# Soil organic matter in riparian floodplain soils: regionalization of stocks and stabilization processes

vorgelegt von

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von der Fakultät VI – Planen Bauen Umwelt der Technischen Universität Berlin zur Erlangung des akademischen Grades

Doktor der Ingenieurwissenschaften

– Dr.-Ing. –

genehmigte Dissertation

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Tag der wissenschaftlichen Aussprache: 9. Februar 2016

Berlin 2016

"Gut Ding will Weile haben"

Deutsches Sprichwort / German saying

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# Acknowledgements

This would not exist without the help of many people. I would like to thank...

- ...**Prof. Dr. Friederike Lang** for supervision, outstanding support throughout the years and especially for being a teacher and recipient for questions about soil and life.
- ...**Prof. Dr. Martin Kaupenjohann** for supervision and for having me introduced into the fascinating world of soils.
- ... *Dipl.-Ing. Jaane Krüger* for having shared thoughts, success, disappointment, stress, leisure and offices for the last 8 years.
- ...a large number of students having crossed my way over the last years and with whom I was allowed to work during their bachelor, master or diploma thesis. Within this group I would like to mention *M.Sc. Gilles Kayser* for his outstanding master thesis which significantly contributes to Chapter E and *Dipl.-Geogr. Theresa Schiller* who supported me in the early years of this project to assess the wilderness of Danubian floodplains.
- ...all staff members who did an extraordinary work in the soil science laboratories in Berlin and Freiburg. Special thanks go out to *Sabine Dumke* and *Sabine Rautenberg* who carried out heaps of measurements contributing to the presented results.
- ...all colleagues in the soil science groups in Berlin und Freiburg for supporting and inspiring me.
- ... *Dr. habil. Arne Cierjacks* and *Prof. Dr. Birgit Kleinschmit* and all those who have been involved in riparian floodplain research at the TU Berlin.
- ...colleagues in the National Park Donau-Auen for supporting my research, especially *Dr. Christian Baumgartner* and *DI Christian Fraissl*.
- ...my parents Sabine und Klaus Graf for support and ongoing faith in all of my decisions
- ...my wife *Ulli Graf-Rosenfellner* for being at my side in the past and for the rest of our lives. You are the reason for everything.

## List of publications / bibliographic information

This thesis contains three articles already published in peer-reviewed international journals (Chapters B, D, and E) and one unpublished manuscript (Chapter C). The published articles are:

Chapter B -

#### Carbon stocks of soil and vegetation on Danubian floodplains

A. Cierjacks, B. Kleinschmit, M. Babinsky, F. Kleinschroth, A. Markert, M. Menzel, U.

Ziechmann, T. Schiller, M. Graf, and F. Lang

Published in:

Journal of Plant Nutrition and Soil Science (2010), Vol. 173, 644 – 653.

DOI: 10.1002/jpln.200900209

http://onlinelibrary.wiley.com/doi/10.1002/jpln.200900209/abstract

Chapter D -

# Soil formation and its implications for the stabilization of soil organic matter in the riparian zone

M. Graf-Rosenfellner, A. Cierjacks, B. Kleinschmit, and F. Lang

Published in:

Catena (2016), Vol. 139, 9 - 18.

DOI: 10.1016/j.catena.2015.11.010

http://www.sciencedirect.com/science/article/pii/S0341816215301594

Chapter E -

# Organic matter distribution in floodplains can be predicted using spatial and vegetation structure data

A. Cierjacks, B. Kleinschmit, I. Kowarik, M. Graf, and F. Lang

Published in:

River Research and Applications (2011), Vol. 27(8), 1048 – 1057.

DOI: 10.1002/rra.1409

http://onlinelibrary.wiley.com/doi/10.1002/rra.1409/abstract

The unpublished manuscript is:

Chapter D -

#### Round robin test on the soil disaggregation efficiency of ultrasound

M. Graf-Rosenfellner, G. Kayser, G. Guggenberger, K. Kaiser, M. Kaiser, C.W. Müller, M. Schrumpf, T. Rennert, G. Welp, and F. Lang

#### **Abstract**

Mineral soils in riparian floodplains are known for large organic carbon (OC) stocks in both top and sub soil. Soil forming conditions unique in these landscapes, mainly determined by flooding and sedimentation, contribute to these large stocks compared to other mineral soils in temperate climates. With regard to the position of soils in the carbon cycle, knowledge on stabilization mechanisms for soil organic matter (SOM) in addition to soil OC stocks is crucial to assess the function of riparian floodplain soils as sinks or sources of carbon. In this thesis, both aspects were investigated in the area of the "National Park Donau-Auen" which is one of the biggest remaining near-natural riparian floodplains in central Europe.

In the soils sampled in the study area a mean OC stock of 177 t ha<sup>-1</sup> was determined and confirms the assumption that riparian floodplain soils are characterized by large OC stocks. Within the study area, soil OC stocks differed with regard to the hydroecomorphological site conditions, which are the result of the site-specific flooding dynamics. These are supposed to change along a gradient with increased distance from the river channel and are indicated by different vegetation types. Sites close to the river dominated by willow forests ("softwood sites") showed lower soil OC stocks (154 t ha<sup>-1</sup>) compared with sites in larger distance to the river which were dominated by hardwood tree-species (oak, ash, elm or maple, "hardwood sites", 186 t OC ha<sup>-1</sup>). The influence of flooding dynamics on OC stocks at these two extremes of the gradient is further underlined by the vertical distribution of soil OC stocks. Close to the river, buried top soils were found with over- and underlying coarsely textured sediments. Periods without sedimentation allow Ah horizons to form, which are subsequently covered by large amounts of sediments in periods with strong aggradation. This can be explained by periodic changes of flooding conditions with large amplitudes at these sites. Opposed, soils distant to the river constantly receive lower amounts of fine sediments rich in OC. Consequently, no buried top soils are found in the sub soil there and flooding conditions can be assumed to be temporally constant.

Furthermore, flooding dynamics also determine stabilization mechanisms for SOM on these sites. Prior to analyzing stabilization mechanisms for SOM, methods for their determination involving ultrasound technique were evaluated with regard to reproducibility and comparability of the results. Results of a round robin test on this topic showed that both were satisfying and ultrasound technique was therefore used to assess stabilization mechanisms for SOM.

In soils formed under dynamically changing flooding conditions at softwood dominated sites, large portions of SOM were stabilized by occlusion into aggregates or were present in a scarcely stabilized fraction as free particulate organic matter (together 20 - 40% of total OC in these soils). These fractions were of minor importance in soils formed under constant flooding conditions (hardwood sites, < 10% of total OC in these fractions). Obviously, free particulate OM (which is usually mostly

found in young top soils because it gets mineralized within years) can sustain for longer times in sub soil under softwood vegetation. Preservation by covering with sediments is likely to contribute to this finding. The importance of stabilization by occlusion into aggregates is remarkable because this mechanism is usually of low importance in coarsely textured soils. Under hardwood forest with constant flooding conditions, fine textured material is deposited and SOM is largely stabilized by sorption on mineral surfaces and aggregation is much less important for stabilization of SOM in these soils.

For the regionalization of OC stocks and stabilization mechanisms for SOM, an approach combining spatial information with vegetation parameters turned out to be suitable. Vegetation parameters like stem number per area unit, mean stem diameter or cover of canopy, herb or shrub layer were included together with spatial information into multiple regression models. These approach allowed to predict site specific flooding dynamics and related OC stocks and SOM stabilization mechanisms as a result.

## Kurzfassung in deutscher Sprache

Mineralische Böden in Flussauen sind für ihre hohen Vorräte an organischem Kohlenstoff sowohl im Ober- als auch im Unterboden bekannt. Hierzu tragen die außergewöhnlichen Bedingungen für die Bodenbildung in Flussauen bei. Diese sind maßgeblich bestimmt durch wiederkehrende Überflutung und Sedimentation. In Anbetracht der Funktion von Böden im Kontext des Klimawandels sind Kenntnisse über Stabilisierungsmechanismen von organischer Bodensubstanz neben der Bemessung der Vorräte an organischem Kohlenstoff unabdingbar für die Bewertung von Auenböden in Bezug auf ihre Senken- oder Quellenfunktion für Kohlenstoff. In dieser Arbeit wurden daher beide Aspekte in einer der letzten und größten sowie annähernd natürlich entwickelten Flussauenlandschaft in Mitteleuropa, dem Nationalpark Donau-Auen nahe Wien (Österreich), untersucht.

