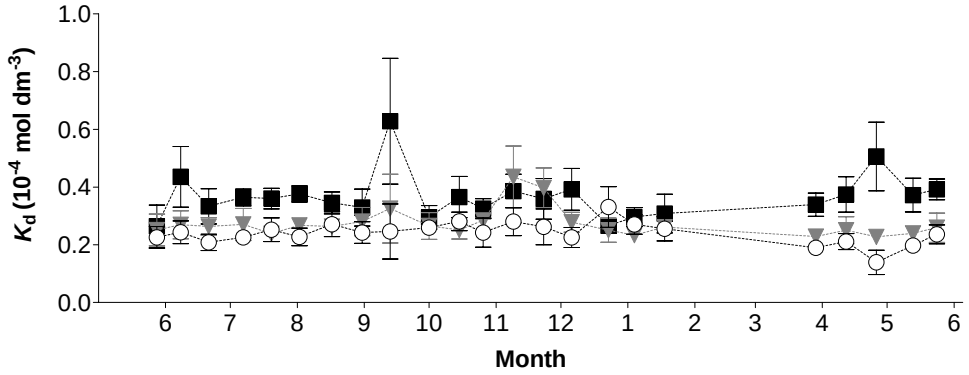

Figure 51 Temporal variation of the dissociation constant of WEOM (K_d) at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

BDOC of meadow and agricultural WEOMa were similar, while bare WEOMa had a slightly lower but highly significant ($p < 0.001$) biodegradability (Figure 52). Amazingly enough, this value was very similar and constant over time. Always about 50% of WEOCa was microbially degradable. It suggests that the quality of potentially available DOM by microbes is not influenced by the vegetation. However, since the total amounts of WEOCa were different among these plots, the absolute substrate values are indeed influenced by vegetation and were always meadow > agricultural > bare ($p << 0.001$) (Figure 53).

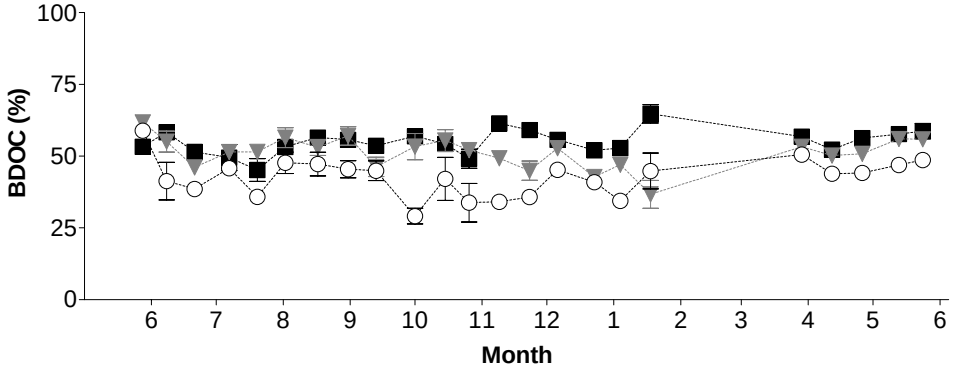

Figure 52 Temporal variation of biodegradability of WEOMa (BDOC) at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

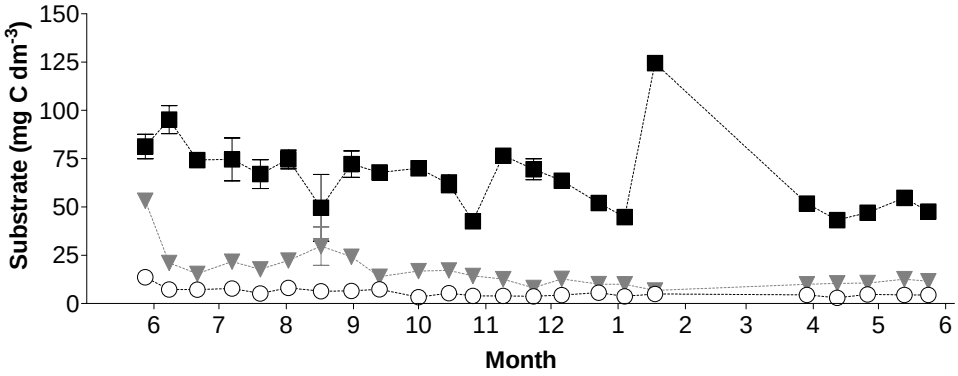

Figure 53 Temporal variation of substrate of WEOMa at three plots in Puch, Bavaria, Germany (A-horizons). Sampling was from June 2004 until June 2005. Symbols: ■ meadow, ▼ agricultural plot with winter wheat, and ○ bare.

In summary,

- Absolute WEOC values at the vegetated plots varied significantly over time. However, the relative values indicated the temporal variations at three plots were similar.
- The quality of WEOM also had temporal variation, but to a considerably lesser degree compared to WEOC.
- The functions of WEOM in regards to biodegradability (BDOC) and B_{max} did not change over time. B_{max} is probably determined by the quality of SOM.
- K_d was basically stable over the whole measurement period. However, more plant input makes the relative ability of WEOM to interact with Cu weaker. However, since vegetated plots had more WEOM, the net effect is that vegetation increases the total amount of WEOM, which can interact with Cu.
- Even though the BDOC was similar at all plots, the absolute substrate amounts were more at the vegetated plots all though the year.

2.4 Effect of the vegetation type on quantity, quality, and functions of DOM

The investigated plots were mainly located in Puch, Bavaria, Germany. This site had agricultural plots (both monoculture and rotation) with following crops: winter wheat (WW), oats, barley, sugar beet (SB), red clover (RC), potatoes, and natural grasses. The catenae in the Murcia region, Spain (*i.e.*, Abanilla, Santomera, and Los Cuadros) had locations with native shrubs and pine trees. The presented data are the mean values of all seasons and from different plots/locations, where the same vegetation was present. For example, the mean value was taken from the data of both pine plots in Santomera and Los Cuadros. For convenience, plant name

will be used instead of WEOM extracted from the plot/location with the plant. For example, WW stands for WEOM from the plot with a WW crop at the sampling period.

Grass and pine had strikingly higher SOC than the agricultural crops; while shrub had the lowest SOC content. These differences were statistically highly significant ($p < 10^{-40}$, Table 14). SOC was similar for the agricultural plots. The least SOC containing shrub location had lower litter input than the locations with other plants (Table 2). Although meadow should not have significant litter input due to the mowing and removal during summer, the plot contained high SOC. This suggests that there was a significant organic matter input from grass roots. In general, more WEOC was available at the plots with more SOC. The plots with developed vegetation such as grass, shrub, and pine had higher values ($p < 10^{-4}$). Since both shrub and pine were in the Murcia region and the rest were in Puch, this can possibly not be explained only by the difference in plant types. For example the pH of WEOM filtrates for shrub and pine was about 8; while the one for Puch samples was about 6 (Table 4, 5). Soil conditions were also different (Figure 4, 5). Nevertheless, the net available WEOC was most likely indeed partially dependent on the vegetation types (Figure 54).

WEOC was similar for all the agricultural crops. However, when only the monoculture plots were compared (*i.e.*, WW, potato, SB), there were differences in WEOC (Figure 55). Even though the investigated plant species were different, the difference in WEOC caused by agricultural plant species has been reported by Chantigny et al. (1997). WEOC concentration was generally higher under legumes than under gramineae species. He pointed out that the difference was the reflection of the different root exudation patterns, since legumes can exude significantly more soluble molecules than gramineae to signal their presence to the rhizobia and to rapidly initiate the formation of root nodules and nitrogen fixation. This kind of process could be observed for red clover, however an influence on WEOC was not observed. Such a difference can hardly be observed until WEOM is extracted immediately after the soil sampling and immediately analyzed, because root derived organic matter is known to be easily metabolized (Barber and Martin, 1976; Xu and Juma, 1993), or such a plant effect can be observed only within the rhizosphere. On the other hand, the results in Figure 55 showed the difference in WEOC caused by the plant species can be observed, when an agricultural plot had the same type of crop for long time. SOC was also influenced by the plant types: WW, SB > SB, potato ($p < 0.05$), even though the trend was not the same as for WEOC. The high WEOC for SB, however, was probably caused more by straw, because this plot had straw while the crop was growing. WEOC/SOC was not different among these plots.

Table 14 Soil organic carbon (SOC), water extractable organic carbon (WEOC), and their ratio at the plots with different types of vegetation (A-horizons). Sampling plot, see Fig. 54. Letters next to figures show the significant difference between plots ($p < 0.05$).

Sampling plot	SOC (g 100 g^{-1})	WEOC (µg g^{-1})	WEOC/SOC (%)
WW	1.3 (a)	14.7 (a)	0.12 (a)
Oats	1.5 (a)	15.4 (a)	0.11 (a)
Barley	1.5 (a)	14.4 (a)	0.09 (a)
SB	1.2 (a)	18.5 (a)	0.15 (a,b)
RC	1.4 (a)	16.1 (a)	0.11 (a)
Potato	1.3 (a)	15.0 (a)	0.12 (a)
Grass	2.6 (b)	58.5 (b)	0.22 (b)
Shrub	1.0 (c)	18.5 (a)	0.20 (b)
Pine	2.6 (b)	50.9 (b)	0.21 (b)

Note: SOC was measured only once in spring 2004 (3 replicates per plot). WEOC is the mean value of seasonal sampling, namely data obtained in spring, summer, and fall 2004 (9 replicates per plot). Therefore the ratio was calculated through dividing individual WEOC by the mean SOC value. The presented ratio values are the mean of 9 replicates.

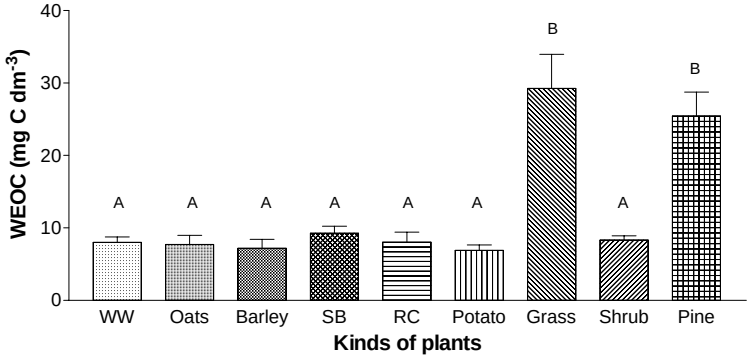

Figure 54 Water extractable organic carbon (WEOC) from pre-incubated soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

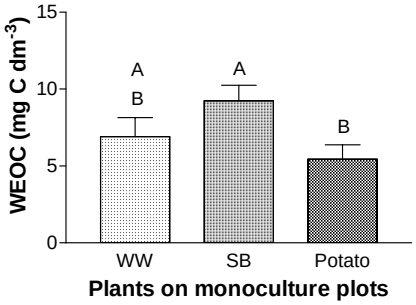

Figure 55 Water extractable organic carbon (WEOC) from pre-incubated soils taken at monoculture plots in Puch, Bavaria, Germany (Ap-horizons). WW: winter wheat, SB: sugar beets. Presented data is the average value of all data of all seasons in 2004 with the standard error of mean. Different letters indicate significant differences ($p < 0.05$).

The quality of WEOM in regards to its optical properties such as Absorptivity and RSF are presented in Figure 56 and 57, respectively. Absorptivity indicates the aromaticity of WEOM. Pine WEOM had the most aromatic WEOM, following that, shrub and three agricultural crops (barley, sugar beet, potato), and then the other agricultural crops and grass. Even though there appears to be a difference among the agricultural crops, the difference was not so significant. This result suggests again that the WEOM under trees including shrub is composed of more aromatic DOM. This is not unreasonable when one considers the high input of lignin containing litter is present in those vegetative environments. The WEOM from the other locations or plots, especially meadow, is probably more strongly derived from rhizoexudation than from litter.

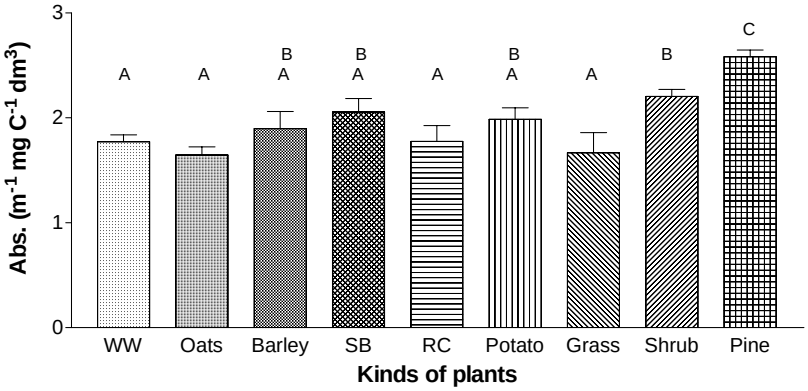

Figure 56 Absorptivity of WEOM (Abs.) extracted from pre-incubated soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

The RSF shows that the investigated WEOM from various plants can be divided into two groups. If only agricultural crops were considered, there appears to be a difference between "above-ground" (WW, oats, barley, and RC) and "underground" crops (potato and sugar beets). There could be a difference in the root exudation pattern between these crops. However, it is only a hypothesis, because no information was available for the root crops in regards to their root exudation pattern.

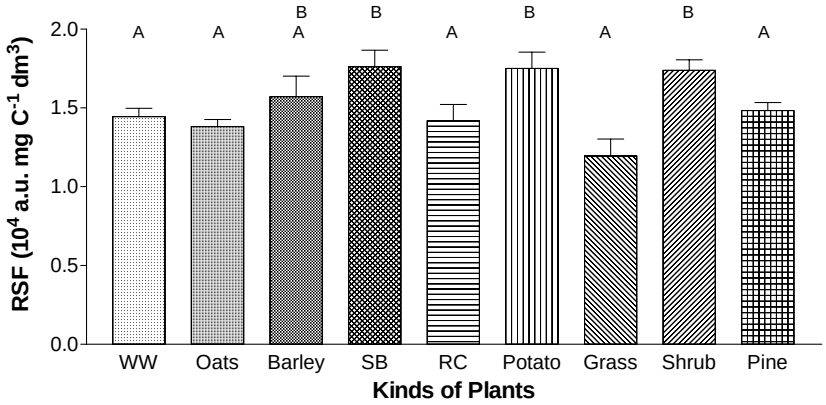

Figure 57 Relative summed fluorescence of WEOM (RSF) extracted from pre-incubated soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

FE indicates that the growth of "natural" vegetation can result in a decrease FE, even though it was only statistically significant for pine (Figure 58). These results most likely reflect the pine forests produced abundant litter, which was left on the location. This was not true for the other vegetation. This litter was presumably enriched in aromatic lignin, which absorbs UV light but does not fluoresce very well. The monoculture crops also had difference between WW and the rest (Figure 59). WW is the only crop, which produces substantial plant litter that is for example about 5 times more than the potato's (Köhnlein and Vetter, 1953). Since this crop had also lower FE, this difference could be reflecting the difference in the amount of litter input.

