

# Hydration of humic and fulvic acids studied by DSC

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**Abstract** Qualitative and quantitative aspects of hydration of four humic acids (HA) and three fulvic acids (FA) originating from different sources were investigated. DSC experiments at subambient temperatures were carried out in order to monitor differences in ice behavior originating from freezable water surrounding humic molecules. It was found that kinetic effects play a significant role in hydration processes of both HA and FA. In fact, the hydration took part over 21 days which was detected as a progressive decrease in ice melting enthalpy. Simultaneously, the peak shapes and positions changed indicating structural changes in the physical structure of the humic substances. In case of FA, the dependency of melting enthalpy on water concentration showed a linear trend resembling a complete hydration previously observed for water-soluble hydrophilic polymers. In contrast, the melting enthalpy of some HA increased in a step-like way with increasing water content, suggesting preservation of original hydrophobic scaffold during the hydration. The differences between the rather young FA and the rather old HA lead to the conclusion that water can play a significant role in processes of humification. We assume that separation of hydrophobic

and hydrophilic domains and thus increase in nanoscale heterogeneity represents an important physical contribution to the overall humification process. It was also demonstrated that the higher content of oxygen in humic molecules is not the only indicator of higher water holding capacity. Instead the porosity of humic matrix seems to contribute as additional parameter into these processes.

**Keywords** Humic and fulvic acids · Hydration · Water · Differential scanning calorimetry · Humification

## Introduction

Humic substances (HS) represent a complex mixture of various molecules formed in nature as a by-product of biomass and dead animal bodies' decomposition. Chemically, HS can be visualized as a mixture of both substituted aromatic and aliphatic molecules forming various physical structures stabilized by intermolecular interactions [1]. The primary structure reflects the conditions of formation, such as parental material, climate conditions, and character of present microorganisms. In soils, their role is, among others, closely linked with soil stability, sorption processes, water holding capacity, and cell biology of soil living organisms [2, 3]. The most important fractions of natural organic matter (NOM) are fulvic acids (FA) and humic acids (HA); FA are fractions of NOM soluble at all pH values, while HA are soluble only in alkaline solutions [2].

Hydration is the crucial factor playing role in biological function of molecules in both living and natural systems. Water represents an important medium for nutrient transport, cell membrane processes, induces biologically active conformation of biomolecules, etc. It is also the case of NOM. Due to the presence of solid particles, molecular

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assemblies or single molecules, water properties in terms of vapor pressure, enthalpy, entropy, viscosity, and density will differ from nanosite to nanosite in the OM matrix [3]. In general, water can be subdivided into free water which physical structure is not influenced by the presence of additional molecules. Another type is bound water, whose properties are more or less modified by the respective interaction partners [4]. Consequently, supplementary types of water can also be considered, resulting in distinguishing of four water categories: (i) free water: water non-associated with solid particles and including void water not affected by capillary force, (ii) interstitial water: water trapped inside crevices and interstitial spaces of flocs and organisms, (iii) surface (or vicinal) water: water held on to the surface of solid particles by adsorption and adhesion, (iv) bound (or hydration) water [5–7]. Using differential scanning calorimetry (DSC), three types of water belonging to above groups can be distinguished [7]: free water ( $W_f$ ) whose melting/crystallization temperature and enthalpy are not significantly different from those of bulk water [water type (i)]; water species exhibiting large differences in phase transition enthalpies and temperatures known as freezing-bound water [ $W_{fb}$ ; water type (ii)]; and non-freezing water ( $W_{nf}$ ) which is strongly associated with the molecule and shows neither crystallization exotherms nor melting endotherms on DSC curves [water types (iii) and (iv)] [8, 9].

Recent studies have shown that hydration of NOM results in physicochemical changes of soil organic matter (SOM) [10–12]. It may strongly affect its sorptive properties, such as sorption kinetics and binding of hydrophobic organic compounds [13–16], swelling [10], increased flexibility [17], alterations in conformation or changes in ionization status of polar functional groups [12]. These hydration-driven changes can have an impact on nutrient or pollutant retention and transport of solutions (e.g., hydrophobic pollutants) through the organic matter of soils and sediments [13–15].