Der mittlere Kohlenstoffvorrat in den untersuchten Böden betrug 177 t ha<sup>-1</sup> und bestätigt damit die Annahme, dass sich Böden in Auenlandschaft durch hohe Kohlenstoffvorräte auszeichnen. Innerhalb des Untersuchungsgebietes unterschieden sich die bestimmten Vorräte jedoch in Hinblick auf die jeweiligen hydroökomorphologischen Standortbedingungen, welche von der dort vorherrschenden Überflutungs- und Sedimentationsdynamik bestimmt werden. Es wird weithin angenommen, dass diese sich entlang eines Gradienten mit steigender Entfernung vom Fluss verändern und durch unterschiedliche Standortvegetation angezeigt werden. An Weichholzauenstandorten in Flussnähe wurden im Mittel geringere Kohlenstoffvorräte im Boden (154 t ha<sup>-1</sup>) festgestellt als an Hartholzauenstandorten (186 t ha<sup>-1</sup>) in größerer Distanz zum Fluss. Der Einfluss der Überflutungsund Sedimentationsdynamik auf die Kohlenstoffvorräte wird zusätzlich durch die Unterschiede in der vertikalen Verteilung der Kohlenstoffvorräte in Bodenprofilen an diesen Standorten hervorgehoben.

An flussnahen Standorten konnten im Unterboden ehemalige Oberbodenhorizonte bestimmt werden, die durch dicke Sedimentschichten aus Material mit grober Textur begraben worden sind. Offensichtlich konnten sich diese Oberböden während längerer Perioden ohne Sedimentationsereignisse entwickeln und wurden danach bei sehr starken Sedimentationsereignissen begraben. Dies lässt sich durch wiederkehrende Veränderungen in den Überflutungs- und Sedimentationsbedingungen mit starken Amplituden bezüglich dieser Parameter erklären. Im Gegensatz dazu konnten in Böden in größerer Distanz zum Fluss, die durch zeitlich konstante Sedimentation von geringen Mengen an feinem Material gekennzeichnet sind, diese begrabenen Oberböden nicht im Unterboden gefunden werden.

Die Überflutungs- und Sedimentationsdynamik hat ebenfalls einen Einfluss auf die Mechanismen für die Stabilisierung der organischen Bodensubstanz. Vor der Durchführung der Analysen wurden in einem Ringversuch die Reproduzier- und Vergleichbarkeit der verwendeten Standardmethode zur Bestimmung der Stabilisierungsmechanismen unter Einbeziehung von Ultraschall zur Disaggregierung überprüft. Hierbei konnte gezeigt werden, dass die verwendete Methode die zuvor angezweifelten Qualitätsmerkmale der Reproduzier- und Vergleichbarkeit zufriedenstellend erfüllt und die Nutzung von Ultraschall für die Bestimmung der Stabilisierungsmechanismen somit geeignet ist. In Böden, die unter sich dynamisch ändernden Überflutungs- und Sedimentationsbedingungen gebildet werden, wurden relativ große Mengen an organischer Bodensubstanz durch Einschluss in Aggregate stabilisiert oder lagen als gering stabilisierte freie, partikuläre organische Bodensubstanz vor (zusammen 20 - 40 % des gesamten organischen Kohlenstoffs in diesen Fraktionen). Diese Fraktionen wiesen in Böden, die sich bei konstanten Überflutungs- und Sedimentationsbedingungen gebildet haben, einen wesentlichen geringeren Anteil auf (< 10 % des gesamten organischen Kohlenstoffs in diesen Fraktionen). Offensichtlich bleibt die freie, partikuläre organische Bodensubstanz (die gewöhnlich in großen Anteilen nur in jungen Oberböden vorliegt, da sie innerhalb weniger Jahre mineralisiert wird) auch in Unterböden von Weichholzauenstandorten über längere Zeit erhalten. Für den Schutz dieser Fraktion vor Mineralisierung scheint die Überlagerung mit großen Mengen an Sediment verantwortlich zu sein. Darüber hinaus ist auch der relative Anteil an durch Einschluss in Bodenaggregate stabilisierter organischer Bodensubstanz an diesen Standorten bemerkenswert, da dieser normalerweise bei Böden mit grober Textur nur eine untergeordnete Rolle spielt. In den Böden der Hartholzaue mit feiner Textur, die sich unter konstanten Überflutungs- und Sedimentationsbedingungen entwickelt haben, ist die Stabilisierung der organischen Bodensubstanz durch Sorption an Oberflächen der klar dominierende Stabilisierungsmechanismus. Der Einschluss in Aggregate spielt hier nur eine untergeordnete Rolle. Für die Regionalisierung der Kohlenstoffvorräte und der Stabilisierungsmechanismen der organischen Bodensubstanz wurde ein Ansatz, der sowohl räumliche als auch Vegetationsparameter für die

Identifikation und Vorhersage der Überflutungs- und Sedimentationsdynamik an den jeweiligen Standorten der Aue verwendet, entwickelt. Forstliche Standortparameter wie die Stammzahl, der Brusthöhendurchmesser aber auch der Baumkronendeckungsgrad sowie die Dichte der Kraut- und Strauchschicht wurden in Regressionsmodellen mit räumlichen Informationen zusammengeführt. Diese Modelle erlauben durch diesen neuen Ansatz die Vorhersage der Überflutungs- und Sedimentationsdynamik und den damit verbundenen Bodenkohlenstoffvorräten und Stabilisierungsmechanismen für organische Bodensubstanz.

### A Introduction and synthesis

#### A.1 Background and rationale

#### A.1.1 Climate change and soils in carbon cycle

The global and regional climate change caused by increasing concentrations of CO2 and other greenhouse gases (GHG) in the atmosphere including its expected aftermaths are among the most important issues of mankind in the upcoming century (Cubasch et al., 2013). The need to understand processes and mechanisms involved in this phenomenon compels with regard to lives threatened by natural disasters and economical costs induced by climate change (Hallegatte et al., 2016). Limiting the increase of CO<sub>2</sub> emissions from burning fossil fuels and sequestrating more carbon on a long term seem to be crucial. The importance of soils for the latter becomes evident when regarding distribution of carbon stocks between different terrestrial reservoirs: about 2,500 Pg C are found in soils as soil organic carbon (SOC, 1,550 Pg C) and inorganic carbon (SIC, 950 Pg C). The biotic reservoir, which comprises total terrestrial biomass, contains only 1/5 (560 Pg C) of the mass of carbon in soils while 760 Pg C are found in the atmosphere (Lal, 2004a). This means that soils may sequestrate large amounts of carbon and provide the potential to mitigate the predicted increase of atmospheric CO2 concentrations by sequestration - if related processes are understood and soils are treated and managed accordingly. On a global scale, the potential for annual sequestration of carbon in soils is numbered with 0.9 +/- 0.3 Pg C a<sup>-1</sup> and can diminish the predicted annual increase of CO<sub>2</sub> in the atmosphere by 30 % (Lal, 2004b). Although carbon reservoirs in soils and terrestrial biomass are rather small compared to the largest known carbon reservoir (oceanic carbon: 38,000 Pg C), they are of special concern because they are potentially more labile (Batjes, 1996). Therefore, soils bear the risk of an unwanted additional release of large amounts of carbon due to wrong management practices (Lal, 2004b). This is underlined by findings which show that as much as half of the increase in CO<sub>2</sub> emissions recorded in the last two centuries has been bound formerly within terrestrial ecosystems (Houghton and Goodale, 2004).

It is obvious that stocks and concentration of OC in soils are only two variables which describe its potential for sequestration of OC. In addition, the grade of stabilization of soil organic matter (SOM) against mineralization by microorganisms is a major factor controlling the outflow of carbon from soil to the atmosphere. Increasing knowledge on mechanisms determining the stabilization of SOM with respect to the properties of different soils and ecosystems but also to different demands of land use is crucial to tap the full potential of soils to sequestrate carbon and avoid unwanted release. The grade of stabilization of organic matter against mineralization in a certain reservoir can be described

by its mean residence time (MRT). The MRT is defined as the timespan organic matter stays in a certain reservoir from entering the reservoir (for soils in forms of detritus, root exsudates or excretions) to being transferred to another (e.g. as  $CO_2$  as a product of mineralization). Within the terrestrial biological carbon cycle, which is shown in Figure A-1, SOM has the longest mean residence time (MRT) and slowest turn-over rates compared to OM in living biomass or detritus. The variety of stabilizing mechanisms in soils reveal that the MRT of SOM can vary between 10 and >1,000 years (von Lützow et al., 2006). This further emphasizes the possible function of soils to sequestrate OC on a long term on the one hand but also the risk of quick release of carbon on the other.