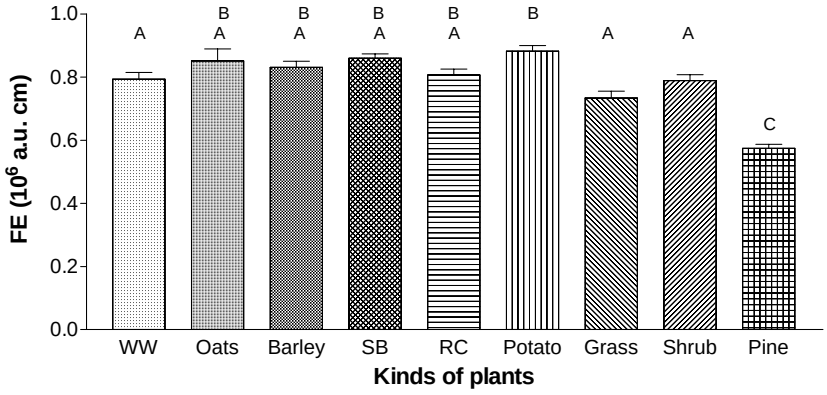

Figure 58 Fluorescence Efficiency of WEOM (FE) from pre-incubated soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

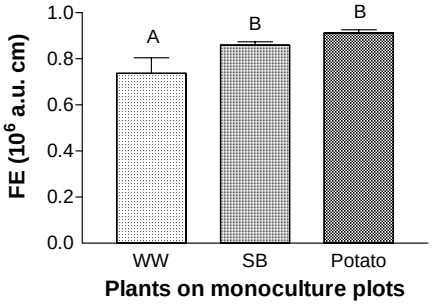

Figure 59 Fluorescence Efficiency of WEOM (FE) extracted from pre-incubated soils taken at monoculture plots in Puch, Bavaria, Germany (Ap-horizons). WW: winter wheat, SB: sugar beets. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

HIX could be a good indicator of root input, since this parameter shows the degree of condensation of molecules (*i.e.*, higher C/H ratio). HIX is lower if there was a significant root input, since root exudates are known to be composed of relatively uncondensed, simple organic matter and it fluoresces in the short emission wavelength region (Figure 21 (a)). HIX in Figure 60 shows clearly that meadow WEOM is composed of more simple WEOM. Pine WEOM was presumably also composed of enough root exudates; however this plot might have had at the same time a large portion of humified DOM. Since HIX is the ratio between two ranges in a fluorescence emission spectrum, which can be assigned to simple (uncondensed) and complicated (condensed, humified) organic matter, it is not surprising that the pine WEOM had relatively high HIX value.

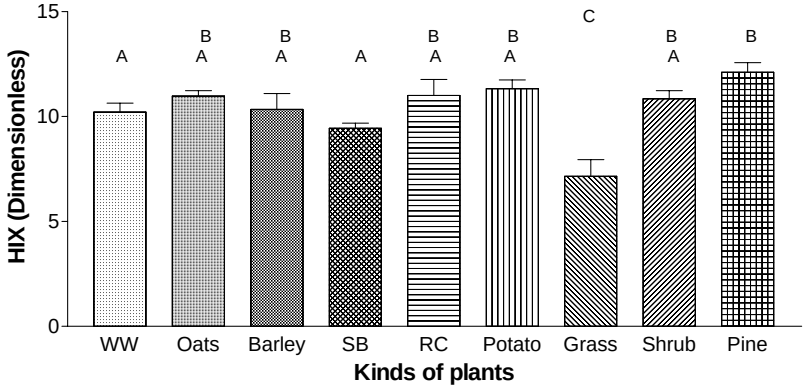

Figure 60 Humification Index of WEOM (HIX) extracted from pre-incubated soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

The relative lack of affinity of meadow WEOM for Cu compared to the WEOM developed under other vegetation is quite pronounced (Figure 61). On the other hand, pine WEOM had a greater binding capacity for Cu. This result suggests again that pine WEOM is more composed of humified DOM, while the meadow WEOM is mainly composed of simple WEOM.

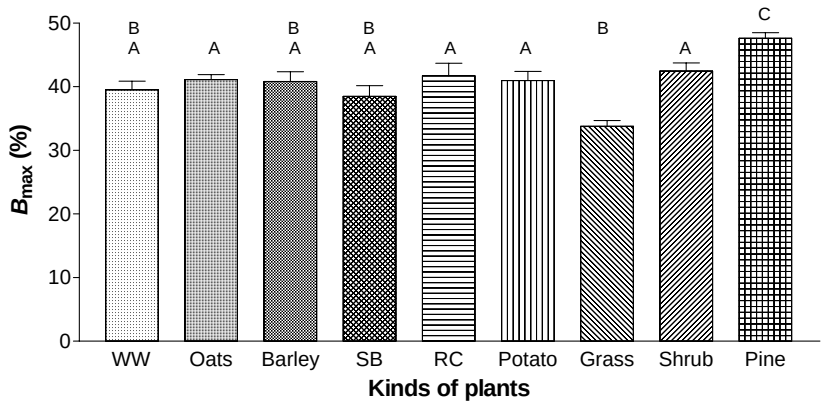

Figure 61 Maximum binding capacity of WEOM (B_{max}) extracted from pre-incubated soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

Both grass and pine WEOM interacted with Cu less rapidly (higher K_d values, Figure 62). It was therefore confirmed that more plant input results in weaker interaction with Cu.

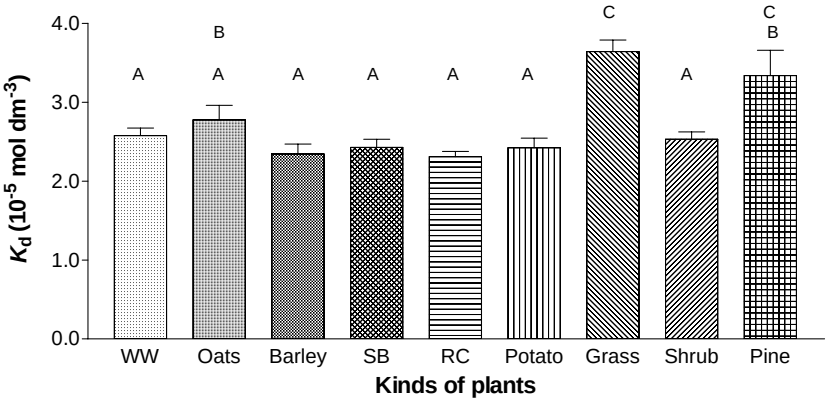

Figure 62 Dissociation constant of WEOM (K_d) extracted from pre-incubated soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

Even though the results presented so far showed some differences between different kinds of plants, the BDOC was not at all affected (Figure 63).

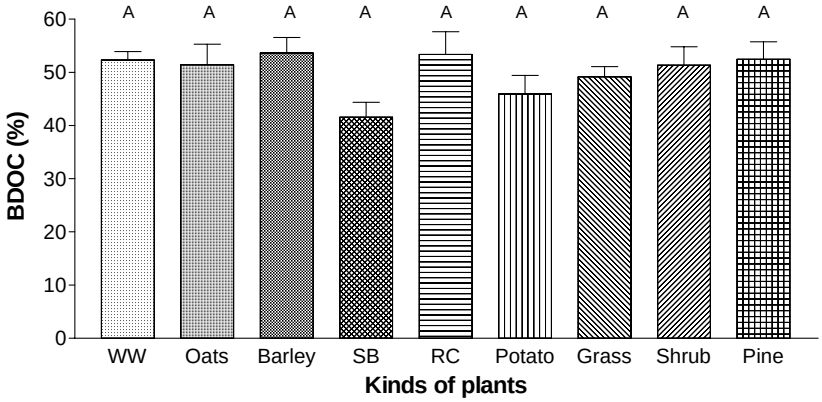

Figure 63 Biodegradability of WEOMa (BDOC) extracted from air-dried soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

It implies that the WEOM functionality as a substrate in bulk soil was similar. This is somewhat surprising in that trees are the source of substituted phenols such as tannin, which can suppress microbial activity. One can conjecture that they are efficiently incorporated in the solid matrix, such as the humic acids, of soils. It must also be kept in mind that in this study mainly non-rhizosphere soil was sampled. Within the rhizosphere significant differences may certainly exist. In regards to the absolute amounts of potential substrate (Figure 64), grass resulted in the significantly highest amounts, followed by that of pine. This reflects the fact that grass and pine resulted in the highest WEOC concentrations.

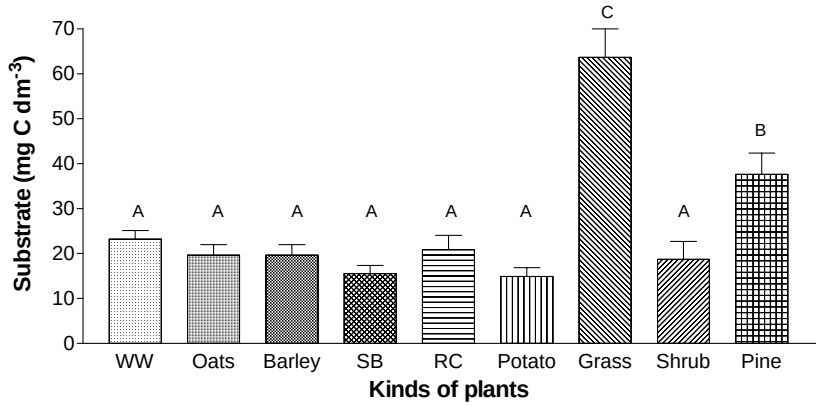

Figure 64 Substrate of WEOMa extracted from air-dried soils taken at the plots/locations, which had different plant species (A-horizons). WW: winter wheat, SB: sugar beets, RC: red clover. Presented data is the average value of all data of all seasons in 2004 with standard error of mean. Different letters indicate significant differences ($p < 0.05$).

In summary,

- WEOM in the pine forests and grassland was significantly different from the WEOM in the agricultural plots and shrub locations in regards to their quantity, quality, the ability to interact with Cu, and the absolute substrate amount for microbes.
- WEOM in the pine forests was composed of more humified DOM, while WEOM in the grassland was composed of simple DOM such as root exudates. This may reflect the higher litter (leaves and branches) input of the former.
- The biodegradability of WEOCa was the same irrespective of vegetation type.
- Among agricultural WEOM, there appeared to be a difference in RSF between "above-ground" and "underground" crops. These crops may have different root exudation patterns.

General summary of vegetation effect on quantity, quality, and functions of DOM

The major effects of the presence of vegetation on WEOM properties are;

- More WEOC,
- More substrate amount for microbes,
- Lower FE, and
- Lower K_d.

WEOC changed over time, especially at the sampling plots with vegetation in an absolute sense, even though the relative variation was similar at all intensively investigated plots in Puch. On the other hand, the quality indicator FE did not change over time. K_d did not change over time to a large extent, either. However, this parameter was sensitive to the plant input such as leaving remnants on the ground. Otherwise the other properties of WEOM were not so strongly affected by vegetation. If there were, the difference did not have any particular trends.

The difference of WEOM properties caused by different kinds of plants was observed only when one compared agricultural crops and shrubs to grass and pine. The WEOM from the various investigated agricultural crops as well as shrubs was similar for most parameters. Among agricultural crops, only RSF showed difference between "above-ground" and "underground" crops. They might have different root exudation patterns.

In short, the presence of vegetation had a significant influence on WEOM through increasing the amount of background SOM, providing fresh organic matter from plant roots as well as plant litters. The quality and the functions of WEOM appeared to be controlled by the

input through these three factors, namely, litters, roots, and SOM. Therefore the difference in type of plants must play a role in controlling WEOM quality as well, because each plant has different feature and different root exudation patterns. Overall, BDOC was the parameter, which was least influenced by vegetation. It suggests that the microbially potentially available WEOM in non-rhizosphere soils is almost the same quality in any ecosystems, even though the absolute substrate amounts are dependent on the vegetation conditions.

3 Effect of agricultural practices

3.1 Effect of agricultural practices on quantity, quality, and functions of DOM (Rotation vs. monoculture)

Effect of agricultural practice (monoculture vs. rotation) on WEOM properties was investigated at Puch. Selected were:

- Winter wheat (WW) monoculture and rotation plots
- Potato monoculture and rotation plots

These agricultural practices have been performed over 50 years. Presented data here are the mean values of nine replicates per sampling plot (three replicates per plot at each sampling time, in May, July, and October 2004) with the standard error of mean.

The potato rotation plot had significantly higher SOC ($p < 0.01$) and WEOC ($p < 0.05$) than the monoculture counterpart (Figure 65 (b)). However this difference was not observed at the WW plots (Figure 65 (a)), suggesting that the influence of the agricultural practices can be or can not be observed as a function of plant types. Since potato and WW were growing in the same row of the rotation plots, where five different agricultural crops were alternatively grown every year, both rotation plots had similar WEOC. However, monoculture plots also had similar SOC, WEOC, and their ratio, even though the crops produce different amount of litter. According to Köhnlein and Vetter (1953), WW produces about 5 times more litter than potato does on the same loam soil under the same climate. Similar WEOC/SOC suggests that the organic matter availability was similar at all plots irrespective of vegetation type and agricultural practices.

Table 15 Soil organic carbon (SOC), water extractable organic carbon (WEOC), and their ratio at monoculture and rotation plots in Puch (A-horizons). Letters next to figures show the significant difference between plots with same vegetation ($p < 0.05$).

Vegetation	Sampling plot	SOC (g 100 g^{-1})	WEOC (µg g-1)	WEOC/SOC (%)
Winter wheat	Monoculture	1.20 (a)	13.8 (a)	0.12 (a)
	Rotation	1.40 (a)	15.5 (a)	0.11 (a)
Potato	Monoculture	0.98 (a)	10.9 (a)	0.11 (a)
	Rotation	1.60 (b)	19.0 (b)	0.12 (a)

Note: SOC was measured only once in spring 2004 (3 replicates per plot). WEOC is the mean value of seasonal sampling, namely data obtained in spring, summer, and fall 2004 (9 replicates per plot). Therefore the ratio was calculated through dividing individual WEOC by the mean SOC value. The presented ratio values are the mean of 9 replicates.

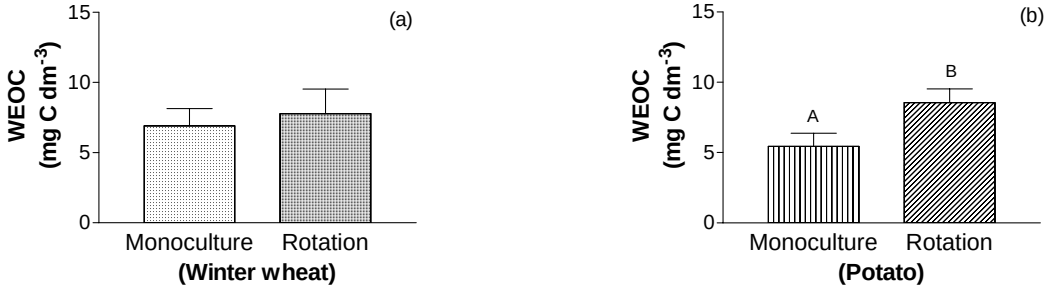

Figure 65 Water extractable organic carbon (WEOC) at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 9 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

Absorptivity (Figure 66) of the WEOM from WW monoculture plot was higher than the rotation counterpart. Even though the difference appeared to be present at the potato plot, the difference was not statistically significant ($p < 0.05$). The results from the WW plots indicate that monoculture produces a WEOM, which is relatively enriched in chromophores.