Furthermore, the hydration kinetics of SOM is influential factor for transport and sorption processes in soil. Nevertheless, knowledge about wetting and swelling processes, which both control the overall hydration kinetics, is limited [10]. Many studies have shown that the sorption of organic substances in SOM depends among the others on the water content, number of drying and wetting cycles, hydration time, and the type of water binding [10]. The quality of studied organic material and its pore size distribution plays a significant role in hydration kinetics as well [12]. After the absorption of water, size of inner pores of organic matter will increase due to swelling while size of external ones decreases. Hydration process of soils can be divided into two steps. The first step includes the wetting of mineral and organic soil components. After wetting, water

is distributed within the pore volume. Possibly, swelling of SOM and clay minerals as well as hydration of salts may follow [10]. This dynamic process is based on the assumption that the structure and properties of organic matter is similar to that of hydrogels since there is still a certain parallelism with their properties [12]. This is rather questioned by the observation that in a peat and SOM matrix water becomes a short-term plasticizer and long-term anti-plasticizer [18]. Furthermore, the structure of HS does not seem to be that reversible considering their supra-molecular assembly, differences in polarity or mechanical properties (elasticity) of individual components.

In this work, we studied the character of hydration water in water/humic substance systems. The goal was to determine both quantitative and qualitative aspects of hydration of HS in solid and liquid phase via its freezing–melting behavior and to explore the differences in properties of water in contact with humic matter using DSC. The working hypothesis of this study is that the hydration level is a crucial factor influencing both state of water in humic matrix as well as character of physical structure of respective HS. We assume that the degree of humification, i.e., history of HS development, should have a strong influence on hydration processes of these materials. Last but not least, the similarity of hydration mechanisms with more homogeneous hydrogels or biopolymers is questioned as well.

## Materials and methods

### Materials

The following standard samples were purchased from the International Humic Substances Society (IHSS) and were measured as received: Suwannee river II HA 2S101H (SRHA), Suwannee river II FA 2S101F (SRFA), Elliot soil HA 1S102H (ESHA), Elliot soil II FA 2S102F (ESFA), Pahokee peat HA 1S103H (PPHA), Pahokee peat II FA 2S103F (PPFA), Leonardite HA 1S104H (LHA). Elemental analysis of investigated samples (Table 1) was obtained from official IHSS web site (<http://www.ihss.gatech.edu/elements.html>), where also other structural details can be found.

### DSC to study the freezing–melting behavior of hydrated HS

Approximately 2 mg of each sample was placed in an aluminum DSC Tzero pan and excess of water was added. Surplus water was allowed to evaporate slowly at room temperature until the desired water content was obtained.

**Table 1** Elemental composition in % (w/w) of dry, ash-free IHSS samples (<http://www.ihss.gatech.edu/elements.html>)

IHSS samples	C	H	O	C/O	C/H
LHA	63.8	3.70	31.3	2.04	17.3
SRHA	52.6	4.28	42.0	1.25	12.3
SRFA	52.3	4.36	43.0	1.22	12.0
ESHA	58.1	3.68	34.1	1.71	15.8
ESFA	50.1	4.28	42.6	1.18	11.7
PPHA	56.4	3.82	37.3	1.51	14.8
PPFA	51.3	3.53	43.3	1.18	14.5

Subsequently, the pans were hermetically sealed. DSC was performed using the TA Instruments Q200 equipped by a rapid cooling system (RCS) in order to study the melting process of ice formed by freezable water. An empty hermetically sealed pan was used as reference. The measurements were conducted ranging from 40 to  $-90$  °C at 3 °C/min, and then from  $-90$  to 30 °C at 5 °C/min under the flow of nitrogen (50 mL/min). The temperature scale and heat of transition were calibrated using distilled water and indium as standards, respectively. Each measurement ran through three immediately subsequent identical cycles to test reproducibility of ice melting and to investigate potential changes in the hydrated HS structure due to the freezing–melting cycles. In this way, it was also verified that sample pans were well hermetically sealed so no water was evaporating and that formation of ice, due to its volume expansion, does not cause the HS supramolecular destruction. Selected samples were prepared in triplicates

and no significant difference in the DSC record and respective melting enthalpy was observed.