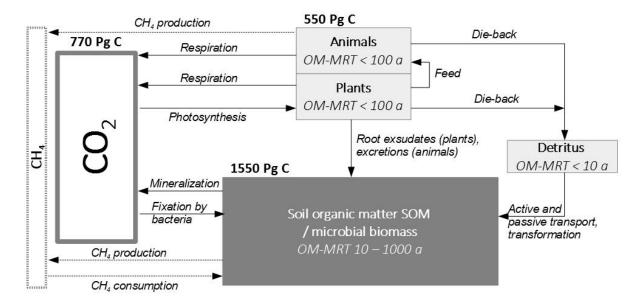


Figure A-1: Reservoirs and pathways for carbon in the terrestrial biological carbon cycle for temperate climates including mean residence times of organic matter (OM-MRT) in the different reservoirs. Areas of boxes in this figure representing soil organic matter, living biomass (plants and animals) and  $CO_2$  are proportional to the amount of C in these reservoirs (this is not applicable for  $CH_4$  and detritus because amount of C in these pools is far too small to figure). Figure is drawn according to Kandeler (2010), Lal (2004a), von Lützow et al. (2006).

#### A.1.2 Stabilization of soil organic matter – pools, mechanisms, methods

The stability of SOM against mineralization is determined by different mechanisms to which it is exposed in soil. If SOM is stabilized by one or more of these mechanisms, the probability to get mineralized is decreased to a certain extent which is defined by the persistence of the stabilizing mechanism. Soil organic matter with similar stabilizing mechanism and MRT are usually summarized in distinct pools. This enables the description of the continuum of probabilities for SOM mineralization. Furthermore, these pools can be used in computing models for modelling the development of OC stocks as well as transfers between pools (such as *RothC*, e.g. *Zimmermann et al.*, 2007).

The different stability of SOM in different pools is indicated by the wide range of determined carbon ages of SOM particles or molecules in a soil sample. As stated in Figure A-1, MRT of SOM can differ

from less than 10 years to more than 1,000 years. Usually, three pools are distinguished: the labile pool (MRT < 10 years), the intermediate pool (MRT 10 – 100 years) and the stabile pool (MRT 100 – 1000 years). It is obvious that the share of SOM in each pool contributes to the overall stability of SOM and is determined by the share of different stabilizing mechanisms. Three superordinate mechanisms are supposed to govern stabilization of SOM (e.g. *Oades, 1988; Christensen, 1996; Sollins et al., 1996; Knicker and Hatcher, 1997; von Lützow et al., 2006; Jastrow et al. 2007*):

- (1) Interactions with surfaces or metal ions, such as ligand exchange, formation of polyvalent cation bridges or complexation of organic molecules with metal ions.
- (2) Occlusion of SOM within soil aggregates or encapsulation within macromolecular matrices resulting in spatial inaccessibility of SOM for mineralizing microorganisms or enzymes.
- (3) Chemical recalcitrance as an intrinsic property of organic molecules (primary recalcitrance) or as a property of organic molecules after a certain transformation (secondary recalcitrance, such as formation of charred material after fire). Both result in selective preservation of molecules or parts of OM.

Schmidt et al. (2011) widened the concept for stabilization of SOM: They proposed that a reduced probability of SOM mineralization has to be regarded as a complex function of physicochemical and biological conditions in the surrounding environment. This concept also includes other well known mechanisms contributing to stabilization of SOM, for example the preservation due to oxygen deficiency.

With regard to the definition of soil aggregates, the role of environmental conditions for SOM stabilization further underlines the pivotal role of aggregates for SOM stabilization (*Sollins et al.*, 1996). Aggregates are defined as clusters made of mineral soil constituents usually formed around organic particles. Soil aggregates exist in sizes from micrometers to centimeters and are hold together by forces much stronger than those occurring between adjacent aggregates (*Martins et al.*, 1955). It is obvious that aggregates form the environment around organic matter and are of high importance for its stabilization, consequently. Thus, understanding formation and stability of soil aggregates is one focus of research on SOM stabilization.

For the quantification of SOM present in distinct pools different experimental methods are available. Usually several pools with different levels of protection against mineralization are obtained according to their physical or chemical properties or a combination of both (*von Lützow et al., 2007*). One of these methods is fractionation of SOM with regard to its density which allows to differentiate between three pools (*Golchin et al, 1994; Gregorich and Beare, 2008*): free light fraction of particulate organic matter in the labile pool which is not or only scarcely stabilized, occluded light fraction of particulate organic matter in the intermediate pool which is stabilized by occlusion into aggregates and organic matter associated with mineral surfaces which contributes to the stable pool.

With regard to the determination of SOM in the intermediate pool a controlled aggregate breakdown and release of formerly occluded SOM is for example part of many experimental methods. Application of ultrasound is supposed to be the preferable method for controlled aggregate breakdown because it is assumed that the required energy can be estimated by a calorimetrical calibration (*North, 1976*). Disadvantages of this method have also already been discussed in literature. According to different authors (*Koenigs, 1978; Christensen, 1992; Schmidt et al., 1999*), the most striking drawback is that reproducibility of disaggregation results gained involving ultrasound dispersion is questionable. Substantial differences in the properties of the ultrasonic devices used (such as oscillation frequency and amplitude) as well as a lack of standardized procedures are stated as the most important reasons for that (*Christensen, 1992; Amelung and Zech, 1999; Mayer et al., 2002; Mentler et al., 2004*). However, the application of ultrasound can be regarded as the accepted standard procedure for controlled disaggregation in experiments on SOM stabilization by occlusion into aggregates.

#### A.1.3 Riparian floodplains – formation and relevance for carbon cycle

Riparian floodplains are areas at river margins which have recently been or were in the past subject to flooding dynamics. These unique ecosystems are formed under the influence of regular inundation during flooding events and sedimentation as well as erosion of soils. The importance of riparian floodplain soils for global and regional carbon cycles is due to these processes: they are (mostly) mineral soils with very high OC stocks in both, top and sub soil (Bai et al., 2005). High stocks which are found are contributed to two different pathways for OC into the soil: Firstly, allochthonous OC originated from top soils eroded in the river catchment is brought to the riparian floodplains along with sediments (Pinay et al., 1992; Cabezas and Comín, 2010). Secondly, soils in floodplains are rich in nutrients which are also brought along with sediments and reveal the high net-primary production of riparian floodplain ecosystems. This causes a high input of autochthonous OC to floodplain soils along with litter from on-site vegetation (Tockner and Standford, 2002). Riparian floodplains cover only 4 – 6 % of global land surface (Mitra et al., 2005) but annual OC-accumulation rates are assumed to be 10 – 20 times higher than rates of other terrestrial ecosystems (Van der Valk, 2006). With regard to the latter, the superordinate position of riparian floodplain soils in carbon cycles is obvious. However, the influences of flooding dynamics on stabilization mechanisms for SOM are not known yet and even publications on quantification of OC stocks in these soils are scarce.

Despite their known function in carbon cycles recent riparian floodplains are only barely found in densely populated areas like central Europe nowadays. *Tockner and Stanford (2002)* showed that 90 % of the riparian floodplains in Europe and North America are "cultivated" today and not subjected to flooding dynamics anymore. In addition to the need for fertile agricultural land, reasons

for the decline of riparian floodplain areas in the last 150 years were river channelization and construction of dikes for flood protection (*Blackbourn, 2006; Lair et al., 2009*).