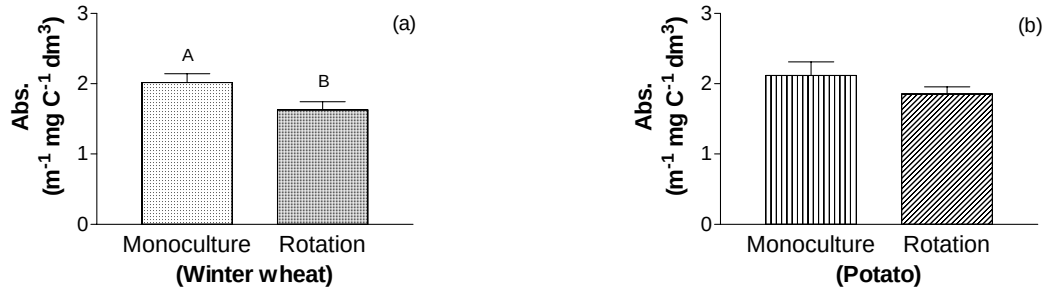

Figure 66 Absorptivity of WEOM (Abs.) at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 9 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

RSF at the WW monoculture plot was again higher than the rotation counterpart. Again, the potato plots appear to have the same trend, but the difference was statistically not significant ($p < 0.05$). This result suggests that the monoculture results in a WEOM, which is relatively enriched in fluorophores (Figure 67).

There was no statistically significant difference ($p < 0.05$) in FE in both crop systems (Figure 68). Monoculture had significant difference between WW and potato. This difference might have been caused by the difference in litter production as was mentioned in section VI 2.4.

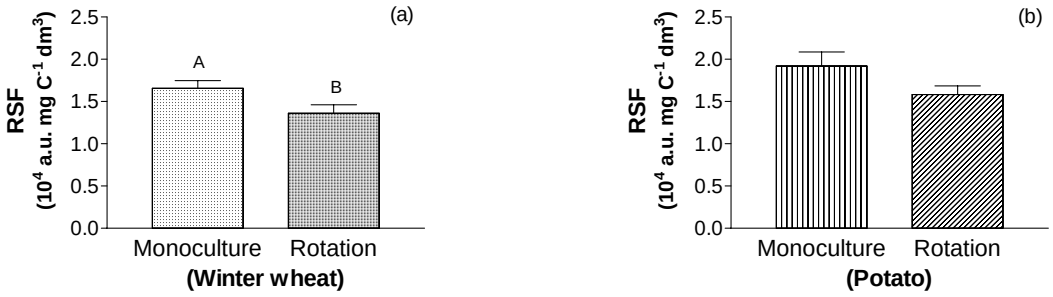

Figure 67 Relative summed fluorescence of WEOM (RSF) at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 9 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

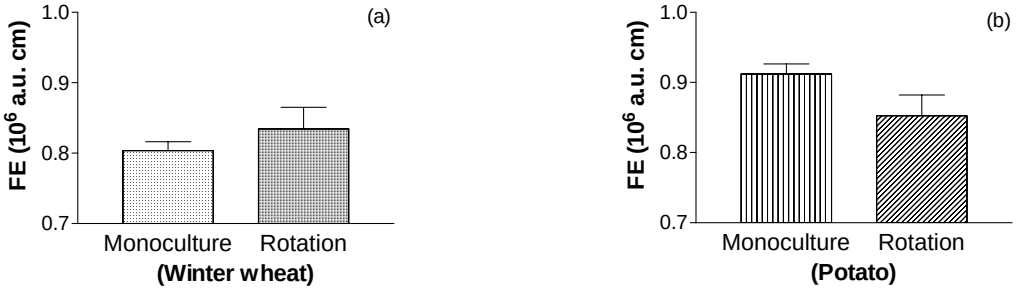

Figure 68 Fluorescence Efficiency of WEOM (FE) at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 9 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

HIX was different at potato plots: rotation > monoculture (Figure 69), suggesting the rotation plot had more humified WEOM. This difference was not observed at the WW plots.

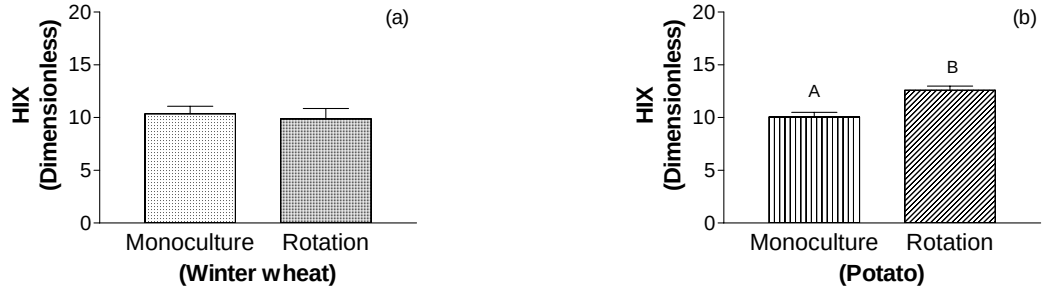

Figure 69 Humification Index of WEOM (HIX) at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 9 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

The maximum Cu binding capacity of WEOM (B_{max}, Figure 70) was higher at the WW monoculture plot than the rotation counterpart. Such difference was not observed at potato plots.

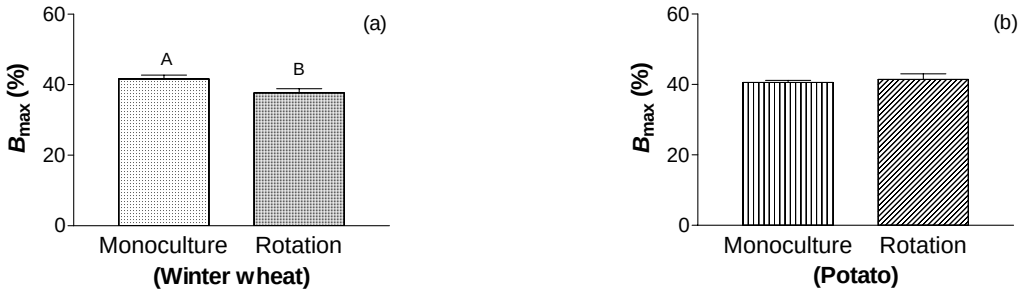

Figure 70 Maximum binding capacity of WEOM at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 3 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

The dissociation constant (K_d, Figure 71) of potato rotation WEOM was lower than the one at the monoculture counterpart. This means that the rotation potato WEOM had efficient interaction with Cu. However, such difference was not observed at the WW plots.

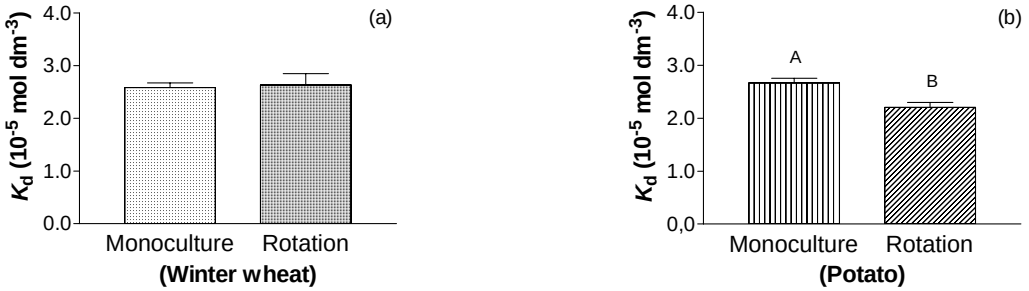

Figure 71 Dissociation constant of WEOM at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 3 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

Biodegradability was not influenced by the agricultural practices at all (Figure 72). The absolute substrate amounts at potato rotation plot had higher value than the monoculture counterpart (Figure 73). Since the BDOC at all plots was similar, the difference in absolute substrate amount was the reflection of the difference in absolute amount of WEOM between the potato plots, which is in turn the reflection of the difference in the litter production among crops.

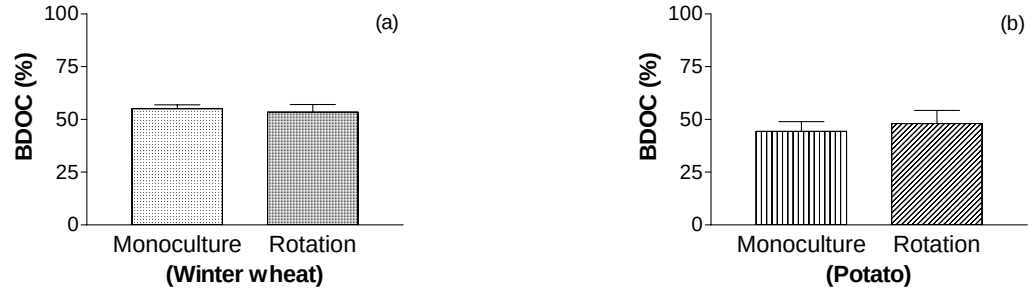

Figure 72 Biodegradability of WEOMa (BDOC) at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 9 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

Figure 73 Substrate of WEOMa at the monoculture and the rotation plots in Puch, Bavaria, Germany (Ap-horizons). (a): plots with winter wheat; (b): plots with potatoes. Presented data are the mean values of 9 replicates with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

In summary,

- The affected parameters by the agricultural practices at potato plots (*i.e.*, WEOC, HIX, K_d, and substrate) were different from those of the WW plots (*i.e.*, Absorptivity, RSF, and B_{max}). The remaining parameters did not show any difference caused by the different agricultural practices.
- Monoculture enriched both chromophores and fluorophores in a WEOM at the WW plot. This WEOM had also higher Cu interaction ability (low K_d).
- Potato rotation plot had higher WEOC and substrate value, because the BDOC was similar at both plots. This plot had also the WEOM, which is more humified and can interact with Cu efficiently compared to the monoculture counterpart.
- Substrate amount at WW plots was higher than at the potato plots. This might reflect the difference in the litter input between these two crops, even though this kind of difference was not found in section VII 1.4, where a larger number of crops were compared.
- The influence of two agricultural practices, either monoculture or rotation, on WEOM was small. However, potato had significant difference in WEOC, which is presumably caused by the difference in litter production.

3.2 Effect of agricultural practices on quantity, quality, and functions of DOM (Biological vs. Conventional)

The other agricultural practices investigated here were the so called "biological (BA)" and "conventional (CA)" agricultures. The chief difference between these two practices is the type of fertilizers, which was applied, and whether or not pesticides were used. "Biological" agriculture utilizes only natural organic matter such as plant residues and/or manures, while "conventional" agriculture utilizes chemical fertilizers and pesticides. Two Italian agricultural sites (in Tuscany and Basilicata) and one Spanish agricultural site (in Manejo) were investigated. The Italian plots had durum wheat; while the Spanish plots had broccoli. Since broccoli at the Spanish plots was harvested after the summer sampling, no fall samples were provided. Therefore, the presented data here are the mean values of nine replicates per sampling plot (three replicates per plot at each sampling time, in March, August, and December 2004) at the Italian sites, while six replicates (in March and October 2004) at the Spanish site with the standard error of mean. Since different kinds of organic manures were applied (*i.e.*, green manure to Italian sites; while a mixture of green and animal manure to Spanish site), the data obtained for these sites can also give the insight into the effect of animal manure as oppose to green manure on WEOM.

SOC was similar for all plots (Table 16). The available WEOC was significantly higher for Manejo compared to Italian samples ($p < 10^{-7}$). Therefore, the ratio of WEOC and SOC was also significantly high in Manejo ($p < 10^{-9}$). Since there are a number of explanations for these differences between Italian and Manejo sites, the comparisons should be restricted to within each site.

Irrespective of the different agricultural practices, which had been carried out over 5 years, no difference was observed for SOC, WEOC, and their ratio in Tuscany and Basilicata. Therefore, one can conclude that the green manure does not have strong impact on the organic matter content and its availability in these sites over the investigated time period. Manejo plots, however, had significant difference in WEOC ($p < 0.001$) and WEOC/SOC ($p < 0.005$), even though SOC was not significantly different. BA contained more WEOC and had higher WEOC/SOC than the CA counterpart. This high WEOC availability at the BA was presumably caused by the usage of organic fertilizer. The mixture of goat manure and grape residue had apparently a strong impact, by providing more readily water soluble organic matter. Therefore, the type of fertilizer appeared to be important controlling factor of the available amount of WEOC.

Table 16 Soil organic carbon (SOC), water extractable organic carbon (WEOC), and their ratio at biological and conventional agriculture plots at three different sites (A-horizons). Letters next to figures show the significant difference within each site ($p < 0.05$).

Sampling site	Sampling location	SOC ($g\ 100\ g^{-1}$)	WEOC ($\mu g\ g^{-1}$)	WEOC/SOC (%)
Tuscany	BA	0.77 (a)	11.5 (a)	0.15 (a)
	CA	0.95 (a)	14.6 (a)	0.15 (a)
Basilicata	BA	0.69 (a)	8.4 (a)	0.12 (a)
	CA	0.81 (a)	11.5 (a)	0.14 (a)
Manejo	BA	0.94 (a)	95.7 (a)	1.02 (a)
	CA	0.68 (a)	25.9 (b)	0.38 (b)

Note: SOC was measured only once in spring 2004 (3 replicates per plot). WEOC is the mean value of seasonal sampling, namely data obtained in spring, summer, and fall 2004 (9 replicates per plot). Therefore the ratio was calculated through dividing individual WEOC by the mean SOC value. The presented ratio values are the mean of 9 replicates.

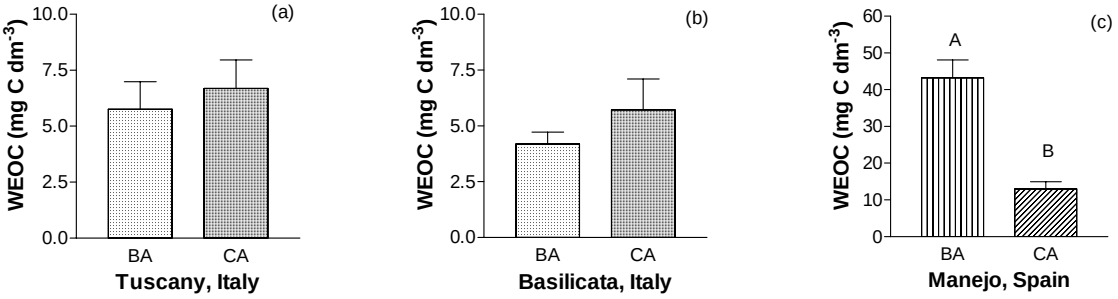

Figure 74 Water extractable organic carbon (WEOC) at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

The difference in Absorptivity was again only observed at the Spanish site: BA > CA (Figure 75). This is not unreasonable, since organic fertilizers, especially the animal manure, will contain optically active material while inorganic ones do not.

RSF showed the same trend as Absorptivity had at only the Spanish site (Figure 76). Italian sites did not show any differences.

FE was hardly influenced by the difference of the type of fertilizers at all sites. (Figure 77). Even the Spanish site did not show any difference.