#### Determination of water content by thermogravimetric analysis

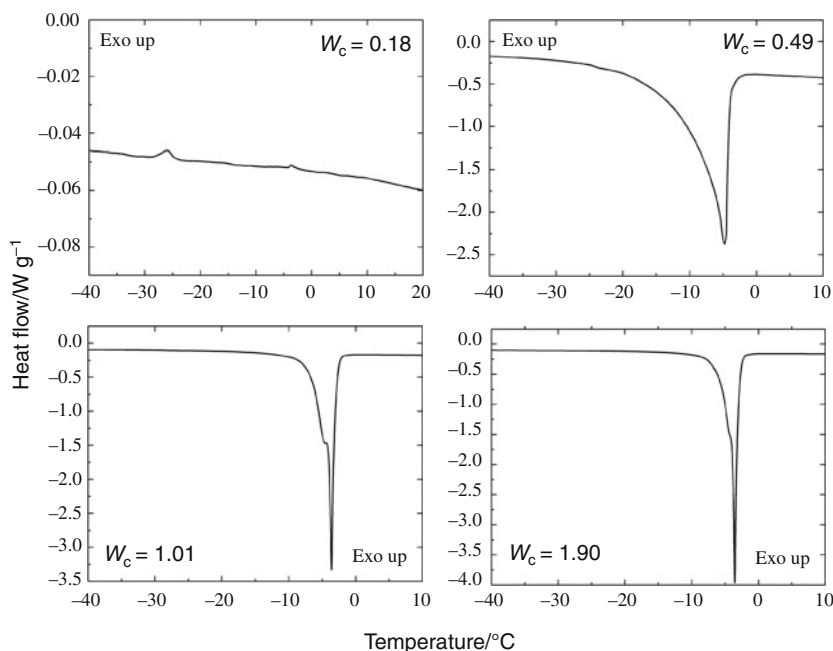
TGA Q5000IR (TA Instruments, New Castle, USA) was used to determine the moisture content of purchased IHSS standard samples and to obtain the precise concentration of water ( $W_c$ ) in the samples. Experiments were carried out in open Pt pans. The temperature range of the experiments was from 25 to 180 °C at 3 °C/min under the flow of nitrogen.

The  $W_c$  nomenclature used in this study is based on the ratio between mass of water with respect to the dry mass and is defined as grams of water per gram of dry sample.

## Results and discussion

### Hydration state after 1 day of HS-water contact time

In this part, hydration of IHSS samples in the  $W_c$  range between 0.1 and 2.0 (g/g) were investigated. The DSC records were obtained 24 h after the first contact between water and HS. Representative examples of heating curves of FA and HA originating from Suwannee river of different  $W_c$  are given in Figs. 1 and 2, respectively. The records are plotted in the temperature range from  $-40$  to 10 °C since at lower temperatures no thermal events were detected.