In addition to their function in carbon cycle, riparian floodplain ecosystems are characterized by a high biodiversity and are home to several rare species adapted to special conditions related to flooding dynamics and therefore deserve protection (Naiman and Décamps, 1997; Tockner and Standford, 2002). Beside the influence of these special conditions on biota, also landscape and soil formation are determined by flooding dynamics. Usually, the formation of landscapes and soils can be regarded as temporally decoupled processes (Stahr et al., 2008). This is different for riparian floodplains, where landscapes are (re-)formed permanently due to sedimentation and erosion caused by regular flooding. Continuous landscape formation has implications for soil formation and has to be regarded as a soil forming factor unique in these landscapes. Piégay and Schumm (2003) presented a model for the formation of riparian landscapes by dividing the river hydrosystem into three main spatial gradients: in addition to the lateral gradient (distance to main river channel), hydroecomorphological site conditions and (as a result) soils and vegetation differ also along a longitudinal gradient (upstream/downstream) and a vertical gradient (altitude above mean water level). With regard to differences in flooding dynamics along the lateral gradient (distance to main river channel), their influence on landscape and soil formation can be described as follows (Figure A-2).

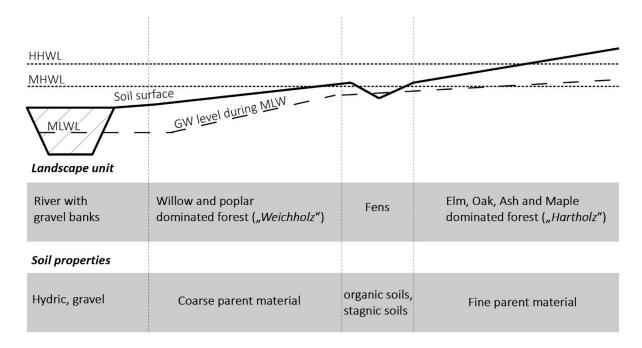


Figure A-2: Conceptual model for formation of riparian landscapes along the lateral gradient (distance to main river channel) as results of differences in hydroecomorphological site conditions, modified according to Stahr et al. (2008). (MLWL is mean low water line, MHWL is mean high water line, HHWL is high high water line, GW is groundwater).

Areas within a distance from 50 to 100 meters from river are regularly flooded and consist of gravel banks. These sites are only scarcely vegetated due to regular sedimentation and erosion. In addition, inundation during flooding disturbs development of plants. During flooding, water flow velocity is too fast for particles < 2 mm to be deposited and therefore soils consisting of fine earth are not present. The adjacent landscape unit more distant to the main river channel is characterized by frequent flooding and a water flow velocity which allows deposition of coarse sediments < 2 mm as parent material for formation of sandy soils. Forest vegetation on these sites is dominated by tree species such as willow or poplar. These species show a high tolerance for inundation and flexible branches that prevent damages caused by floating debris. The low specific wood density of these tree species is the reason why these areas are named "Weichholzaue" (weich = soft, holz = wood) in German. Unfortunately, the translation to English often used ("softwood forest") is confusing because the density threshold for softwood is lower in the English classification system, thus "softwood" usually summarizes the group of gymnosperm tree species such as coniferous trees, which are never found in riparian floodplains. However, the term "softwood" is used in two publications presented within this thesis and I prefer to continue using this term to avoid confusion with regard to chapters B and E. Within the publication presented in chapter D, I described the same landscape units according to dominating tree species (WiP sites = willow and poplar) which might be preferable.

With increasing distance to the main river channel, the water flow velocity during occasional flooding events (sometimes only once a year) is slow enough to allow fine sediments to settle. As a result, loamy soils are found in these areas which are characterized by forests that consist of elm, oak, ash and maple species. The German term "Hartholzaue" (hart = hard) used for this landscape units further refers to the higher wood density of these tree species compared to the tree species in softwood forests. Consequently, the term "hardwood forest" is used for this landscape unit within this thesis which is in agree with other authors (e.g. Mitsch and Gosselink, 2015). Depending on ground water level, also fens or soils with stagnic properties can be found preferentially in sinks or old beds of side branches of the river between the two former described landscape units. Vegetation found here needs to withstand conditions as present in permanently water-logged soils.

#### A.1.4 "Nationalpark Donau-Auen": An example for riparian floodplains in central Europe

An increased understanding of the function of floodplains as retention areas during flooding events and the need for protection of their unique ecosystems has changed policy in recent years.

Remaining recent riparian floodplains have been protected and more and more retention areas have been reactivated by relocation of dikes and sanctuaries in the vicinity of rivers. An example for such areas is the *Nationalpark Donau-Auen* in river margins of the Danube near Vienna, Austria. With its size of roughly 9,300 ha the national park is among the biggest riparian wetland areas subjected to near-natural flooding dynamics in Europe (map of national park and investigation area is given in Figure A-3).

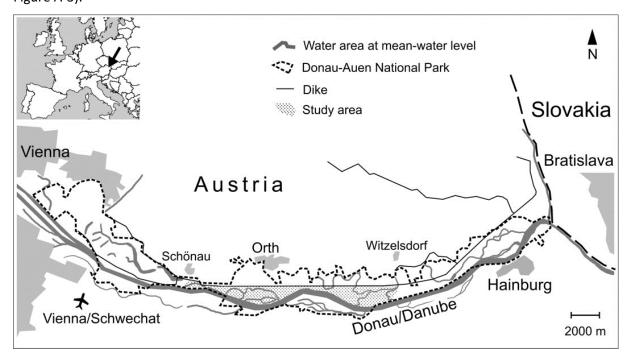


Figure A-3: Overview of "Nationalpark Donau-Auen" and position of the study area used for most investigations presented in this thesis (figure taken from publication in chapter B)

Due to the short time since the establishment of the national park in 1996 and the fact that the water discharge of the Danube is strongly influenced by human activities, flooding dynamics present cannot be called "natural". Since the 1830s the Danube has been channelized systematically and the river system in the area of the national park shifts from a meandering state with a lot of anabranches to an area with decreased dynamics in fluvial forming processes (*Lair et al., 2009*). This has a strong influence on aggradation and erosion of material (*Lair et al., 2009*) but also on water levels in the river as well as groundwater table position (*Hohensinner et al., 2008*). The latter is the reason why fens or ground-water influenced soil types cannot be found in the area nowadays (this would be expected according to the presented model for formation of riparian landscapes). However, compared to other riparian floodplains adjacent to large rivers in central Europe, the area is still subject to flooding dynamics which can be regarded in certain areas as near-natural and therefore

deserves protection. This near-natural state is further underlined by the presence of large areas of riparian forests which cannot be found elsewhere in central Europe in this size. This has two reasons: riparian forests are protected because forestry activities have been restricted in the area since the founding of the national park (*Bundesgesetzblatt, 1997*) and the presence of flooding dynamics as the prerequisite for this forest type to grow and sustain.

The riparian floodplains within the national park area are therefore a suitable place to study the influence of these dynamics on soil OC stocks and stabilization. This was shown in a number of independent studies on various soil and ecology related aspects carried out in the area (*Graf et al. 2007; Lair et al., 2009a; Lair et al. 2009b; Zehetner et al. 2009; Suchenwirth et al., 2012; Rieger et al., 2013; Rieger et al., 2014*). Consequently, an area of 1300 ha situated within the national park on the northern river bank between the villages of Schönau and Witzelsdorf south of the Marchfeld dike was chosen for most of the investigations presented in this thesis.

#### A.2 Objectives and structure

According to the presented background, soils in general and riparian floodplain soils in particular are of high interest with regard to their function in carbon cycle. Hence, overall goal of my thesis is to link determined soil OC stocks and stabilization mechanisms for SOM with described landscape units but also with hydroecomorphological site conditions responsible for their formation. To achieve this, a quantification of soil OC stocks and their differences when comparing landscape units in riparian floodplains is mandatory as the first step. This will provide information on the spatial distribution of OC in soils within the study area. Subsequent determination of stabilization mechanisms for SOM in soils situated in different landscape units will provide insight into the influence of flooding dynamics on these mechanisms. Prior to analyzing stabilizing mechanisms, known methods for their determination involving ultrasound technique need to be evaluated with regard to the reproducibility of the results gained.

With a known spatial distribution of soil OC stocks and stabilization mechanisms for SOM a regionalization of these parameters within the study area will be possible. Indicators sufficiently predicting these parameters can be determined. The known model for formation of riparian landscapes can be evaluated if it sufficiently describes soil and landscape formation in the study area and is suitable to predict spatial distribution of soil OC stocks and stabilization mechanisms for SOM.