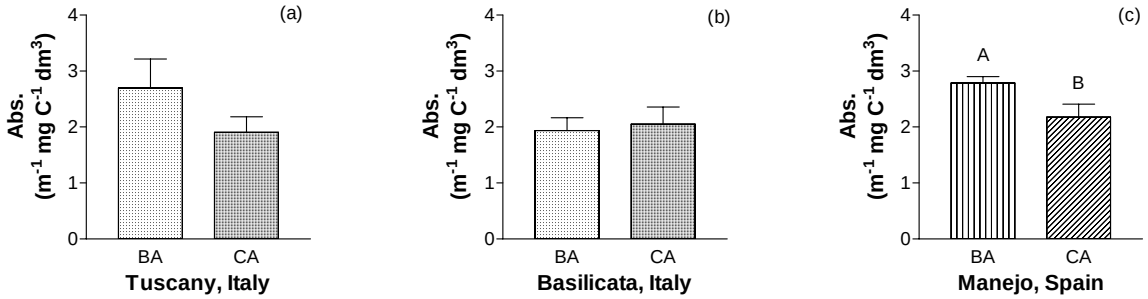

Figure 75 Absorptivity of WEOM at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

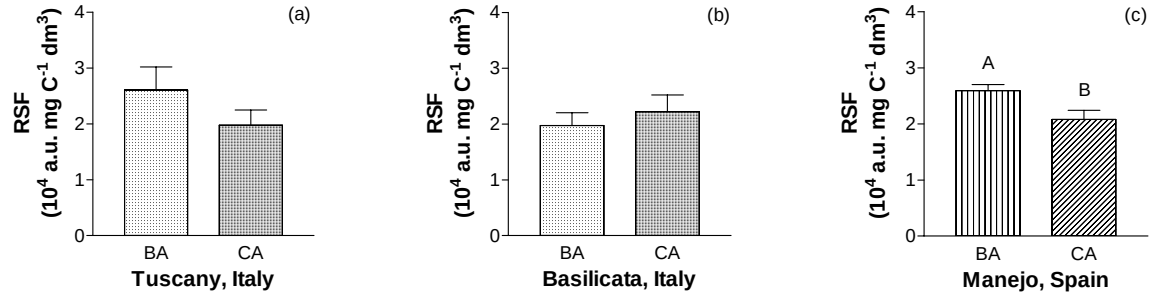

Figure 76 Relative summed fluorescence of WEOM (RSF) at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

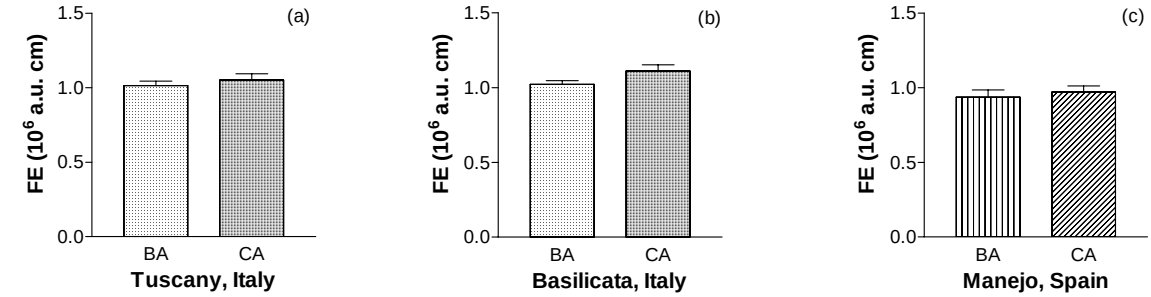

Figure 77 Fluorescence Efficiency of WEOM (FE) at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

HIX had difference at Basilicata and Manejo sites, but the trend was different (Figure 78). Basilicata had the trend: CA > BA, while Manejo had the inverse trend: BA > CA. The difference at the Manejo site was especially significant, because BA WEOM had higher HIX (*ca*. 15); while CA WEOM had lower HIX (*ca*. 6). Most of samples analyzed in this dissertation had HIX range in between these two values, indicating that the Manejo BA plot had very humified organic matter, which is probably derived from the animal manure; while the Manejo CA plot had less humified one.

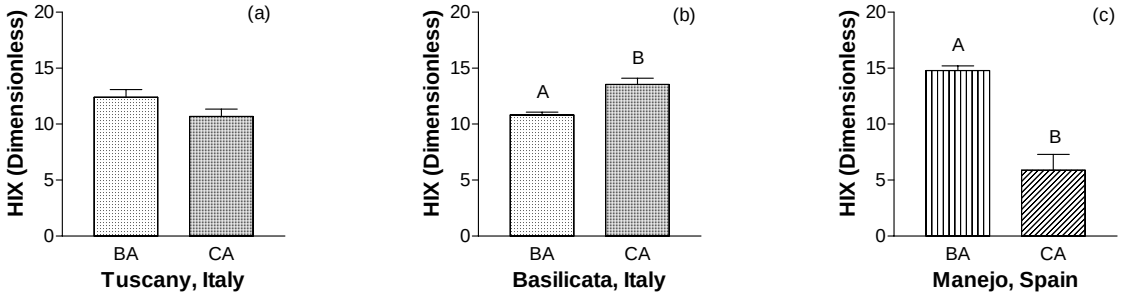

Figure 78 Humification Index of WEOM (HIX) at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

The maximum Cu binding capacity of WEOM (B_{max}) showed the difference at Tuscany and Manejo sites: BA > CA (Figure 79). However, such difference was not observed at Basilicata site. Since these two sites had different soil texture between BA and CA plots (Figure 6), this difference might have been caused by the soil condition, since the Basilicata site does not have a difference in soil texture.

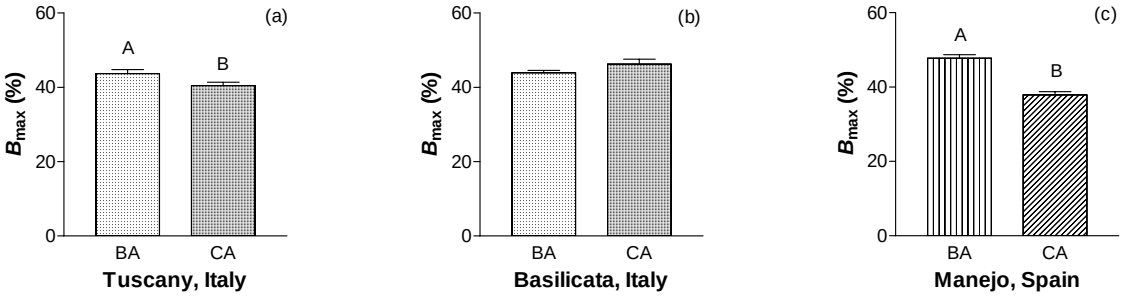

Figure 79 Maximum binding capacity of WEOM (B_{max}) at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

K_d was influenced at Basilicata and Manejo sites: CA > BA (Figure 80). These differences are corresponded to the difference in HIX. More humified WEOM has more efficient (lower K_d) interaction with Cu. Again, Manejo BA WEOM, which had very high HIX, has very low K_d value.

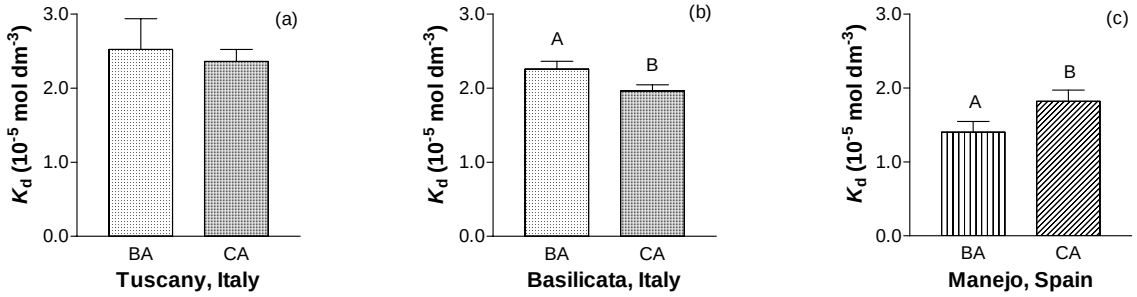

Figure 80 Dissociation constant of WEOM (K_d) at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

BDOC in Manejo was significantly different between the two different practices (Figure 81). BA had significantly lower value than CA WEOMa. This result confirmed the explanation for Figure 74. High WEOC content at the Manejo BA plot was indeed because of the low biodegradability. This is somewhat surprising and would indicate that the added organic material is rapidly brought into a state (either through sequestration or metabolism), where it is no longer rapidly utilizable, or originally more refractory DOM existed. HIX indicated that the WEOM at BA plot in Manejo site was composed of more humified WEOM.

Because of the difference in BDOC at Manejo site, the substrate amount was similar at both plots (Figure 82), even though the soil at the BA plot contained significantly high WEOCa (data not shown). The remaining two Italian sites did not show any differences, suggesting the green manure behaves in the same manner as the chemical fertilizers.

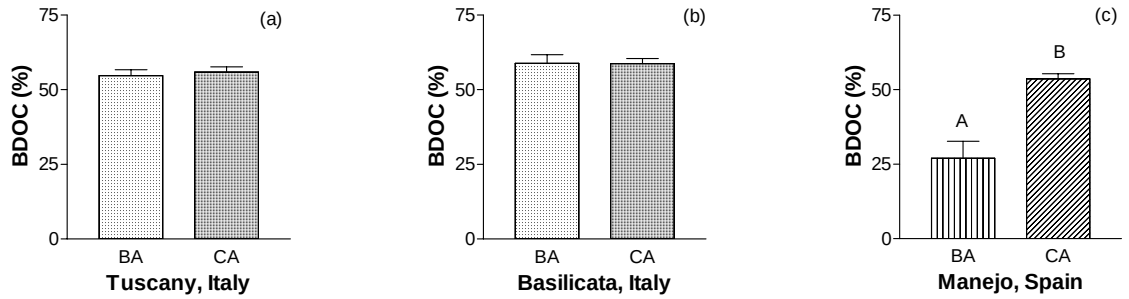

Figure 81 Biodegradability of WEOMa (BDOC) at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

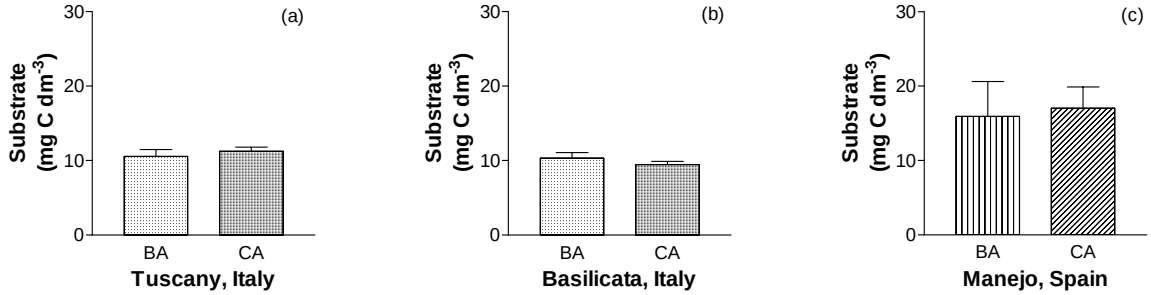

Figure 82 Substrate of WEOMa at the biological and the conventional agricultural plots in three different sites (Ap-horizons). (a) Tuscany, Italy, (b) Basilicata, Italy, (c) Manejo, Spain. Durum wheat was cropped in all Itaian plots; while broccoli was cropped in Manejo plots. Green manure was applied to the Italian BA plots; a mixture of grape residue and goat manure was applied to the Manejo BA plot. Chemical fertilizers were applied to all CA plots. Presented data are the mean values of 9 (Italian sites) or 6 replicates (Spanish site) with the standard error of mean. Letters in the graphs show the difference is significant ($p < 0.05$).

In summary,

- The quantity, quality, and functions of WEOM from two plots at the Manejo site were significantly influenced by the two different agricultural practices; while the WEOM properties in the other two Italian sites were hardly affected. The impact of animal manure on WEOM as opposed to the green manure appeared to be stronger.
- Since the Manejo BA plot had higher amount of WEOM, which had higher B_{max} (more ability to interact with Cu) and low K_d (more efficient interaction with Cu), it might have had significant influence on the heavy metals *in situ*.
- The difference in soil texture between BA and CA plots at Tuscany and Manejo sites appeared to have an influence on the WEOM function in regards to the ability to interact with Cu (B_{max}), which may be influenced by the soil texture.

General summary of effect of agricultural practices on quantity, quality, and functions of DOM

The effects of the following agricultural managements on WEOM properties were investigated: monoculture vs. rotation and "biological" vs. "conventional" agriculture.

The results from the comparison between monoculture and rotation showed that the affected parameters of potato WEOM were different from the ones of WW WEOM. Therefore, the impact of the agricultural practices on WEOM is dependent on the types of crops growing on that plot. Potato plots were more affected in regards to their absolute amounts such as WEOC and substrate, and also some quality parameters such as HIX and K_d; while WW plots were more in regards to their quality. It was observed at the meadow (grass) in section VI 2.4 that HIX and K_d, which become low and high, respectively, when there are significant plant input (especially root input for low HIX), suggests that potato monoculture might have had constantly high root input *in situ*. Since this is an "underground crop", more DOM might have been released to grow. The monoculture practice enriched optically active DOM, which has a higher interaction capacity with Cu in the WW plots.

Winter wheat	Potato
Absorptivity: mono > rot	WEOC : rot > mono
RSF: mono > rot	HIX: rot > mono
B_{max} : mono > rot	K_d : mono > rot
	Substrate: rot > mono

mono: monoculture, rot: rotation

The results from the comparison between "biological" and "conventional" agricultures showed the animal manure had a strong impact on many parameters. The WEOM at the Manejo BA site had significantly high and efficient interaction with Cu, more humified and less biodegradable, therefore more accumulation in soil. The green manures applied to two Italian sites had little effect on WEOM, suggesting that they behave in the same manner as the chemical fertilizers did.

The agricultural practices indeed had influence on WEOM quantity, quality, and its functions. The choice of suitable agricultural practice for a crop as well as the appropriate fertilizer appeared to be of importance to decrease the ecological impact and perform sustainable agriculture.

4. Effects on quantity, quality, and functions of DOM through applying stress to plants

4.1 Effect of glyphosate application on quantity, quality, and functions of DOM

The effect of an herbicide, glyphosate in conjunction with soybean plant presence on the quantity, quality, and functions of DOM was investigated with soil samples taken from the lysimeters at the GSF (Neuherberg, Bavaria, Germany). Glyphosate-tolerant soybean plants were grown in two lysimeters and the glyphosate was applied to one of them once in 2004 and twice in 2005.

2004

The soil samples analyzed in 2004 were from the soil depth 5-20 cm. The effect of glyphosate was minimal for most parameters. Only FE in summer (Figure 86) and HIX in fall (Figure 87) showed differences between the glyphosate applied WEOM and the non-applied counterpart. The glyphosate stayed on the top 5 cm of the soil in the lysimeters for the entire year (S. Grundmann, personal communication). For this reason, the soil samples from the upper layer and the lower layer (5-20 cm) were taken and investigated in 2005.

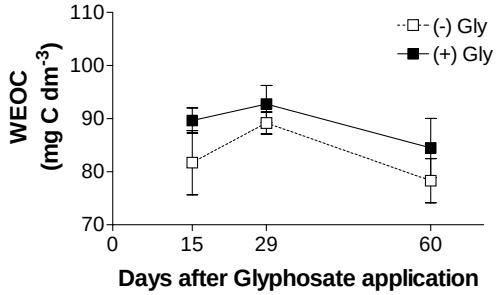

Figure 83 Change in water extractable organic carbon (WEOC) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean.