**Fig. 1** DSC heating curves of ice melting in hydrated SRHA measured after 1 day of hydration

**Fig. 2** DSC heating curves of ice melting in hydrated SRFA measured after 1 day of hydration

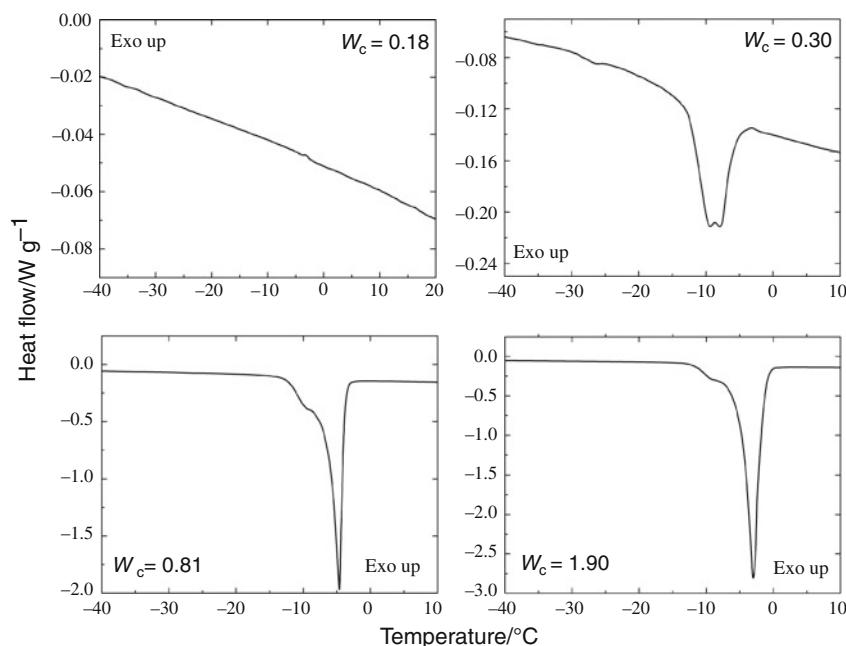


Figure 1 shows the melting behavior of hydrated SRHA after a water contact time of 1 day. For  $W_c = 0.18$ , no endothermal phase transitions were detected, only several small exothermal peaks associated with system restructuring. This is in accordance with the assumption that at very low water content, all water molecules are present in the form of non-freezing water, i.e., all of them are restricted by the intimate contact with the humic molecules surface and thus they cannot participate in ice formation [12]. Increasing the water content to  $W_c = 0.49$  resulted in an endothermal event around  $-5\text{ }^\circ\text{C}$  which indicates the melting of ice originating from the presence of freezable water. A further increase in water content to  $W_c = 1.01$  and  $W_c = 1.90$  increased the peak area (see the changes in Y-axis range) and peak temperature were slightly shifted to higher values. Simultaneously, a small shoulder appeared in both thermograms indicating overlapping processes which can reflect melting of different types of freezing-bound water. With this respect, it is noteworthy that the peak width decreased with increasing water concentration which implies either decrease in heterogeneity of ice structures with increasing water content or occurrence of additional processes during the ice melting (e.g., transition from cubic to hexagonal ice, see e.g., Ref. [19]).

The hydrated SRFA samples (Fig. 2) show a comparable qualitative melting behavior as SRHA. Like for SRHA, the peak temperature decreased with increasing water content and the melting peaks split similarly to those for SRHA, although the shape of the double peak differs from that of SRHA. However, unlike SRHA, SRFA reveals also a small endothermic peak around  $-25\text{ }^\circ\text{C}$  at concentration  $W_c = 0.30$ , and the main melting peak occurred around  $-10\text{ }^\circ\text{C}$ .

As freezing-bound water represents a kind of water/ice whose structure is affected by the interaction with humic molecules, mainly by polar groups and by the distribution of pores in the physical structure [12], the differences between the melting enthalpy of ice in SRHA and SRFA show that SRFA has a stronger impact on the ice structure than SRHA.

The other IHSS samples of HA and FA investigated in this study showed similar behavior and therefore their thermograms have not been plotted.

In none of the investigated samples, thermograms obtained from three subsequent freezing–melting cycles differed from each other. This suggests that the freezing–melting process of the water did not affect the physical structure of hydrated FA and HA. This is in contrast to polysaccharide Konjac mannan where peak number was reduced in the second and third heating cycle [9].

#### Hydration state after 21 days

Figure 3 reports the change of the DSC melting curves of SRFA at  $W_c = 0.51$  during the hydration period up to 21 days. The record from the first day displays a peak with maximum around  $-5\text{ }^\circ\text{C}$ . In fact, the position of the peak did not significantly change during the period of hydration. In contrast, the melting enthalpy decreased confirming the incorporation of larger amount of water in hydration, i.e., progressively larger amount of total water content is present as non-freezing water. Further, as can be seen mainly at 4th and 10th day, there appeared an exothermal peak preceding the ice melting which can be attributed to the cold crystallization resulting from the crystallization of

glassy water [20, 21] which is typical (among others) for water/biopolymer system [22]. These observations confirm that during hydration not only new surfaces are wetted but also the change in the physical structure of HS takes part. This causes the existence of “restricted” water which is reflected by appearance of cold crystallization on DSC exotherm. The peak broadening suggests an increase in heterogeneity in the sample, potentially caused by an increase in the distribution of inner places and surfaces in humic matrix, such as cavities and holes in which water molecules experience a variety of interactions and physical states.