For the regionalisation of OC stocks stabilization mechanisms for OM in riparian floodplains soils, the following questions need to be answered:

- How large are soil OC stocks in the study area compared to other terrestrial ecosystems and how are they spatially distributed?
- Methods for determination of stabilizing mechanisms for SOM usually include the application of ultrasound for controlled aggregate breakdown. Are results gained by this method reproducible even if different devices or procedures are applied?
- Which stabilizing mechanisms of SOM are relevant in riparian floodplain soils and how are they spatially distributed?
- Which environmental drivers control stabilization mechanisms and finally OC stocks in riparian floodplain soils?
- Which parameters indicate spatial distribution of OC stocks and stabilizing mechanisms?

Four publications are presented in this thesis in chapters B to E which address these questions. Figure A-4 provides an overview on topics presented by questions treated in this thesis and the chapter which provides most of the information to answer the respective questions. Hypotheses to be tested are presented in the respective publications.

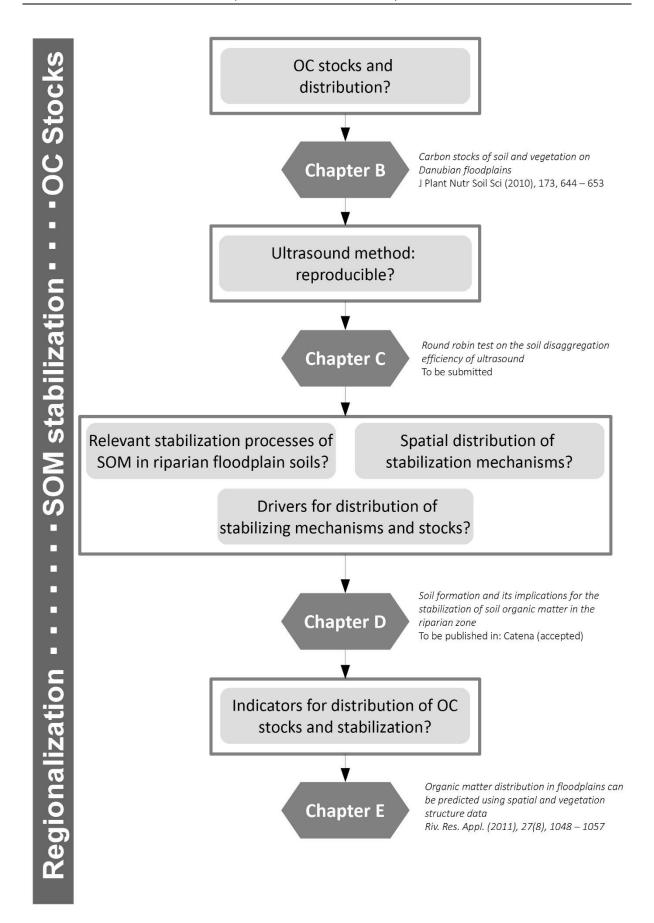


Figure A-4: Overview of objectives and questions forming structure of this thesis.

First, results of a survey on OC stocks in soils and vegetation in the investigation area are presented Chapter B. The results underline the assumption that OC stocks in soils are huge compared to other terrestrial ecosystems. However, to assess the function of floodplain soils in the carbon cycle, stabilization mechanisms of SOM need to be determined (as stated in section A.1.2). For investigation of SOM stabilized by occlusion in soil aggregates, ultrasound is often applied for controlled aggregate breakdown. With regard to the function of ultrasonic devices, a satisfying reproducibility of the established methods is questionable. Hence, a round robin test on soil disaggregation efficiency of ultrasound has been carried out prior to the experiments on stabilization mechanisms (Chapter C). Results of the round robin test show a certain variety of disaggregation efficiency (especially for strongly aggregated soils) but underline that the reproducibility is adequate even if different ultrasonic devices or procedures are applied. Therefore, a study on stabilization mechanisms relevant in soils in the study area has been carried out involving a method to determine differently stabilized fractions of SOM which includes ultrasonic disaggregation (Chapter D). Results show that stabilization mechanisms of SOM differ among landscape units in investigated floodplain. It is shown that stabilization mechanisms for SOM are determined by flooding dynamics, similar to other soil properties and vegetation structure. Stabilization of SOM by sorption on mineral surfaces is of high importance in fine textured soils distant to the river. Close to river, coarsely textured soil is found with surprisingly high portions of OM stabilized by occlusion into soil aggregates. In these soils, huge sedimentation which covers former top soil layers also contributes to stabilization of SOM. Finally, different spatial and vegetation parameters were evaluated with regard to their predictive power for soil OC stocks and stabilization mechanisms of SOM (Chapter E).

In the following sub sections A.3.1 to A.3.4 the summarized results of the chapters are evaluated with regard to the questions listed above. Sub section A.3.5 will suggest topics for future research developed out of the presented results.

#### A.3 Extended summary and synthesis

#### A.3.1 Soil OC stocks in the study area and their spatial distribution

OC stocks of soils in the investigation area were determined within two studies presented in this thesis. All presented results confirm the assumption that **riparian floodplain soils are rich in organic carbon and show high OC stocks in consequence**. In the first study OC stocks were determined in soils sampled on 76 sites (see chapter B). Means of soil OC stocks down to 1 m depth were found between 154 t ha<sup>-1</sup> to 212 t ha<sup>-1</sup> depending on the landscape unit sampled (overall mean: 177 t ha<sup>-1</sup>). One extreme value for mineral soil was found with 354 t ha<sup>-1</sup> of soil OC stocks. These results are in line with or even exceed presented soil OC stocks in riparian floodplains in general (*Batjes, 1996; Cabezas et al., 2009*), but also for the soils analyzed in the same area (*Haubenberger and Weidinger, 1990; Rieger et al. 2014*). *Hofmann and Anders (1996)* numbered soil OC stocks under non-riparian lowland forests between 50 and 150 t ha<sup>-1</sup> that emphasizes the extraordinary position of soils in riparian floodplain forests compared to soils of other forest ecosystems. As assumed in the introduction, the flooding dynamics as the main driver for soil formation in riparian floodplains which are not present in other landscapes obviously contribute to the large soil OC stocks.

According to the model for formation of riparian landscapes presented, forest vegetation indicates differences in hydroecomorphological site conditions as a result of differences in flooding dynamics which are supposed to change along with distance from the main river channel. Therefore, different soil properties but also different OC stocks were expected in soils formed at sites representing different landscape units. To assess the horizontal distribution of soil OC stocks, soils were sampled at sites differing with regard to the dominant vegetation classes. These vegetation classes were identified via aerial photographs prior to sampling and at the site during the campaign and form differentiable landscape units:

- "softwood forest" dominated by Salix alba;
- "cottonwood forest" dominated by Populus alba, Populus x canadensis, Populus nigra;
- "hardwood forest" dominated by Quercus robur, Fraxinus excelsior, Acer campestre, Ulmus spec.;
- "reforestations" dominated by tree species similar to "hardwood forest" but obviously reforested and differentiated by stand structure as well as lower tree height and stem circumference at breast height;
- "meadows and reeds" without forest vegetation.

The high number of landscape units indicated by five vegetation classes reveals that human disturbances prior to formation of the national park still strongly influence the present vegetation

structure. This is shown by the large number of areas identified as reforestations or by the occurrence of forested cottonwood stands which would be present in mixed stands together with willows in floodplain forests developed without any human disturbances. Further differences between the actual spatial distribution of these landscape units and the conceptual model are indicated by the determined distances of the landscape units from the main river channel. In contrast to the conceptual model, these do not differ significantly from each other with exception of landscape unit with softwood forest vegetation. The latter was found in the direct proximity of the main river channel with a significantly lower distance to it than the other landscape units. This scattered spatial pattern of landscape units as well as soil OC stocks is shown in Figure A-5 and gives first indications that spatial gradients alone may not sufficiently describe distribution of landscape units and OC stocks.