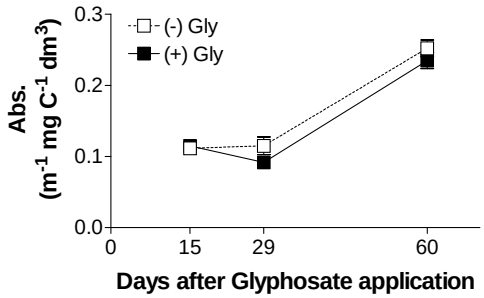

Figure 84 Change in Absorptivity of WEOM (Abs.) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean.

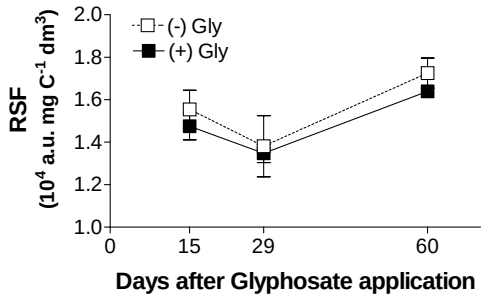

Figure 85 Change in relative summed fluorescence of WEOM (RSF) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean.

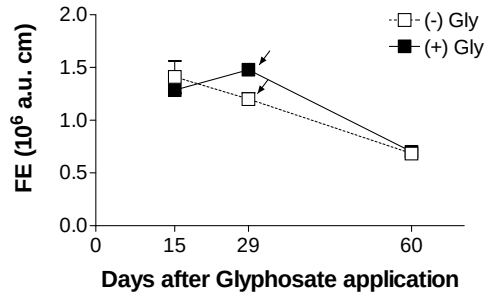

Figure 86 Change in Fluorescence Efficiency of WEOM (FE) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

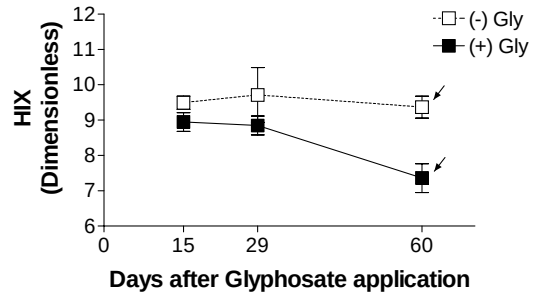

Figure 87 Change in Humification Index of WEOM (HIX) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

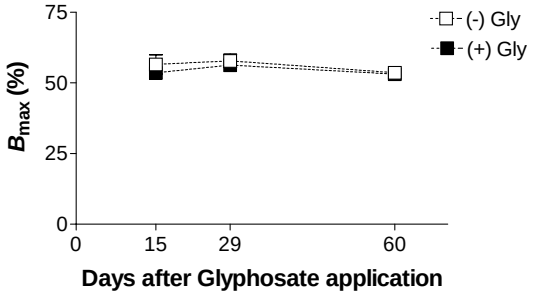

Figure 88 Change in maximum binding capacity of WEOM (B_{max}) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean.

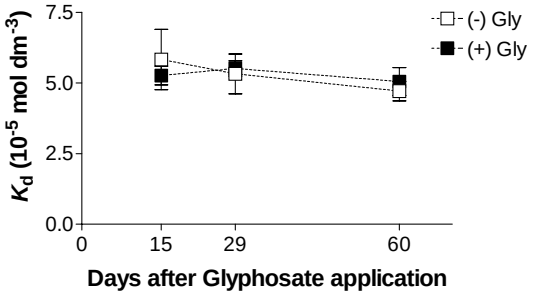

Figure 89 Change in dissociation constant of WEOM (K_d) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean.

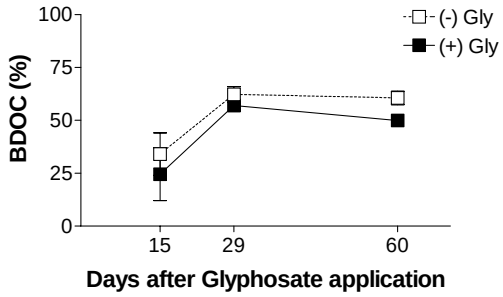

Figure 90 Change in biodegradability of WEOMa (BDOC) at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean.

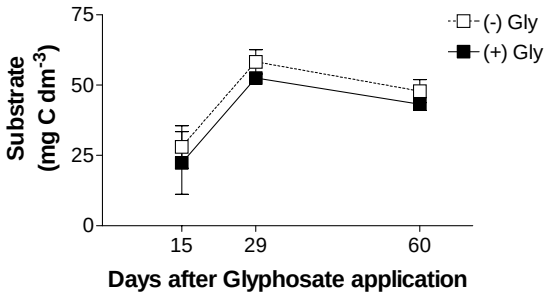

Figure 91 Change in substrate of WEOMa at the soil depth 5-20cm in the glyphosate applied and the control lysimeters after the glyphosate application. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean.

2005

Year 2005 had cool summer; therefore the germination of soybeans did not occur until the 3rd seeding in the middle July, and the plant growth was not as good as in 2004. The height of the plants was lower and only half of the nodules compared to 2004 were present. The metabolism of the glyphosate was less than observed in 2004 (S. Grundmann, personal communication). Nevertheless, the soil samples were taken from the lysimeters 6-times in 2005.

There was hardly any difference between WEOM, which was extracted from soils taken at 0-5 cm and 5-20 cm (Figure 92). WEOC/SOC was not significantly different between treatments, either (Table 17). The only difference, which was observed in 2005 between the 2 lysimeters, was FE (Figure 95 (b)). This constantly higher FE at the glyphosate applied lysimeter was not observed in the upper layer, where the applied glyphosate must have been accumulated. Therefore, the glyphosate *per se* did not result in the FE increase at the depth of 5-20 cm. Furthermore glyphosate does not fluoresce. This difference therefore might have been caused by the post treatment of the lysimeters: harvested plants in 2004 were air-dried and chopped off into 2 cm pieces and mixed with soils at lysimeters in November 2004. Unfortunately there is no record how well the plants were grown in that year and how much plants were brought back to the system. There might have already been a difference in these.

There was an increase in WEOC at the end of August. This increase was associated with the plant growth after the successful seeding in the middle of July. WEOC, however, decreased after the 2nd glyphosate application, implying that the plants were stressed by the glyphosate. The weight of harvested soy (Figure 101) clearly shows that the plant growth was significantly restricted by the glyphosate, even though the planted soy was “glyphosate-tolerant” one. The difference was especially obvious for the amount of sprouts. This suggests that the glyphosate restricted plant growth by directly affecting the plants. This might have resulted in the difference of root activity, therefore the difference between two lysimeters was more clearly observed in the deeper layer, where more plant input occurs through root activity. The WEOC/SOC reflected this fact (Table 17). The soil taken at the glyphosate applied lysimeter could release less WEOC than the control. Since the organic matter that is released from plants does not have much optically reactive material, the Absorptivity and RSF decreased. Also, FE and HIX decreased on the same sampling day (Figure 95 and 96). This was again caused by the plant growth.

Table 17 Soil organic carbon (SOC), water extractable organic carbon (WEOC), and their ratio at the glyphosate applied ((+) Gly) and the control ((-) Gly) lysimeters (A-horizons). Letters next to figures show the significant difference ($p < 0.05$).

Sampling location	SOC (g 100 g^{-1})	WEOC (µg g^{-1})	WEOC/SOC (%)
(-) Gly	1.2	24.7 (a)	0.21 (a)
(+) Gly	1.2	22.7 (a)	0.19 (a)

Note: SOC was measured only once in 2004 before the experiment carried out (3 replicates). WEOC is the mean value of all obtained samples, irrespective of the difference in depth (30 replicates per lysimeter). Therefore the ratio was calculated through dividing individual WEOC by the mean SOC value. The presented ratio values are the mean of 30 replicates.

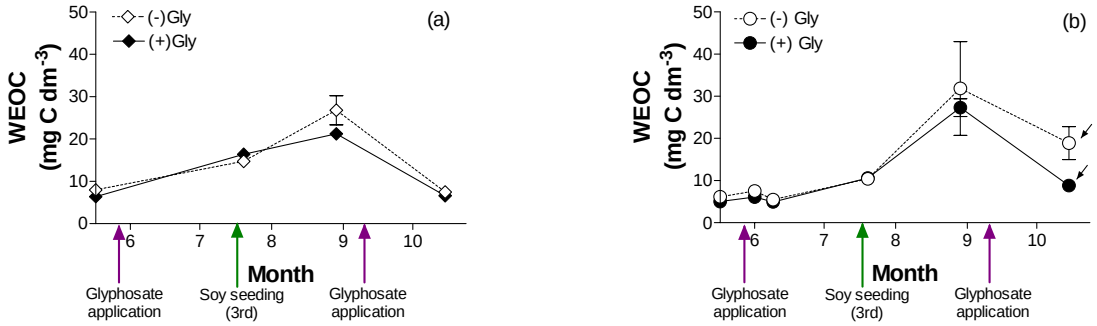

Figure 92 Change in water extractable organic carbon (WEOC) over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

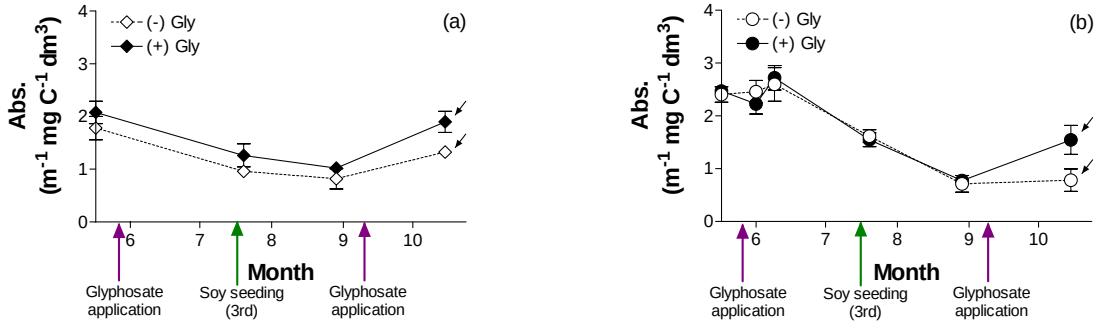

Figure 93 Change in Absorptivity of WEOM (Abs.) over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

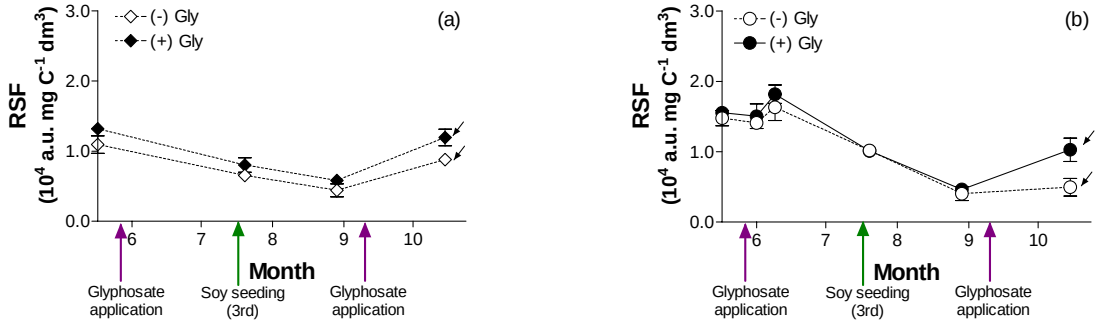

Figure 94 Change in relative summed fluorescence of WEOM (RSF) over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

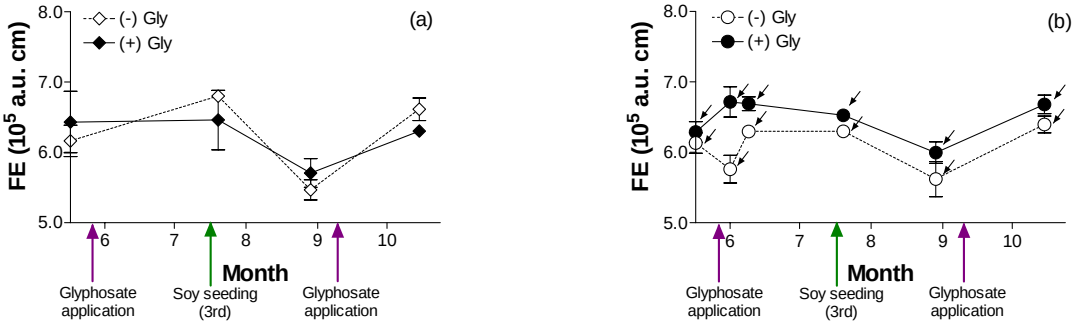

Figure 95 Change in Fluorescence Efficiency of WEOM (FE) over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

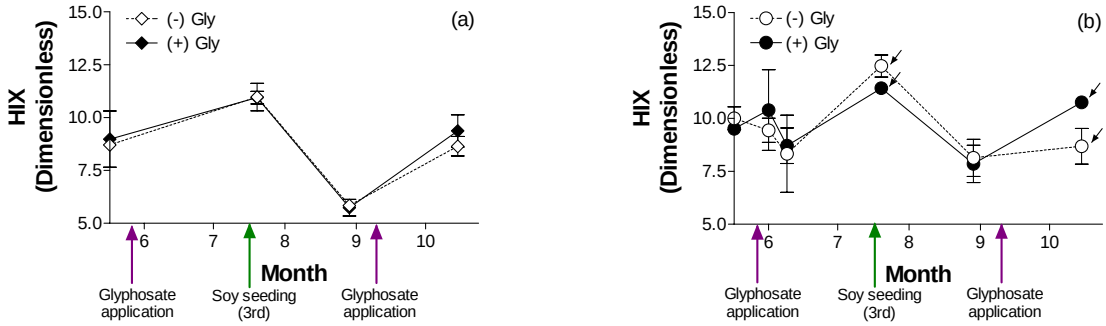

Figure 96 Change in Humification Index of WEOM (HIX) over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

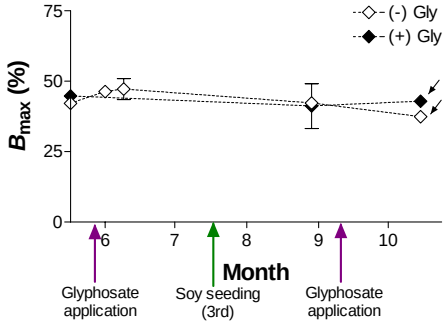

Figure 97 Change in maximum binding capacity of WEOM (B_{max}) over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

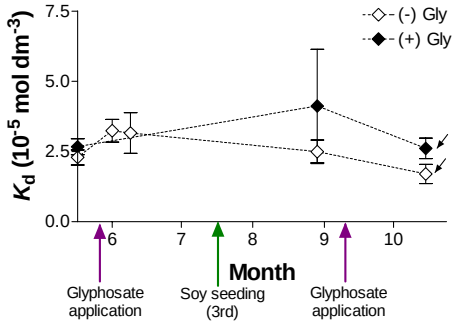

Figure 98 Change in dissociation constant of WEOM (K_d) over time over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

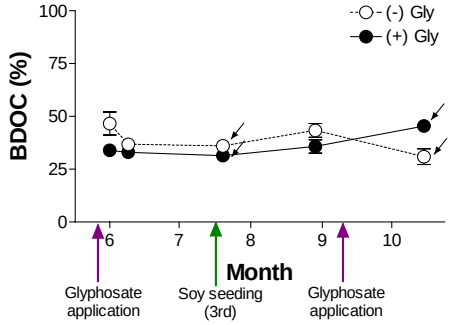

Figure 99 Change in biodegradability of WEOMa (BDOC) over time over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

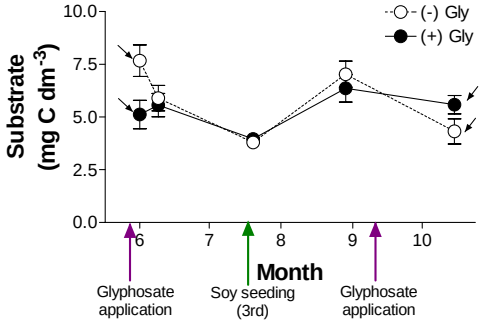

Figure 100 Change in substrate of WEOMa over time at the soil depth 0-5cm (a) and 5-20cm (b) in the glyphosate applied and the control lysimeters. (+) Gly: glyphosate applied lysimeter, (-) Gly: the control. The presented data are the mean values of 3 replicates with the standard error of mean. Arrows show the difference is significant ($p < 0.05$).