Similar records were obtained for the SRHA sample, but the melting enthalpy decrease was more intensive in case of SRFA. Assuming that the extent of this decrease indicates a more intensive interaction between HS and water, this observation can be explained by the higher polarity of SRFA than SRHA (see oxygen content and C/O in Table 1) resulting in a higher hydrophilicity and in faster wetting and swelling of SRFA than SRHA.

Unlike the more homogeneous hydrophilic biopolymers, where the hydration processes are usually completed within hours to days (even in case of insoluble ones), the kinetics of hydration in HS evidently plays a more important role as well as the hydration mechanism appears to be different which is in line with the recent results implying that hydration of e.g., peat can be as long as several months [10, 12].

As reported in our recent work, hydrophobic hydration plays a crucial role in chemistry of HA; FA are more polar and their influence on mobility of water molecules is generally larger [23]. The motion of water confined in

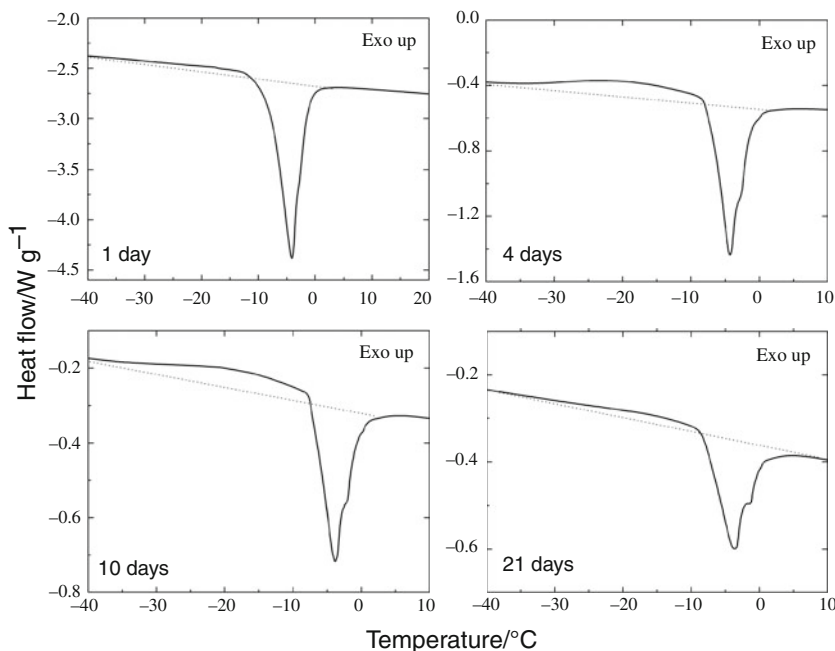
polar cavities or bound on the polar surface is more restricted and therefore the formation of ice is associated with formation of less perfect crystals or even amorphous ice than in case of bulk water or water on hydrophobic surface [24]. This explanation agrees with the observation of cold crystallization for the SRFA. When the HS/water system is cooled rapidly, part of water might remain in a supercooled state due to above-mentioned restrictions in the form of so-called glassy water [20, 21]. On heating, the molecular motion of glassy water is enhanced and water changes to crystal ice [9]. That phenomenon was only minor in records of the first day and last day (Fig. 3), which points to the importance of kinetic factor while considering hydration processes of HS, i.e., wetting and swelling. It also indicates that the structure has been stabilized during the preceding hydration period.

### Hydration kinetics

In order to evaluate the time development of hydration more quantitatively and draw qualitative conclusions on hydration kinetics, the endothermic peaks were integrated to obtain the melting enthalpies. Figure 4 representatively shows the melting enthalpies for four selected  $W_c$  of Leonardite HA (LHA), Suwanee river HA and FA (SRHA and SRFA, respectively) as a function of time.