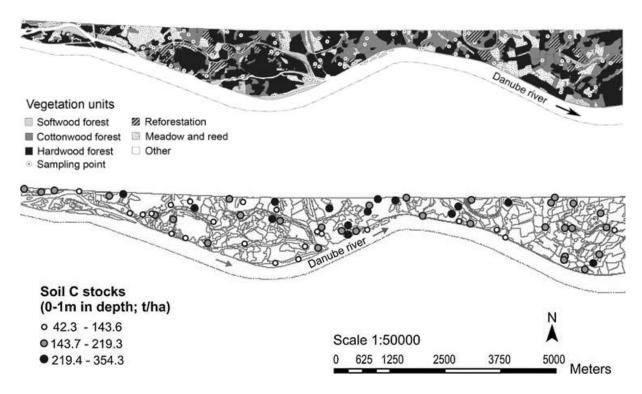


Figure A-5: Spatial distribution of landscape units identified according to dominant vegetation and soil OC stocks. Figure is re-assembled from figures presented in Chapters B and E.

However, soil properties and OC stocks determined were found to differ in the soils of the five landscape units. As expected, coarsely textured soils were found under softwood forest while a loamy texture was more often determined under hardwood, cottonwood and reforestations which is in agreement with the conceptual model. Similar trends for differences in soil OC stocks were observed. Lower mean soil OC stocks were determined under softwood vegetation (mean of soil OC stocks: 154 t ha<sup>-1</sup>) while they were higher under cottonwood and hardwood forests or reforestations (means of soil OC stocks between 176 and 186 t ha<sup>-1</sup>). Highest stocks were found under meadows and reeds (mean soil OC stock: 212 t ha<sup>-1</sup>), a landscape unit which is not present in natural riparian

landscape and also a result of human disturbances. The lower soil OC stocks under softwood vegetation compared to higher soil OC stocks under hardwood vegetation was confirmed by investigations on additional four sites situated in these landscape units presented in Chapter D.

Obviously, differences in flooding dynamics also determine the horizontal distribution of soil OC stocks along with other soil properties in recent riparian floodplains.

Differing mean OC stocks in soils found under the five landscape units showed a large variability within soils of the respective unit. This contributes to the result that the detected differences were not found to be statistically significant. The missing significance in correlation between vegetation and soil OC stocks can be explained with the idea that vegetation species distribution does not indicate the hydroecomorphological site conditions during formation of soils and time needed to build up soil OC stocks in every case. This is attributed to human influence on vegetation structure in the past (as stated above) and to the slow response of vegetation species distribution to changing flooding dynamics. The latter likely occurred due to shifting river beds in the past which strongly influence flooding dynamics and soil forming conditions as a result but are not indicated by the present vegetation (*Fiebig et al., 2009; Lair et al., 2009b*).

The vertical distribution of OC stocks presented in Chapters B and D further emphasizes the exceptional position of riparian floodplain soils within the group of mineral soils and provides insights into the influence of flooding dynamics on soil formation. Opposed to other mineral soils, highest portion of OC stocks were not found in top soils: OC stocks in sub soil exceeded the stocks determined in Ah horizon by factor 2-3. A more detailed sampling approach on selected sites presented in Chapter D allowed to differentiate characteristics of the vertical distribution of OC within soils under softwood vegetation compared to soil under hardwood vegetation. These landscape units were chosen because they represent the extreme of the gradient of flooding dynamics in riparian floodplains according the conceptual model presented. Soils under softwood vegetation show a discontinuous decreasing trend for OC concentration and OC stocks in a high number of distinguishable soil horizons with increasing soil depth. A continuous decrease of these parameters was found for soils formed under hardwood vegetation with a low number of soil horizons compared to soils formed under softwood vegetation. These differences between soils under softwood and hardwood vegetation are shown in Figure A-6.

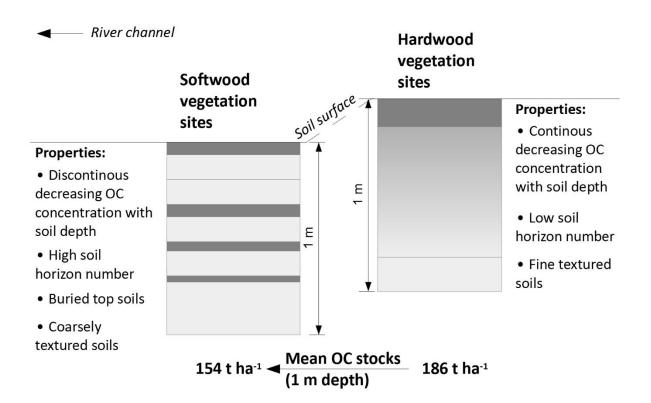


Figure A-6: Differences of soils under softwood and hardwood vegetation. Higher OC concentration in soil is represented by darker grey shading.

It can be assumed that OC of allochthonous origin contributes significantly to OC in sub soil in both cases because this is a major pathway for OC into the soil in riparian floodplains. With regard to softwood vegetation sites close to the river I found that sediments and soils were much coarser and OC contents were lower than at hardwood vegetation sites what contributes to the higher OC stocks found in the latter. This was expected due to the differences in water flow velocity during flooding events. In soils under softwood vegetation, former Ah horizons (mainly containing autochthonous OC) now covered by coarse sediment material are still visible in sub soil. These horizons are enriched in OC compared to the material above and below. The presence of buried Ah horizons further indicates that longer periods without sedimentation allowed the formation of an Ah horizon. This implies that flooding conditions reflected by sedimentation intensity change dynamically at these sites. In contrast, no such former Ah horizons were found in sub soil under hardwood forest vegetation. Here, a continuous aggradation of low amounts of fine sediments does not cover Ah horizons with large amounts of sediments. Consequently, former Ah horizons are not visible in sub soil and flooding conditions are constant and obviously do not change with the same amplitudes present at softwood sites.

Summarizing, horizontal and vertical distribution of soil OC stocks in riparian floodplains are determined by site specific flooding conditions and differ by trend between landscape units. Large differences in soil properties (e.g. soil texture) as well as the different processes contributing to OC

stocks in subsoil (sediments and buried Ah horizons) suggest that stabilization mechanisms for SOM in soil formed under different flooding dynamics may show pronounced differences.

#### A.3.2 Soil disaggregation efficiency of ultrasound

The reproducibility of disaggregation results gained by application of ultrasound to soil samples in suspensions was evaluated in a round robin test with nine different participating laboratories. My results show that soil disaggregation efficiency is comparable despite the use of different ultrasonic devices used and procedures applied by the participating laboratories (see chapter C). The extent of disaggregation was tested by determining masses of particle size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  as well as by comparing size distribution of particles <  $63~\mu m$  after disaggregation. A low number of outliers from the population was identified using two different statistical tests within determined masses of particle size fractions  $63-200~\mu m$  and  $200-2000~\mu m$ : Less than 10~% of the determined masses were identified as outliers. The number of outliers for parameters describing particle size distribution <  $63~\mu m$  was even lower with less than 3~% of these parameters were identified as outliers. I can deduce that disaggregation results gained by using ultrasound for aggregate breakdown in suspension are reproducible despite of the use of different devices or protocols.

The statistical methods applied are frequently used for evaluating round-robin test results (Wilcox, 2010). Both statistical methods compare single values with the range of values defined by the results gained from all participating laboratories. The first test is based on standard deviation, the second on inter-quartile range. It is obvious that both tests do not provide information on the variation of the results. It is only tested whether a value belongs to a population with regard to certain criteria or not. In general, the variation of results was found to be larger after disaggregation of soils with stable aggregates compared to soils with aggregates characterized by a lower stability. Similar to results for outliers, variation was found to be higher for masses of particle size fractions > 63 µm compared to parameters describing particle size distribution < 63 μm. In preliminary intra-laboratory tests for which always the same ultrasonic device and procedure was used the mean coefficient of variation was 12 %. It was found to be larger (mean coefficient of variation = 38 %) for results gained in different laboratories in the round robin test (even if determined mean values were similar to those determined in the intra-laboratory test). This difference was most obvious for disaggregation results of the soil with stable aggregates. The most likely reason for the difference in variations is an increased bias caused by unavoidable errors due to different operators in the round robin test. In addition, an influence of differences in ultrasonic devices and the procedures on disaggregation efficiency cannot be excluded but seem to be low. Device parameters which may influence disaggregation efficiency are oscillation frequency and amplitude as well as dimensions of the metal

tip used for application of ultrasound into the soil-water-suspension ("sonotrode"). Devices used in laboratories for which a larger number of outliers was detected differ in these parameters from those with low number or no outliers in the results.