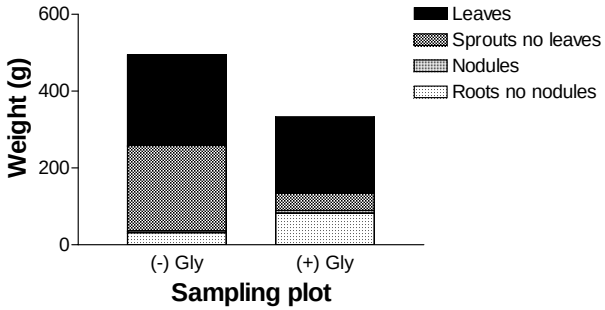

Figure 101 Influence of glyphosate on the plant growth in 2005. 1 soy plant was taken at each lysimeter after the harvest in the middle of October. The weight was measured after let them dry for 5 days in a hood. (+) Gly: soy from the glyphosate applied lysimeter, (-) Gly: soy from the control. Data was provided by S. Grundmann.

In summary,
the glyphosate itself appeared to hardly influence the quantity and quality of DOM. However, the glyphosate application caused differences in the soil, through affecting the plant growth. As a result, WEOC showed differences between the glyphosate applied and not applied soils. Constantly higher FE at the lower layer of the glyphosate applied lysimeter in 2005 might have been caused by the glyphosate application, but this is not clear. Nevertheless, the experiment showed that the DOM quantity and quality are more influenced by the plant activity than the cause such as glyphosate.

4.2 Effect of ozone fumigation on quantity, quality, and functions of DOM

The effect of ozone, which was adjusted to double the concentration of ambient air, via the activity of beech trees on quantity, quality, and functions of DOM was investigated with soils from eight lysimeters in the GSF, Neuherberg, Bavaria, Germany. Four of them were exposed to the double concentration of ozone in ambient air ((+) O_3), while the other four were exposed to ambient air ((-) O_3).

The influence of the ozone concentration was minimal. The values of SOC, WEOC, and their ratio were all similar irrespective of the treatments (Table 18). The increase in WEOC was observed in November (Figure 102). At the sampling time in November, there were already litter on ground from the beech trees. Therefore, this increase was probably caused by the litter input. The increased state lasted also in December. The litter input from the beech trees still continued at that time. The cool temperature might also have lowered the microbial activity; therefore the consumption of DOM was lower than before November.

Table 18 Soil organic carbon (SOC), water extractable organic carbon (WEOC), and their ratio at the ozone fumigated ((+) O_3) and the control ((-) O_3) lysimeters (A-horizons). Letters next to figures show the significant difference ($p < 0.05$).

Sampling location	SOC (g 100 g^{-1})	WEOC (µg g^{-1})	WEOC/SOC (%)
(-) O_3	2.7 (a)	52.6 (a)	0.19 (a)
(+) O_3	3.0 (a)	53.8 (a)	0.18 (a)

Note: SOC was measured only once in 2004 after the experiment carried out (3 replicates per lysimeter). WEOC is the mean value of all obtained samples (18 replicates per lysimeter). Therefore the ratio was calculated through dividing individual WEOC by the mean SOC value. The presented ratio values are the mean of 18 replicates.

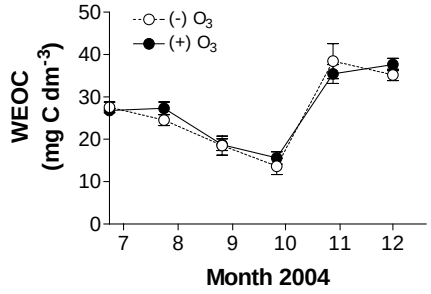

Figure 102 Change in the water extractable organic carbon (WEOC) over time. WEOM was extracted from soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

There was no influence of ozone to WEOM quality. Absorptivity (Figure 103) and RSF (Figure 104) changed from October to November. However, this just reflected the increase of WEOC in that month.

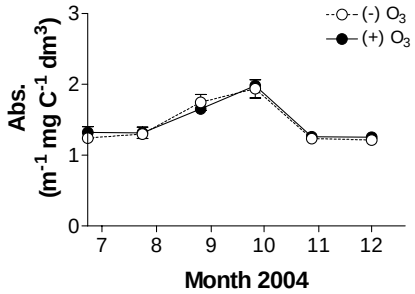

Figure 103 Change in the Absorptivity of WEOM (Abs.) over time. WEOM was extracted from soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

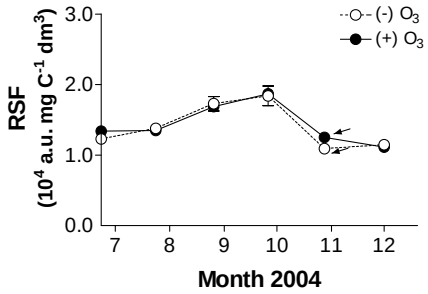

Figure 104 Change in the relative summed fluorescence of WEOM (RSF) over time. WEOM was extracted from soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

FE and HIX showed slight differences, however they were observed only in November and December (FE, Figure 105) and in August (HIX, Figure 106). The difference in FE was not consistent.

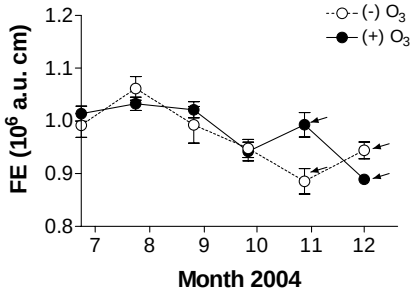

Figure 105 Change in the Fluorescence Efficiency of WEOM (FE) over time. WEOM was extracted from soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

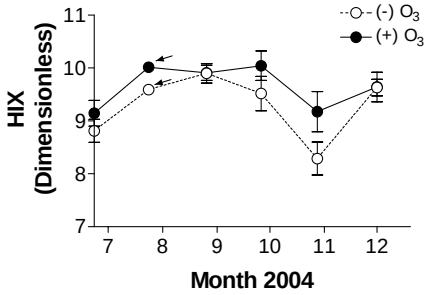

Figure 106 Change in the Humification Index of WEOM (HIX) over time. WEOM was extracted from soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

Functions of WEOM appeared to be influenced by ozone fumigation. Both B_{max} (Figure 107) and K_d (Figure 108) appeared to be lowered under the ozone enriched atmosphere. The difference in B_{max} was, however, not statistically significant except in August. Even though the influence of ozone fumigation was not significant, the values of B_{max} here were significantly lower than the other B_{max} obtained in the other sections of this dissertation. The soil used for this experiment was litter-rich forest soil. The nature of DOM in such a litter-rich forest soil might be somehow different from the others. Since the values here were significantly lower, WEOM in this lysimeter had less capacity to interact with Cu. This difference suggests again that this B_{max} value may be a function of the type of soil.

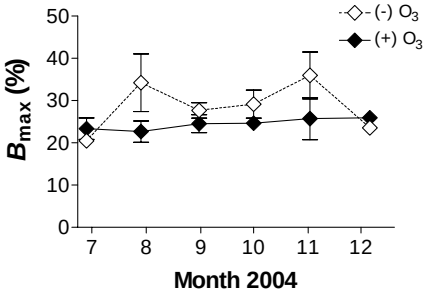

Figure 107 Change in the maximum binding capacity of WEOM (B_{max}) over time. WEOM was extracted from soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

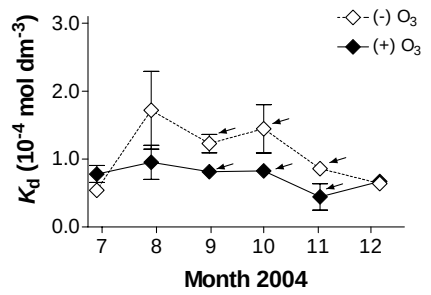

Figure 108 Change in the dissociation constant of WEOM (K_d) over time. WEOM was extracted from soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

K_d had significant differences in September through November. Always WEOM from the lysimeters exposed to the ozone enriched air had lower K_d, *i.e.*, stronger interaction with Cu.

Based on the results in section VI 2, K_d is influenced by the plant input: the more plant input, the higher K_d value. This result in Figure 108 may reflect the difference of the amount of root exudates or plant litter input. Presumably beech trees exposed to double ozone containing air had been stressed and released less amount of root exudation or produced less amount of litter. However, it is still an assumption.

The other function, biodegradability of WEOM and substrate availability, was not influenced by the ozone (Figure 109 and 110). There was significant difference in July for substrate value, otherwise no difference was observed.

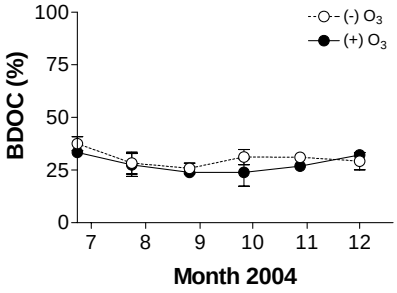

Figure 109 Change in the biodegradability of WEOMa (BDOC) over time. WEOMa was extracted from air-dried soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

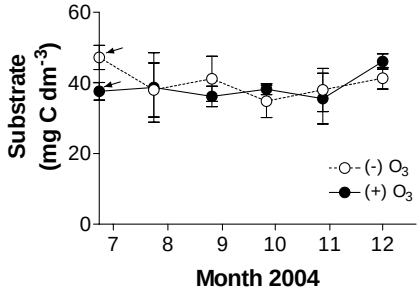

Figure 110 Change in the substrate of WEOMa over time. WEOMa was extracted from air-dried soils taken at lysimeters (A-horizons). (+) O_3: lysimeter exposed to the air containing double ozone concentration of the ambient, (-) O_3: the control.

In summary,

- Ozone fumigation of beech trees did not influence on the quantity and quality of WEOM.
- The ability of WEOM interacting Cu appeared to be influenced by ozone, but the difference was observed for only a limited time.

The same experiment was performed also in 2003 at the same lysimeters and found that the ozone fumigation did not influence *in situ* microbial functionality, because the litter degradation was not affected, even though the photosynthetic performance of beech trees was reduced and the microbial community structure and function were affected, with a tendency towards a lower diversity and a significant reduction in the potential nutrient turnover (Schloter et al., 2005). The data on WEOM presented here of course can not show such differences. However, if the microbial function of consuming organic matter like litter degradation was the same irrespective ozone fumigation, the WEOM was presumably microbially processed in the same way at all lysimeters. Therefore, the difference was hardly at all observed in this fraction.

General summary of effects on quantity, quality, and functions of DOM through applying stress to plants

The influence of the factors, which affect the plant growth such as glyphosate application and ozone fumigation, on the WEOM properties was investigated. The influence was not clearly seen in both case studies. The glyphosate application lowered FE of WEOM, but it was not observed in the other case study with the ozone fumigation. The interacting ability with Cu appeared to be affected by the ozone, but the difference was not significant. The net result here was that WEOC was hardly influenced by the stress factors to plants, but the quality can be influenced, even though the impact appeared to be small. However, the results obtained here were in the second and third year of the glyphosate study and also in the second year of the ozone fumigation study, the effects of these treatments may only be able to be seen in a number of years later.

VII. Discussion

We have to manage soils to maximize their ability to serve as a sink for carbon, buffer for ground water quality, and basis for food production and biodiversity. In order to keep or improve the functions of soils, we have to understand DOM properties. Even though it composes only a small part of total SOM pool, DOM plays various ecological roles in soils, because it is dynamic and the link between the geosphere and the hydrosphere/atmosphere, and also the link between the abiosphere and the biosphere. The functions of DOM are dependent on its quality, which is in turn a function of its sources. The prime source of DOM, vegetation, was considered in this dissertation.

The effect of vegetation on DOM quantity, quality (*i.e.*, optical properties), and its functions in regards to Cu interaction and substrate availability was investigated through analyzing WEOM, which was extracted from pre-incubated soils that were taken on fields in various ecosystems with various vegetation conditions in pan-Europe. WEOM was thought to reflect the DOM under stable *in situ* conditions. Therefore, the common vegetation effects on DOM properties, which may be present in the world, could be investigated through analyzing this WEOM from various sites.

The prime purpose of this dissertation is to intensify our knowledge this little researched organic matter pool, especially non-forest fields, in that how vegetation affects DOM properties. The conceptual model (Figure 1) is to be used for clarifying this with the results presented in section VI.

In the conceptual mode, DOM-R is the DOM in the rhizosphere; while DOM-B is the DOM in the bulk soil. The analyzed WEOM is practically equivalent to DOM-B. Even though "SOM" means in general soil organic matter, SOM here represents solid organic matter imbedded in the soil's matrix. 10 pathways are presented. Pathways ① and ③ show the vegetal input from roots (*i.e.*, rhizosphere): root input through microbial modification and direct root input, respectively. Pathways ④ and ⑥ show the vegetal input from plant litter: direct litter input and litter input through microbial modification, respectively. Pathways ② and ⑤ are both DOM input from soil microbes. DOM-B should also go into or come from SOM pool (⑦). Pathways ⑧ through ⑩ show recycling or percolation of DOM-B, however, these pathways are not to be discussed here. The C-loss as a consequence of mineralization of DOM (*i.e.*, CO_2) and the loss of volatile DOM (*e.g.*, small fatty acids) into the atmosphere are not considered.

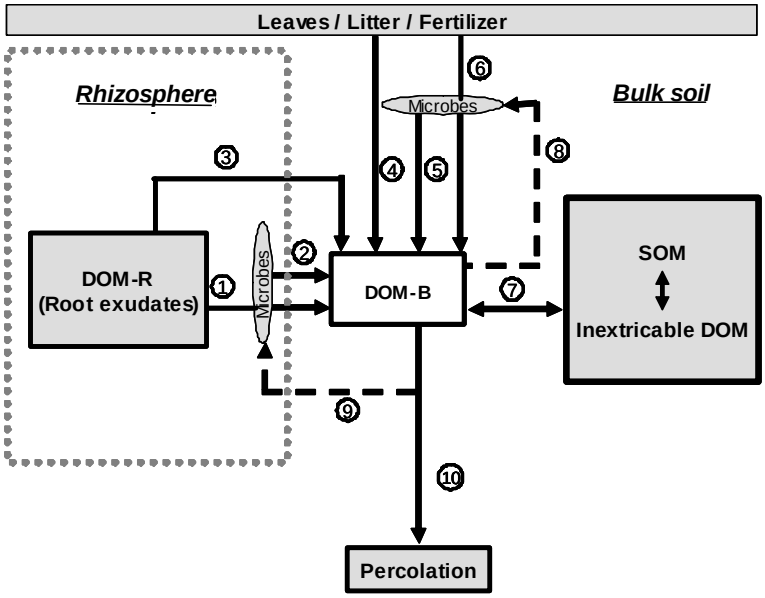

Figure 1 A conceptual model of the impact of vegetation on DOM. DOM-B is bulk soil DOM and is practically equivalent to WEOM. SOM here indicates solid organic matter imbedded in the soil's matrix. The dashed pathways are not components of this dissertation. DOM losses to the atmosphere are not considered.