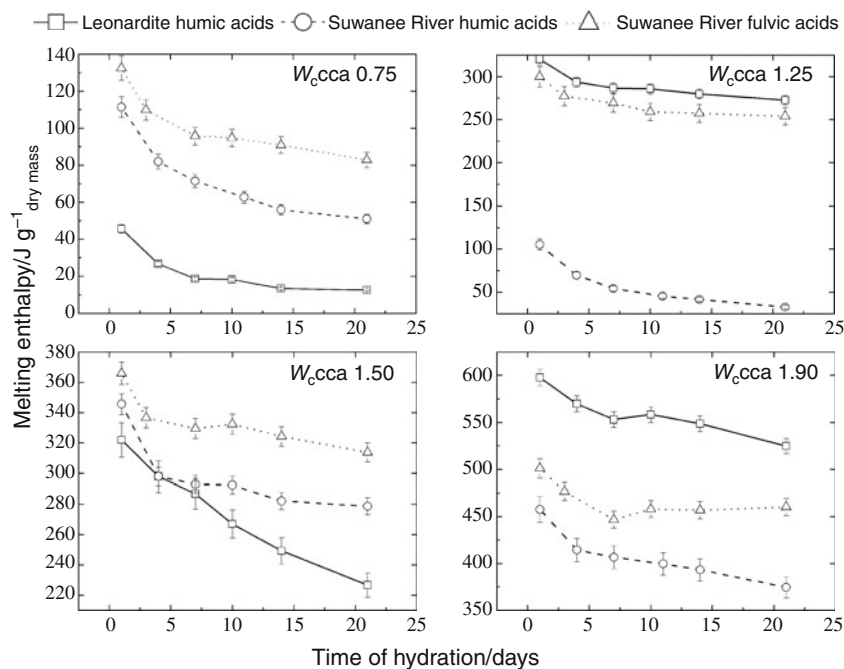
In general, the melting enthalpy decreases with increasing hydration time, although the decrease functions differ qualitatively between the three samples and between the four water contents. During the first 7 days, melting enthalpy decreased fast in all samples. After that time period, the decrease became more moderate. This seems to

**Fig. 3** DSC heating curves of ice melting in hydrated SRHA at a water content of  $W_c = 0.51$  at four time points between 1 and 21 days





**Fig. 4** Development of melting enthalpy of ice in hydrated LHA, SRHA, and SRFA samples as function of hydration time for four different water contents

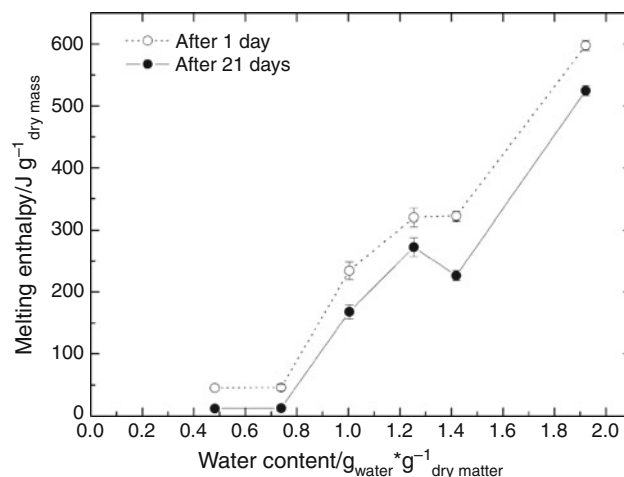


be in line with the recent statement that the wetting of SOM proceeds in two steps [10]. The first step includes wetting of the surfaces and after that the water is distributed to the pore volume. In contrast, Jaeger et al. [12] described hydration of peat as a three step process including breaking of hydrogen bonds, water diffusion, and reorientation of molecular chains during hydration. These differences are most probably due to significantly larger heterogeneity of the whole peat sample in contrast to the purified HS used in our study.

Figure 5 shows the melting enthalpy as a function of the water content for 1 and 21 days of hydration of LHA. As expected, the melting enthalpy increases with increasing water content. However, unlike the hydration of biopolymers (compare for example with Ref. [22]), this dependence does not show a linear trend. Instead, the melting enthalpy increases in two stages. It increases from  $W_c = 0.8$  to  $W_c = 1.2$ , remains constant (1 day), respectively, even decreases significantly (21 days) at  $W_c = 1.4$  and then continues the increase.