The satisfying reproducibility and low variation of disaggregation results gained in intra-laboratory tests show that application of ultrasound is still the method of choice for controlled aggregate breakdown in soil-water suspension. Even for soils with low aggregate stability, differences in particle size distribution after application of low (30 J ml<sup>-1</sup>) and high (400 J ml<sup>-1</sup>) amounts of energy were detectable. Therefore, the basic concept that applied ultrasonic energy can be used as a proxy for the extent of disaggregation can be confirmed. With regard to this thesis I can conclude that the application of ultrasound during experiments for determining SOM pools with different stabilizing mechanisms is a suitable method to determine differences.

With regard to the reported larger variation of results in the round robin test a need to standardize devices and procedures is compelling. As a first step, crucial parameters which may influence disaggregation efficiency (ultrasonic power, ultrasonic device properties) should be listed along with the presented results. This is in agreement with first studies on this topic recently published by others: *Poeplau and Don (2014)* pointed out that ultrasonic power significantly decreased disaggregation efficiency if chosen to low. They suggested to use ultrasonic powers > 45 W for disaggregation because lower ultrasonic power results in worse disaggregation even if the same amount of energy is applied. Also a possible influence of the oscillation amplitude on disaggregation results, as indicated by the presented data, was first discussed by *Mayer et al. (2002)*.

#### A.3.3 Stabilization of organic matter in soils of the study area

With the methods applied for determination of stabilizing mechanisms occurring in the investigated riparian floodplain soils I was able to differentiate between three major pools of SOM: Firstly, particulate OM, which is not or only loosely attached to other soil constituents, forms the free-light fraction (f-LF). Most of SOM in this pool is scarcely stabilized against mineralization and characterized by a fast turnover rate and short mean residence time (< 10 years) consequently (if recalcitrant compounds contribute largely to the molecule structure of OM in this fraction it may show slower turnover). Secondly, particulate OM stabilized by occlusion into soil aggregates was determined (occluded light fraction, o-LF). A wide range of turnover rates characterizes OM in this pool which has an intermediate position. The variability of turnover rates in this pool is attributed to the different characteristics and stabilities of soil aggregates which determine the grade of protection against mineralization (10 to >100 years). Thirdly, SOM stabilized by sorption on mineral surfaces was determined (heavy fraction, HF). This pool is supposed to comprise SOM with the longest mean residence time in soil and contributes to the stable pool (>100 years).

An overview of distribution of OC among the different pools in two soils sampled under softwood vegetation (WiP-1 and WiP-2) as well as in two soils sampled under hardwood vegetation (AMEO 1 and AMEO 2) is given in Figure A-7 (which is taken from the publication used for Chapter D). As already shown for other mineral soils under forest vegetation (Grünewald et al., 2006; Don et al., 2009; Grüneberg et al., 2013), most of the SOM is stabilized by interactions with mineral surfaces in the investigated soils from a riparian floodplain. However, the portion of SOM stabilized in this stable pool is lower in soils formed under softwood vegetation (50 to 76 % of total OC) compared to those formed under hardwood vegetation (84 to 97 % of total OC). The larger portion of SOM in this pool with slow turnover rates is one possible reason why OC stocks in soils under hardwood vegetation were found to be higher than those found under softwood vegetation. It is likely that the finer texture of these soils is responsible for the higher relevance of these stabilization processes under hardwood vegetation because the amount of OM stabilized on surfaces was shown to increase with increasing available surface areas. With regard to SOM stabilized by occlusion into aggregates, this process is obviously of higher importance for stabilization of SOM in soils under softwood vegetation (23 % of total OC was found in occluded SOM) than in soils under hardwood vegetation (6 % of total OC in occluded SOM). With regard to the coarser soil material under softwood vegetation this is surprising because coarsely textured soils are generally known to be less aggregated than fine soils (Kaiser et al., 2012). The important role of aggregation in these soils may be a result of an increased activity of earthworms (as shown for coarsely textured floodplain soils in the direct proximity to rivers by Bullinger-Weber et al., 2007) and can also be caused by the presence of arbuscular mycorrhizal fungi under willow and poplar stands (Mardiah et al., 2014). As occluded OM contributes to the intermediate turnover pool of SOM, these findings may further explain that OC stocks under softwood vegetation differ less than expected when compared to soil OC stocks under hardwood vegetation. The portions of barely stabilized SOM present as particulate OM in the free light fraction are in general higher in soils under softwood vegetation as in soils under hardwood vegetation, but differences in horizontal distribution are less distinct than for the other two stabilization mechanisms determined.

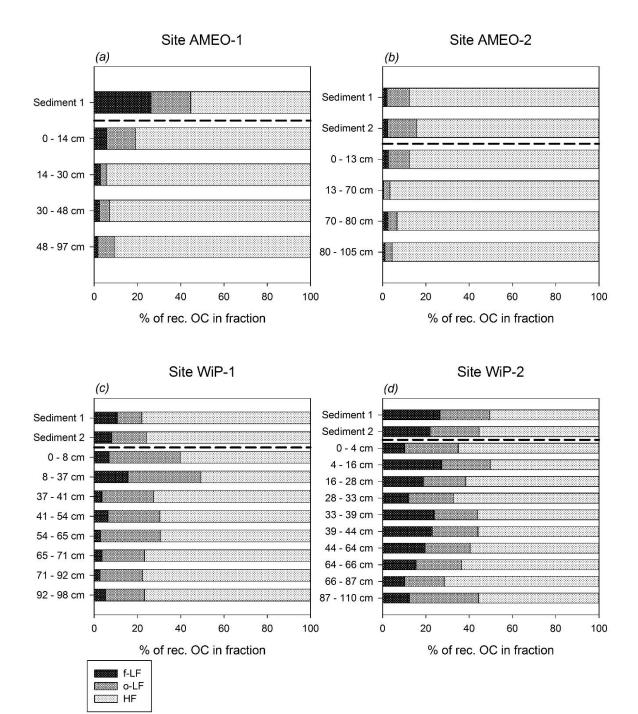


Figure A-7: Relative distribution of OC among free light (f-LF), occluded light (o-LF) and heavy fractions representing pools of OC with increasing turnover rates (f-LF > o-LF > HF) in freshly deposited sediments and different soil horizons of two sites (AMEO 1 and AMEO 2) sampled under hardwood forest vegetation and two sites (WiP 1 and WiP 2) sampled under softwood forest vegetation. The Figure is taken from Chapter D.

When comparing the vertical distribution of barely stabilized particulate OM differences between the two landscape units are revealed. Under softwood sites, significant portions of total OC were found throughout the soil profile to be present in the labile pool. In contrast, only top soils under hardwood sites show large amounts of OC in this fraction. The latter case can generally be expected for the vertical distribution of scarcely stabilized particulate OM in soils not situated in riparian floodplains. As this fraction represents the labile pool with a short turnover time, its OM is usually mineralized shortly after deposition and is only scarcely found in sub soils (which are usually much older than the mean residence time of the labile pool in soil). With regard to special conditions for soil formation in riparian floodplains it is likely that former top soil horizons rich in OC in the labile pool are preserved by being covered with sediments during flooding: This has already been stated earlier with regard to the covered former top soil horizons contributing to soil OC stocks. Obviously this additional process contributes to the preservation of large portions of OM in the labile pool as well as OC stocks in sub soil especially under sites with softwood vegetation. The preservation of OM by soil material covering is also discussed to be relevant for OC budgets in colluvium. Here, it is assumed that OM covered by more than 20 cm of eroded material is preserved to a large extent (Lal, 2005). However, it is obvious that the labile pool of OM in sub soils under softwood vegetation contributes to the total OC stocks and is stabilized due to special conditions of the surrounding environment. This is in agreement with the already presented model of Schmidt et al. (2011) and cannot be described by the traditional mechanisms for SOM stabilization mentioned in A.1.2. The question if classical approaches to determine pools with different mean residence times are appropriate to assess the potential of soils such as those in riparian floodplain for long term sequestration of OC. Anyway, most important for the occurrence and distribution of stabilization or preservation mechanisms of SOM in riparian floodplain soil is the characteristic of flooding dynamics as the hydroecomorphological driver for formation of soils with distinct properties. All parameters discussed to be responsible for the determined horizontal and spatial distribution of stabilizing mechanisms are related to these special conditions for soil formation in riparian floodplains.

A.3.4 Regionalization of soil OC stocks and stabilizing mechanisms for SOM: indicators for prediction.