1. Vegetation effect on quantity of DOM

Figure 22 shows that the presence of vegetation results in an increase in DOM (or more precisely in WEOC) quantity. This increase must be caused through pathways ① through ⑥. Since the investigated WEOC was presumably mainly DOM-B in the model, WEOC could be increased directly through these pathways. However, results of the detailed temporal variation study at Puch (Figure 44) suggested that temporal variation is not affected by the vegetation conditions, even though many past studies suggested that there is significant temporal variation (Zsolnay, 1996; 2003; Kalbitz et al., 2000b). Such temporal variation might have been caused by some other factors, but not by vegetation conditions. Even though no evidence of the direct influence of vegetation on WEOC was observed, the long term vegetation effect could be observed with a "field approach" as was carried out here.

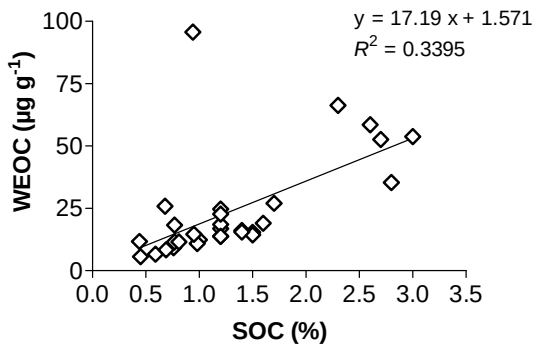

Figure 111 The relationships between water extractable organic carbon (WEOC) and total organic carbon (SOC) contents in soils. The data are presented in Table 13, 14, 15, 16, 17, 18 in section VI.

A positive correlation between SOC and DOC/WEOC has been reported (Saviozzi et al., 1994; Delprat et al., 1997; Gregorich et al., 2000). Based on the data presented in this dissertation, a positive correlation between WEOC and SOC was also present (Figure 111, $p < 0.0001$). The obvious outlier was the Manejo BA (SOC 0.94 %, WEOC 95.7 μg g^{-1}), where the mixture of goat manure and grape residue was applied as fertilizer. This organic manure had obviously strong impact on the WEOC availability. This occurred through pathways ④ and ⑥. Since such significant impact was not observed at the green manure applied plots in Italy, this mixed organic fertilizer at Manejo BA was unique. Its rather refractory nature (Figure 81) might have suppressed the efficient microbial WEOC consumption and made it more extractable. Without this outlier, the other points follow a strong positive relationships ($R^2 = 0.73$).

2. Vegetation effect on quality of DOM

2.1. Tracing source of DOM with UV and fluorescence spectrometry

DOM-B has various sources (① through ⑦). Its quality can be different depending on where the DOM-B mainly comes from. Some of the selected sampling locations have "clear-cut" major sources of DOM. For example,

Case 1) Meadow: significant contribution from rhizodeposition (① though ③).
Case 2) Pine forests: significant contribution from plant litter (④ through ⑥).
Case 3) Bare plot: sole significant contribution from SOM (⑦).

Optical measurements such as UV and fluorescence spectrometry were found as very suitable tools for detecting DOM sources. Some examples are presented here.

DOM in the meadow had a significantly low HIX (case 1). The reason behind is that the root exudates are composed of non-humified organic matter, so relatively uncondensed organic compounds, whose fluorescence emission in the longer wavelength region is relatively not as intense as is typically observed for a humified organic matter (*e.g.*, fulvic acid, Figure 21 (b)). The previous research done at IBÖ by others (Marx et al., 2004) has verified that DOM-R, which was collected by the dipping method, has a HIX value of about 3. HIX at the meadow in Puch was about 6 (Figure 27 (a)). The other investigated areas in this dissertation had a HIX of about 10. Since freshly released DOM from roots is preferentially taken up by microbes (Yevdokimov et al., 2006) and since the microbial activity turns the excreted DOM into one with a higher HIX (Marx et al., 2004), higher HIX values indicate that the fraction of DOM-B, which came though pathway ③, is usually relatively minor. However, the meadow DOM had a low HIX, indicating

that there was a significant input from roots through pathway ③. Such a significant root input is understandable, since the grasses at the meadow had dense and developed roots in the top soil. HIX appeared to be the best parameter, which can trace the root input into DOM-B.

If there was significant litter input (case 2), the major components of DOM-B should be provided through pathways ④ through ⑥. DOM leached from plant litter should be relatively rich in substituted phenols, because plant litter and branches are enriched in these compounds (Kuiters and Sarink, 1986). Phenols, in general, absorb UV light. Therefore, Absorptivity should be a good parameter, which indicates the input of plant litter. The Absorptivity of DOM from the places, where more litter input was present (*e.g.*, pine forest), had higher Absorptivity (Figure 23). The place, where little substantial litter input was present (*e.g.*, bare plots and the frequently mowed meadow), had low Absorptivity.

Microbes themselves can be also the source of DOM (pathways ② and ⑤). Even though they can be a source of DOM during the dry-wet cycles (*e.g.*, Lundquist et al., 1999; Gregorich et al., 2000; John et al., 2003) or during freeze thaw (Zsolnay, 1997), their direct contribution should be minor in moist (pre-incubated) soils. On the other hand, vegetation results in more microbial biomass and can increase their activities *in situ*; therefore they are important as a C sink, which can turn into a source depending on the soil conditions. One can consider the microbes to a DOM processor or filter. The HIX of the microbial biomass obtained by fumigation tends to have a value around 5 (Zsolnay et al., 1999). Unfortunately, this parameter can not distinguish input from microbes or root exudation, since they tend to have similar values. HIX can only indicate whether DOM-B had more input though ③ or not.

The values for the bare plots (case 3) can be used as controls within an investigated site to determine how much the WEOM from the other investigated plots is affected by vegetation.

2.2. Common vegetation effect on DOM quality

By having the bare plot/location within a sampling site, general vegetation effects on DOM could be successfully investigated. The chief DOM source at the bare plot is SOM, which had been accumulated in the past and therefore has been ageing for a relatively long time (⑦), while the other sampling points had significant vegetation input (① through ⑥). Generally speaking, DOM at the vegetated plots had higher Absorptivity, lower RSF, and, therefore, lower FE compared to the controls.

FE was the only parameter, which clearly showed the difference in DOM quality caused by vegetation. This parameter appears to be an indicator of the size of the DOM molecules, since this value becomes lower as DOM molecular size increases (Ewald et al., 1988; Wu et al., 2003). Therefore, larger size DOM molecules might have existed under vegetation at the sites investigated here. An increase in DOM molecular size has been reported when an arable field is turned into meadow (Von Lützow et al., 2002). DOM composed of larger sized molecules has been also found in forest floors as opposed to arable fields (Hughes et al., 1990; Quideau and Bockheim, 1997). Based on this, vegetation results in a relative increase of larger molecules in DOM, which then results in a lower Fluorescence Efficiency. Two things need to be pointed out here. Firstly, the term "large molecular size" does not imply as to how these molecules are larger. This could be due to covalent bonding, but physicochemical attraction is also a possible mechanism. Secondly "larger" does not mean more humified, because the HIX measured here was not increased by vegetation. The most probable explanation for the size alteration of the DOM components is an intermolecular condensation or aggregation caused by organic matter released by plants, which behaves like "glue", which aggregates the DOM components. This organic matter "glue" from plants may be the same material, which can improve the soil aggregate stability. This organic matter "glue" might be preferentially metabolized by microbes, and therefore DOM in bare locations/plot is not as well aggregated. This assumption, however, has to be verified. Nevertheless, FE may be a promising soil quality indicator (Zsolnay, 2005).

3. Vegetation effect on functions of DOM

3.1. Interaction of DOM with Cu

The ability of WEOM to interact with Cu was also influenced by vegetation. Vegetation made K_d value higher, which means that vegetation weakened the ability of DOM to interact with Cu. This is most likely the result of plants supplying less humified DOM to soil, because the preliminary studies of Cu quenching measurement showed that the less humified DOM (based on the location of the emission spectra) is less interactive with Cu. It was also found here that K_d and HIX are generally negatively correlated, namely the more humified DOM can interact with Cu more efficiently. Since microbial activity makes DOM more humified (Marx et al., 2004), plant input through pathways ③ and ④ contributed to this difference. Cu *in situ* interacts less efficiently with DOM, if enough vegetation input through these pathways is present.

On the other hand, the maximum binding capacity (B_{max}) of DOM was hardly influenced by vegetation. This parameter was found to have a positive correlation with HIX in general. Since vegetation does not release humified organic substance, this binding capacity should be

influenced by more DOM dissociated from SOM, whose quality is probably more influenced by soil matrix. Therefore, the difference was hardly observed within a site, which had similar soil condition.

The net influence of DOM on Cu in nature is therefore determined by the absolute amount of DOM present *in situ* in the soil. However since this presence of DOM is very much a function of water content in unsaturated system, the period of time available for Cu-DOM interaction can vary considerably. Under the moist conditions, which usually prevail in the fall, differences in K_d are probably not important ecologically. However in the summer, with its relatively short periods of wet conditions, K_d may be important in reflecting to what extent this interaction can actually take place. Therefore, all in all, vegetation controls directly and indirectly this function of DOM.

3.2. Biodegradability of DOM

The Puch site results showed that the biodegradability (BDOC) of DOM was influenced by vegetation. Meadow DOM was more biodegradable than that of the bare plot. Since the meadow plot must have had significant amount of root exudation (pathway ③), which is more preferentially metabolized by soil microbes (Yevdokimov et al., 2006), this high biodegradability is reasonable. In contrast the DOM of the bare plot, which was mainly leached from SOM (pathway ⑦) and which is more humified and has aged for over 50 years, was less biodegradable. This result indicates that vegetation can provide more biodegradable organic matter and enhance the microbial activity in soil. However, this difference was hardly observed at the other investigated sites. This may be due to source differences: rhizoexudates or the leachate from plant litter. Since the utilized WEOMa for this experiment was extracted from air-dried soils, which have additional contribution from microbes, the difference between vegetation would be diluted unless there was a significant input such as in the case of the meadow.

Since BDOC values tend to be similar, the net ecological effect of vegetation in regards to DOM substrate quantity is in general proportional to the amount of DOM present, showing a direct influence through ③. The meadow, which has both higher and relatively more degradable quantities of DOM, is especially rich in substrate.

4. Temporal variation

The WEOC concentrations vary between the three seasons in which sampling took place. However, there is no consistent pattern except that the seasonal effects are less pronounced in the homogeneous, managed, agricultural plots in Puch as opposed to the unmanaged, highly heterogeneous Spanish locations.

However, with a greater temporal resolution, a seasonal variation could also be seen at the three plots in Puch (Figure 43). There was a definite relative increase of DOM-B in the late fall and early winter. Since the agricultural plot, which had plant residues at that time, had the most significant increase, this increase might have been caused by pathways ④ and ⑥. However, this increase also occurred even at the bare plot, therefore this was not because of the plant residue input, but from temporal fluctuation through pathway ⑦. Temporal variations at the agricultural plot were expected because of the seasonal aspects of plant growth and senescence, however they were not observed. This fact again suggests that microbial activity was so efficient that most of the rhizoexudates were in general turned into DOM-B with a similar quality. If there was more input through pathways ③ and ④, the temporal variation in association with the plant growth would have been more significantly observed. As it is, pathway ⑦ must be the chief source for seasonal variation. It must be repeated here that the seasonal effects of wet-dry cycles and/or freeze-thaw events are not considered here, since the DOM was obtained after a pre-incubation.

5. Effect of vegetation type

The nature of the vegetation, with some striking exceptions such as grasses and pine trees, made no difference in WEOC quantity (Figure 54). The two exceptions were because of the long term vegetative input from the same type of plant. The results in section VI 3.1 (Figure 65) also indicated that if cultivation is performed with the same type of plant for long periods of time (*i.e.*, monoculture), differences in DOM quantity and quality can appear (*e.g.*, potato). This type of difference, however, can only be seen, if the same type of plants had been growing for a long period of time. The short term (*i.e.*, one growing season) vegetation cycles such as in most agricultural regimes in Europe hardly resulted in any differences in WEOM. If DOM-R is obtained from different types of plants, the influence of vegetation type can be observed, because the root exudation pattern as well as the exuded DOM quality is different depending on the plant type (Dakora and Phillips, 2002; Hutsch et al., 2002; Marx et al., 2004). However, the results obtained here show that the influence of DOM-R is very limited. This confirms that the microbes can turn usually DOM-R efficiently into similar material. Although non-optically active material was not characterized, it should be even truer for this class of compounds, since many of them (*e.g.*, fatty acids, amino acids, carbohydrates) are presumably even more labile.

6. Effect of agricultural practices

Two agricultural practices (monoculture vs. rotation and “biological” vs. “conventional”) were investigated. In case of the former, the quality and functions of DOM were little affected

compared to its quantity. The significant difference was found for potatoes: monoculture potatoes had lower WEOC than their counterpart under rotation (Figure 65 (b)). This difference was not observed for winter wheat. The differences found for the potatoes were related to the difference in the litter production between potato and other crops such as winter wheat, red clover, barley, oats, which grow sequentially at the rotation plot, which had potatoes at the investigated time. The plants such as winter wheat, barley, and oats produce plant residue, which is about 5 times more than that of potatoes (Köhnlein and Vetter, 1953). Furthermore, the residues of potatoes have a low C/N ratio and lignin content, which increases their decomposition rate (Bending and Turner, 1999). The impact of monoculture agricultural practices on DOM appears only in the case of very specific crops. Such an impact can be observed, for example, when a low litter producing crop such as potatoes is grown at the same place for long time. The difference occurs due to the difference in plant input mainly through ④ and ⑥, and as a consequence through ⑦. It was also suggested that the crop rotations increase DOM over a number of years (Campbell et al., 1999a; Haynes, 2000). In order to perform sustainable agriculture and to promote C sequestration, a low litter producing crop such as potatoes should perhaps be grown in the rotational sequence, because rotation practice appears to prevent the depletion of SOC.

The comparison between the impacts of either “biological” or “conventional” agricultural practices showed little difference in regards to DOM-B amounts, quality, or functionality at the two Italian sites (section VI 3.2). They were located in climactically different regions, but the biological plots received the same kind of green manure (a mixture of Egyptian clover and faba beans), which appeared to behave within the framework of this dissertation in the same manner as the inorganic fertilizers. It has been suggested that green manure increases the C input to a soil, but its effect on SOM equilibrium may occur too slowly to be observed over the relatively short time of a few years (Robertson et al., 1993, 2000). The results obtained here support this. The agricultural practice of organic farming was adopted only about 5 years ago at both sites. Also, the green manure applied here might have also been rapidly degraded by microbes, since the microbial activity is high under the relatively warm temperature (Marschner and Bredow, 2002) such as the case the Italian sites.