This can be explained as follows: due to LHA porous structure, water is present in different states influencing the ice melting enthalpy. The step-like dependency indicates the mechanisms of hydration: water penetrates the cavity and increases its volume by swelling while the water still remains largely freezable, and the melting enthalpy increases with increasing water content up to  $W_c = 1.2$ . As soon as the water content exceeds the capacity of the cavity, weak interactions stabilizing the domain are broken and water can penetrate further and wet another set of surfaces, which are most probably more hydrophobic as they are wetted only at higher water content. This explains

the stagnation or respective decrease in melting enthalpy around  $W_c = 1.4$ . We assume that the spatial separation between hydrophilic and hydrophobic hotspots of the HA is at least partially induced by the hydration process itself; as a result of the hydrophobic effect, hydrophobic molecules are thermodynamically separated and “packed” away forming porous scaffold. As reported recently, HA are present as aggregates even in diluted solution [23]. In aqueous environment, supramolecular structures consisting of hydrophobic and amphiphilic molecules are stabilized significantly by hydrophobic interactions, such as van der Waals,  $\pi$ - $\pi$  and CH- $\pi$  dispersive forces [1]. The same interactions can be expected to stabilize the scaffold in the solid state or at water concentrations used in this study.



**Fig. 5** Dependence of the melting enthalpy of ice on water content in hydrated LHA

In this way, water changes the structure of LHA, the scaffold stabilizing the structure is preserved and potentially strengthened during hydration as indicated by the stronger expression of the stepwise curve after 21 days of hydration.

Figure 6 summarizes results for the rest of HS investigated in this study. It shows that the melting enthalpy of SRFA increased linearly with water content, while SRHA showed a two-stage behavior like demonstrated for LHA, with the intermediate decrease of melting enthalpy also occurring around  $W_c = 1.40$ .

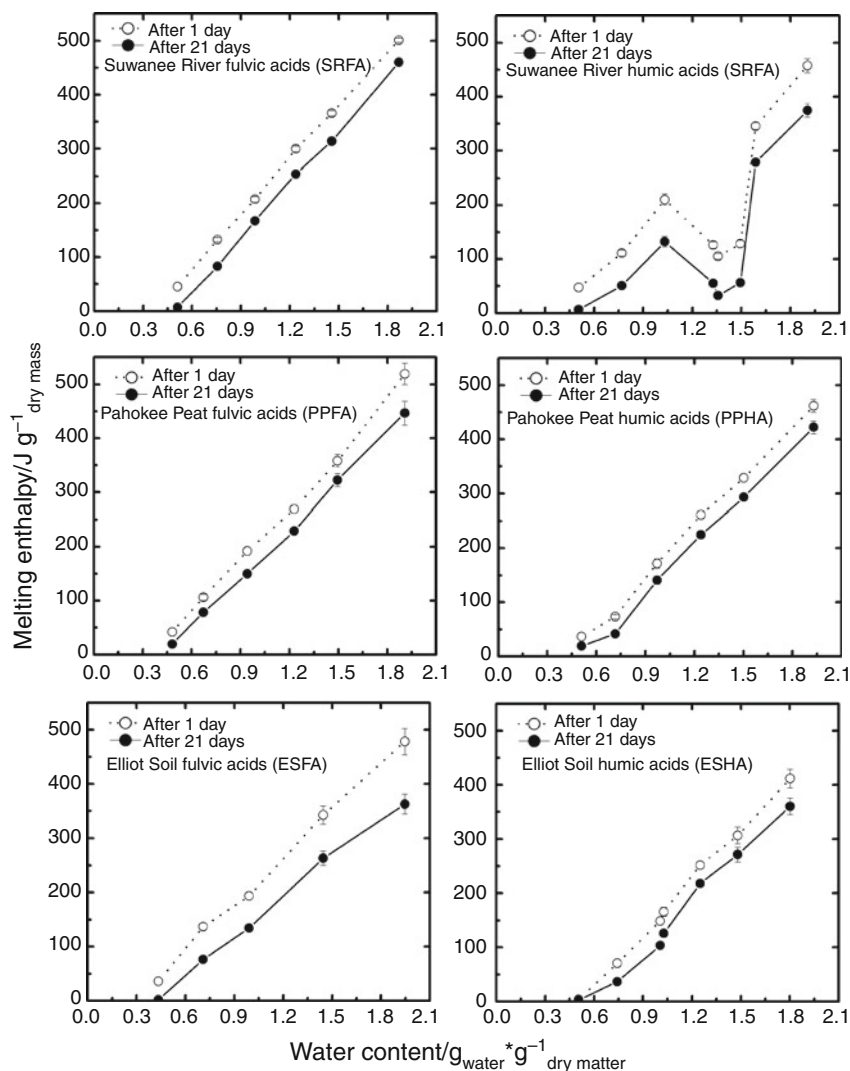
Like SRFA, other IHSS humic and fulvic samples showed a nearly linear dependency between melting enthalpy and water content, similar as for hydrophilic polymers [8, 22] with no indication of the “scaffolding” suggested by our results for the HAs. Extrapolation of the melting enthalpy–water content relations to zero melting enthalpy in Fig. 5 indicates the content of non-freezing water [8, 22]. The content of non-freezing water did not