Even though the agricultural practices did not affect DOM at the Italian sites, this was not true for the Spanish site, where a mixture of grape residues and goat manure was applied to the biological plot. This had a unique impact. It resulted in a strong increase in relatively refractory DOM-B with a very high HIX value. Its refractory nature probably resulted in a shift from pathway ⑥ to ④. The B_{max} was higher at this plot. The decrease in K_d is a relatively unique result

in this dissertation, since HIX and K_d were found in general to be negatively correlated (Figure 31). On a practical level, the higher DOM combined with a higher B_{max} and lower K_d indicates that a fertilizer mixture of grape residues and goat manure could change the heavy metal balances in a field by enhancing the leaching of heavy metals. Such information could be of value in the management of metal contaminated fields in the Mediterranean area. Also, due to the refractory nature of its DOM, the applied organic fertilizer C could possibly remain in the soil for long time. As a result, it may improve soil structure. This aspect could also be important for preventing the depletion of C in soils.

7. Effect of anthropogenic chemical impacts

The effect of applying glyphosate to lysimeter soil showed no consistent effect during 2004. This may have been due to the fact that only 5-20 cm were sampled, and afterwards it was determined that most of the glyphosate remained in the top 5 cm. There was a decrease in HIX in the glyphosate applied lysimeter, but this trend did not persist into 2005. In 2005 at both 0-5 cm and 5-20 cm there was no major alteration in DOM. One exception was the significant ($p < 0.001$, paired t-test) increase in FE in the glyphosate treated lysimeter (Figure 95 (b)). This most likely partially reflected the differences in the soy plant growth, since the FE data at the other sites (*e.g.*, Puch, Spanish catenae) showed that this parameter is lower when more plant input is present. The plant growth in 2005 on the glyphosate applied lysimeter was strongly restricted with the harvested soy plants having less weight than the ones from the control lysimeter (Figure 99). The chief difference was in the sprouts. The significant decrease in WEOC at 5-20 cm after the 2nd glyphosate application suggests that the inhibition of the plant growth was caused directly by glyphosate. Since the difference in WEOC was only observed at 5-20 cm, the amount of DOM released from roots might have been the controlling factor. Overall, glyphosate had a direct impact on plant growth, even though the plants were "glyphosate-tolerant". As a consequence, input through pathways ① and ③ can be restricted because of plant stress. Over the long term, the difference in the input through pathways ④ and ⑥ would control the DOM quantity and quality.

In the case of increased ozone there were no differences between the treated and control lysimeters with one exception. The WEOM from the ozone treated lysimeter reacted more efficiently with Cu (lower K_d) than the controls. This was however only the case during the summer and early fall (Figure 108) and was not accompanied by an increase in HIX. The result should be viewed with caution. It is certainly possible that one year of ozone gassing is simply too short to have a biogeochemical impact.

Overall assessments

Vegetation was found to increase DOM concentration. However it appears to occur rather indirectly, through increasing SOC, which is in turn litter input. The type of major vegetation input, either plant litter or root deposition, also appreared to control the DOC availability. From these points of view, vegetation was found to be important controlling factor of DOC.

Direct vegetation effects were observed for the quality and functions of DOM, only if there was significant plant input without microbial modification (pathways ③ and ④). Such input tends to produce DOM, which depending on the source, has a lower HIX or higher Absorptivity as well as high biodegradability, and low efficiency of Cu interaction. Obviously vegetation can influence the functions of DOM. However, this was found to be relatively minor with the exception of the establishment of forests or meadows.

Agricultural practices and anthropogenic stress to plants influence DOM quantity by controlling the litter production. The effect on DOM was found to be observed only when the same type of vegetation has been present at the same plot for long time. Even though the glyphosate and high ozone concentration stresses had no immediate influence on DOM, some impact could be observed one year later. The proper choice of fertilizer appeared to be significantly important. Green manure as opposed to animal manure was found to be less influential. Animal manure application may change strongly the fate of metal ions *in situ*.

To detect the chief source of DOM in the bulk soil, UV and fluorescence spectrometry were found to be very suitable. The parameters obtained with these tools such as Humification Index and Absorptivity could provide information where most of DOM-B is from. Fluorescence Efficiency was found to be the best parameter, to show the influence of vegetation on DOM quality.

The choice of the proper sampling handling and the DOM extraction conditions was extremely important. WEOM, which was extracted with 10 m*M* $CaCl_2$ solution in a practical manner and is composed of the most of potentially available DOM, was found to be the best estimate of *in situ* DOM. A pre-incubation step was found to be needed to diminish the influence of soil conditions such as moisture content, which are known to influence DOM quantity and quality significantly. By adopting this step, the vegetation effect on DOM under the stable condition in nature could be successfully investigated. On the other hand, such a pre-incubation does not make it possible to estimate the effect of periodic impacts such as wet-dry, freeze-thaw, plowing, *etc*., which may be ecologically very important.

A “Field approach” and seasonal sampling with replicates were found to be essential to find out the general vegetation effect on DOM. Short term investigations, such as in the laboratory and green house can not evaluate such long term vegetation effects. The results here showed that most vegetation effects are short living because of efficient microbial activity. Significant vegetation effect on DOM properties can be observable mainly if there are especially high litter and root inputs.

VIII. Conclusion

Vegetation had an influence on the quantity, quality, and functions of DOM in regards to Cu interaction and substrate availability. With the conceptual model it was possible to visualize through which pathways the DOM properties can be influenced. Even though direct vegetal input, which is not microbially metabolized, can have a strong influence on DOM, these inputs were found to be minor with the exception of areas with well established abundant vegetations such as in meadows or forests. Most of vegetal input was found to go into the bulk soil through the microbial metabolism. Therefore, the influence of different plant species on DOM properties was hardly observed. This is due to the efficient microbial activity and to the buffering effect of the bulk soil through sequestration processes. Vegetation does not appear to directly influence DOM quantity, but rather indirectly through increasing the SOC, which in turn has an effect on DOM quantity. Nevertheless, there was a common influence of vegetation on DOM. It increased the amount of DOM and at the same time lowered its Fluorescence Efficiency, which is probably controlled by altering the intermolecular aggregation of DOM. This may be caused by the organic matter released from plants and may be the key organic matter playing a role for stabilizing soil aggregates.

Overall, even though the direct influence of vegetation on DOM in the bulk soil could be minor; this direct DOM input from plants was found as the key factor, which affects the functions of DOM *in situ*. This direct vegetal input decreases the mobility of heavy metals and increases the microbial activity.

UV and fluorescence spectrometry were found as the best tools for detecting the source of DOM. Furthermore, soil sampling with a “field approach” and with the experimental conditions, which were utilized here, were found to be suitable for revealing the general vegetation influence on bulk soil DOM under natural stable soil conditions.

IX. Summary

Dissolved organic matter (DOM) in soil is organic matter, which is present in soil solution. Although DOM is only a small part of the organic matter in soil (SOM), it is of high relevance because through its mobility it plays various ecological roles. For example, DOM controls the mobility and bioavailability of soil contaminants through directly interacting with them, and DOM itself is also the nutrient and energetic substrate for soil microbes, which directly influences the C-balance between geosphere and atmosphere. The ecological process in which DOM participates is dependent on its location, how much of it is available, and also on its quality. Many factors control the quantity and quality of DOM: soil types and conditions, soil hydrology, microbial activity, climate, land use and management, vegetation, *etc*. These factors do not function independently but are all intertwined. Therefore, in order to understand how DOM functions, we need to know more about its sources and sinks. This dissertation will concern itself with one of the prime sources of DOM in soil: vegetation, the prime source of both water soluble and insoluble SOM. Vegetation can contribute directly to the DOM pool through litter as well as through rhizoexudation. Despite this, the effects of plants, especially over an entire growing season, are still largely unknown, especially in arable fields. Also in order to develop optimal management strategies, we have to know how anthropogenic activities alter the vegetal influences on DOM.

The main focus in this dissertation is to intensify our knowledge of long-term DOM production and alteration through vegetation and to investigate the ecological implications of this both in regards to its potential interacting ability with a contaminant (copper) and its availability as a substrate for microbial processes. The following effects on quantity, quality, and functions of DOM have been investigated with DOM extracted from soil samples, which were taken in different environments such as agricultural sites in Germany, Italy, and Spain; lysimeter experiments in Germany; as well as three natural catenae in Spain. This pan-European site distribution may provide information on common vegetation effects, which may be observed everywhere in the world. With this large site selection, the following effects were investigated in this dissertation: overall effect of vegetation, its temporal variation, and the effect of different plant species; the effects of agricultural practices (monoculture vs. crop rotation, conventional agricultural with mineral fertilizers vs. biological agriculture with green and animal manure); and the effects of plants stresses (lysimeters: glyphosate application on agricultural soil, ozone fumigation of beech plants on forest soil).

The sampling sites were selected with the following attributes:

1. For the effect of vegetation:

   Each site had a "control plot/location", where no or very little vegetation input was present, enabling the general effect of vegetation to be observed. Furthermore the different vegetation at some of the sites and the planting of different crops at the Puch site enabled the effect of plant type to be investigated.

2. For the effect of agricultural practices:

   (a) Winter wheat plots and potato plots in Puch were selected. Both monoculture and rotation with these crops have been performed for over 50 years.

   (b) Two agricultural sites in Italy (Tuscany and Basilicata) and also a site in the Murcia region were selected. Green manure has been applied to the biological plot at the former sites; while a mixture of grape residue and goat manure has been applied at the latter site. Chemical fertilizers have been applied to the conventional plots at all sites.

3. For the effect of stress to plants:

   Soils from the lysimeters of the GSF (Neuherberg, Bavaria, Germany) were investigated. In both cases there were control plots with same type of vegetation were present without glyphosate application or ozone fumigation.

Seasonal or even more frequent sampling with replicates (field approach) was realized in order to determine the influence of the temporal variation and to reveal the general vegetation effect on DOM properties *in situ* over a year. The soil samples were always taken at the A-horizons, since the influence of plant is most profound at this horizon.

Since DOM extraction was performed using the batch method with an aqueous solution (10 m*M* $CaCl_2$), this fraction should be more precisely called water extractable organic matter (WEOM), a fraction which reflects the potential *in situ* state of DOM. In order to extract WEOM and also in order to standardize the soil moisture content, which is known to influence WEOM quantity and quality, the soils were pre-incubated with 50% of their water holding capacity at $4^{o}C$ for 1 week before extraction. However, WEOM used for the potential biodegradability measurements was extracted from air-dried soils, since the efficiency of the microbial activity during the pre-incubation depleted the labile DOM pool too severely.

The characterization of WEOM was done with UV and fluorescence spectrometry, while its interaction with copper was quantified with fluorescence quenching. The potential substrate content of WEOM was determined by measuring its organic carbon content before and after a

week-long incubation under optimal conditions. UV and fluorescence spectrometry were found to be the best tools for characterizing WEOM and also for identifying its chief sources in nature, because they can measure WEOM as it has been extracted without concentration, cleanup, or derivatization. Such rapid and precise optical measuring tools were definitely needed for processing the very large number of samples, which needed to be analyzed. Humification Index (HIX) and Fluorescence Efficiency (FE) were obtained by measuring the optical properties of WEOM. The former is based on the fluorescence nature of organic substances and indicates the intramolecular condensation (*i.e.*, C/H); while the latter is the ratio fluorescence/UV and appears to indicate the intermolecular condensation (*i.e.*, molecular size) of WEOM.

Vegetation had a significant influence on the quantity of DOM. This influence was twofold: direct through rhizoexudation and indirect through plant derived litter decomposition. However, in both cases the WEOM in the bulk soil tends to be altered through efficient microbiological activity into a relatively similar qualitative but not quantitative state. The degree of this alteration was not uniform. For example, the WEOM in the meadow with its low HIX value contained substantial amounts of "fresh" material. It can be postulated that this was the result of extremely high root input and from its higher water content, which could suppress microbiological activity and diffuse out the rhizoexudates further away from roots. The degree of such alteration can impact WEOM function. If there is significant direct input from vegetation, WEOM is composed of less humified organic matter, which is more easily biodegradable but less interactive with Cu.

However, such direct input was found to be minor with the exception of well established forests and meadows. In those cases one has a relatively high plant coverage, and, unlike agricultural plants, which are harvested, the vegetal input is continuous (weather permitting). As mentioned above, in the case of the meadow this direct input was most likely through rhizoexudation. However the pine forests did not have a significantly smaller HIX value, but they did have a significantly higher Absorptivity. This would indicate that in their case, the direct input was rather through litter than rhizoexudation.

The microbial altered WEOM was by no means inert. The fact that WEOM obtained from a plot, which had no major organic input for over 5 decades, was still over 40% biodegradable indicates some kind of protective mechanism must exist. The most likely explanation is that plant derived WEOM is not only efficiently altered by microorganisms, but that it is also efficiently sequestered into the soil matrix. This can also be concluded from the temporal studies. Vegetation undergoes very strong seasonal changes, but such strong seasonal changes were not

present in WEOM quantity, quality, or function. The more detailed temporal studies done at Puch showed that plant presence did not account for any changes detected. Therefore, the release of sequestered WEOM from the soil matrix is most likely responsible for the detected changes. The fact that sequestration is reversible is confirmed by the fact that the bare plots had considerably less WEOM than their vegetated counterparts.

The plots with the "underground" agricultural crops of sugar beet and potato had significantly different relative fluorescence as compared to their "above ground" counterparts. However none of the other parameters or functions differed among the agricultural crops, indicating that WEOM is in general quite similar on farmland. In the case of the potato monoculture plot, there was less substrate available even though the HIX value was significantly lower. This was also reflected in a significantly inferior interaction with copper.

Herbicide and high ozone concentration stresses had no immediate significant influences on WEOM. After one year there was an exception to this. The glyphosate treated plots had consistently significantly higher FE, indicating that the soy plants were stressed in those lysimeters, which was indeed the case. The proper choice of fertilizer appeared to be significantly important. Green manure as opposed to animal manure was found to be less influential. Animal manure application may change strongly the fate of metal ions *in situ*.

Vegetation plays a key role in determining the quantity of DOM. However, the qualitative aspects of DOM appeared to be controlled by microbial processes. However there were exceptions to this when stable vegetation (*e.g.*, pine forest, meadow) dominated. Since DOM was extracted from pre-incubated soils, the results of this study might not have reflected the instant vegetation effects and/or the effects of perturbations such as plowing, drying, or freezing. Nevertheless, this study could investigate the long term vegetation effect on DOM properties and reveal the key vegetal input, which has significant influence on DOM functions.

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Lebenslauf

Persönliche Daten: Junko Akagi
Türkenstraße 79, 80799 München
Geboren am 05.11.1977 in Hyuga, Miyazaki, Japan
Ledig

Schulbildung

1984 – 1990: Grundschule in Hyuga, Miyazaki
1990 – 1993: Mittelschule in Hyuga, Miyazaki
1993 – 1996: Oberschule in Hyuga, Miyazaki

Studium

1996 – 2000: Bachelor-Chemie an der Saga Universität in Japan
2000 – 2002: Magister-Chemie an der Saga Universität in Japan

Promotion

2002 – 2006: Wissenschaftliche Angestellte am Institut für Bodenökologie der GSF-Forschungszentrum für Umwelt und Gesundheit und Doktorandin am Lehrstuhl für Bodenökologie des Wissenschaftszentrum Weihenstephan der Technischen Universität München