differ between the HAs and their related FAs. Rather, contents of non-freezable water were tendentially higher for the HAs. We assume that the more complicated inner structure of HA with higher porosity allows the water molecules to hydrate higher surface than in FA.

#### Discussion and future perspectives

Thermal analysis in natural organic matter characterization has been already recognized; however, in most cases, it included thermal-degradation approaches [25, 26]. Only rare studies employ non-destructive methodology (see e.g., Ref. [18]). In this study, DSC proved itself to be a powerful technique in characterization hydration of organic matter via freezing–melting processes. Our results show that hydration changes the physical structure of HS. The two-stage hydration characteristics deduced from the non-linear dependency between melting enthalpy and water content suggests that hydration, due to hydrophobic effect,

**Fig. 6** Dependence of the melting enthalpy of ice in hydrated Suwanee river, Elliot soil, and Pahokee peat FA, and HA after 1 and 21 days of hydration



separates hydrophobic and hydrophilic microregions in the HAs. DSC results indicate that at specific concentrations and in some cases hydrophobic domains [23] are interconnected by non-specific weak interactions; as a result, “hydrophobic scaffold” is formed resembling a quasi-porous structure with rigid hydrophobic moieties surrounded by flexible, swollen hydrophilic moieties, which penetrate the pore system. This separation effect is furthermore supported by latest results of molecular modeling, which showed that hydrophobic supramolecular structures are more stable in hydrophilic environments and hydrophilic structures, vice versa, are more stable in hydrophobic environment [27]. Like the results of our study, these findings suggest that upon aging and hydration–dehydration processes, hydrophobic as well as hydrophilic microregions in the HA matrix will concentrate more and more, forming successively more distinct hotspots and resulting in an increase in nanospatial heterogeneity in the HA matrix.

In accordance with recent supramolecular concept suggested by Piccolo et al. [28] (see Ref. therein), it can be agreed that humification in soil can be considered as a two-step process: (i) biodegradation of dead-cells components, (ii) hydration–dehydration driven aggregation of the degradation products. In light of the previous discussion, formation of new covalent bonds during the humification process producing humus is not the only alternative. Instead, humification or at least part of it can be considered as the progressive self-association of the mainly hydrophobic molecules resistant to biodegradation supported by repeating wetting and drying. In this way, the hydrophobic scaffold is formed allowing to some molecules to be separated from water and therefore to be excluded from microbiological degradation.

## Conclusions

In this study, both the qualitative and quantitative aspects of hydration of HS were investigated and the differences in properties of water surrounding humic matter were explored. The DSC measurements of HS/water confirmed that the kinetics plays a significant role in hydration processes of HS. Unlike most hydrophilic biopolymers, the dependency of melting enthalpy change of freezable water (ice) on  $W_c$  was not constant after 24 h from preparing the sample because of its incomplete hydration. Therefore, the phase transition behavior was monitored over the period of 21 days. During this period, the melting enthalpy change of melting decreased for all samples. The results confirmed earlier observations that the hydration of HS is not a straightforward process. Mainly until approximately day 7, the rapid decrease in change of melting enthalpy occurred.

After that time period, the diminution became more moderate. A crucial factor in distinguishing between HA and FA seems to be the different solubility of particular molecules. In FA, most of molecules are hydrophilic; therefore, their hydration resembles the hydrophilic biopolymers. In contrast, HA consist of a mixture of molecules of various polarity and therefore different wettability. The more complicated inner structure of HA with higher porosity allows the water molecules to hydrate higher surface than in FA. It seems that the younger (or less humified) the HS are, the easier and faster the change of their physical structure is.

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