With regard to the presented results it is possible to relate soil OC stocks and mechanisms contributing to stabilization of SOM on the respective sites to different landscape units as qualified according to dominant forest vegetation. However, the patchiness of the distribution of landscape units within the study area (as shown in Figure A-5) has already indicated that spatial information alone is not sufficient to predict and regionalize OC stocks and SOM stabilization mechanisms. This is

what was assumed before with regard to the conceptual model for landscape formation presented in section A.1.3.

As shown, the flooding dynamics (which were found to be constant under hardwood vegetation and much more dynamic under softwood vegetation) are the main driver for soil formation and distribution of OC stocks and SOM stabilizing mechanisms. In chapter E, vegetation parameters in addition to spatial information were analyzed for better prediction of flooding dynamics on different sites. In addition to the species composition, parameters like stem number per area, mean stem diameter, density of canopy cover, shrub cover or herb cover were evaluated and found to significantly improve the prediction model for flooding dynamics and soil properties such as soil OC stocks as a consequence. Figure A-8 compares the sites representing the extremes of the gradient in flooding dynamics with vegetation parameters found at the sites.

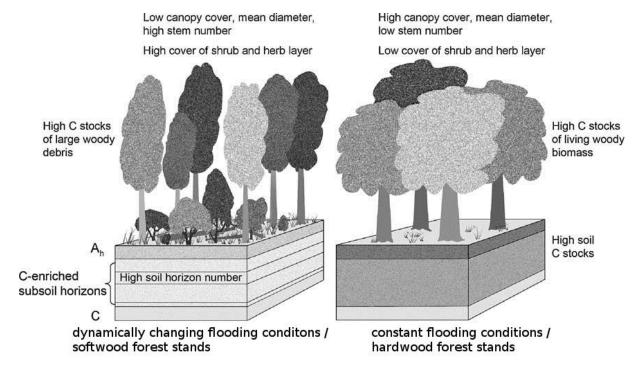


Figure A-8: Sites representing the extremes of the flooding dynamics gradient (highly dynamic sites vs. sites with constant flooding conditions) including vegetation parameters used for prediction (slightly modified figure is taken from chapter E).

As shown in Figure A-8, softwood forest stands in areas with dynamically changing flooding conditions are characterized by a high number of stems with a low diameter and a low canopy cover. The cover of the shrub and herb layers are high on these sites. In addition, large amounts of woody debris is found here. These parameters differ at sites characterized by constant flooding conditions. Here, hardwood forest stands found with low stem numbers, a high stem diameter and a dense canopy cover. In contrast, shrub and herb layers are only barely found and the amount of woody debris is much lower than at sites with dynamically changing flooding conditions. According to these findings, the predictive power of multiple regression models for soil OC stocks was improved by

adding the stem number per area unit and the cover of canopies and herb layer. Similar, the OC concentration in sub soils can be better predicted by using cover of herb, shrub and trees together with spatial information (distance to main river channel) than by using the latter only.

These models finally allow to regionalize data of OC stocks and stabilization mechanisms in riparian floodplains with parameters which can be determined easily by using aerial photographs together with GIS tools and vegetation surveys carried out on the sites. It is likely that the same parameters can be applied to regionalize OC stocks and SOM stabilizing mechanisms in riparian floodplains situated in similar climates with comparable vegetation structure. With regard to riparian floodplains situated in climates that differ strongly from my study area with regard to vegetation structure, these parameters need to be assessed. Anyway, an approach combining spatial and vegetation parameters should always be preferred because this seems necessary due the complexity of flooding dynamics which was shown for my study area will characterize distribution of OC stocks and SOM stabilization mechanisms regardless of the climate.

#### A.3.5 Outlook and future research

The results presented increase the understanding for the function of riparian floodplain soils with respect to the carbon cycle but further provide new starting points for future research on the presented topics. As the presence of large OC stocks in these soils is widely accepted and confirmed by the presented results, knowledge on stabilization mechanisms of SOM in these soils should be further increased. For the moment, the presented studies are the only ones in which these mechanisms were determined in this special kind of soils. However, the spatial pattern of flooding dynamics was found to be very heterogeneous, the conceptual model for the formation of landscape units was found to be only partly sufficient for the study area. Obviously, the complexity of interacting drivers for landscape formation in floodplains may hamper the transferability of results gained in a single area to other riparian floodplains. One example is that it remains unclear if the processes observed are similar when comparing natural and re-activated floodplains. Another aspect related to the transferability of the results is that the soils in the study area were all rich in inorganic carbon due to the calcareous parent material in the river catchment. This soil property is known to strongly affect aggregation (Tisdall and Oades, 1982). Thus, it needs to be shown if stabilization of SOM by occlusion into aggregates is as important in acidic riparian floodplain soils free of calcareous material as it is in calcareous soils under softwood vegetation in the study area. With regard to aggregation as an important process for stabilization of SOM, another factor should be taken into account: the grade of protection against mineralization by occlusion is determined by the stability of the aggregates (e.g. Six et al., 2000). The presented results which were gained with usual methods for determination of stabilizing mechanisms, do not provide information on this property of

aggregates. Therefore, it cannot be excluded that the grade of protection against mineralization is low due to low aggregate stability even when large amounts of OM are occluded. Transferring this idea on my results, it is possible that the low amount of OM occluded in aggregates in soil under hardwood forest vegetation is better protected against mineralization than the high amount under softwood vegetation. Therefore, a parameter like the turnover of aggregates in soils should be evaluated in addition to the amount of occluded SOM. As first step to achieve this goal, new experimental methods including the application of ultrasound for controlled aggregate breakdown may provide useful information on aggregate stability. For instance, experiments with sequential aggregate breakdown (and release of formerly occluded OM) with step-wise increased amount of energies applied by sonication will allow to assign released OM to aggregates with a certain stability. However, this approach will only provide information on mechanical stability of aggregates. If this mechanical stability is proportional to the grade of protection against mineralization needs to be investigated by additional experiments which directly determine mineralization rates of OM occluded in aggregates with different mechanical stabilities. In addition to an assessment of aggregate stabilities, also the structure or "architecture" of aggregates may have effects for their function to protect carbon against mineralization. It is known that most of the aggregates consist of smaller aggregates and are therefore hierarchically structured. First results indicate though that aggregates in some floodplain soils do not show this hierarchical structure what might have an effect on OC stabilization by occlusion in the respective soils.

With regard to the use of ultrasound, the reproducibility of disaggregation results gained by application of ultrasound is satisfying. In the moment no standards for device properties or procedures are available. An influence of parameters such as oscillation frequency or amplitude should be evaluated in further experiments to assure the comparability of results gained with different ultrasound systems also in the future.

Another aspect for future research on OM turnover in riparian floodplain soils is the implantation of techniques to determine to which extent autochthonous or allochthonous sources contribute to OC stocks at different sites. This will provide further insights into the processes behind the building up of soil OC stocks and will supplement findings presented in this thesis.

Finally, OC stocks found in riparian floodplain soils may also give economic incentives for the protection of these areas. Especially in poorer countries environmental or ecological arguments for landscape protection are often not of primary concern for policy makers and people. In most cases this is due to a high economic pressure to cultivate riparian floodplains. If OC stocks in soils and vegetation can be traded similar to CO<sub>2</sub> certificates nature protection goals and economic benefits would not exclude each other anymore. This would finally protect recent floodplains worldwide and provide new arguments for a reactivation of these important landscapes.

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### B Carbon stocks of soil and vegetation on Danubian floodplains

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#### Published in:

Journal of Plant Nutrition and Soil Science (2010), Vol. 173, 644 – 653.

DOI: 10.1002/jpln.200900209

http://onlinelibrary.wiley.com/doi/10.1002/jpln.200900209/abstract

**DOI:** 10.1002/jpln.200900209

J. Plant Nutr. Soil Sci. 2010, 173, 644-653

### Carbon stocks of soil and vegetation on Danubian floodplains

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#### **Abstract**

Riparian forests are assumed to play a crucial role in the global carbon cycle. However, little data are available on C stocks of floodplains in comparison to other terrestrial ecosystems. In this study, we quantified the C stocks of aboveground biomass and soils of riparian vegetation types at 76 sampling sites in the Donau-Auen National Park in Austria. Based on our results and a remotely sensed vegetation map, we estimated total C stocks. Carbon stocks in soils (up to 354 t ha-1 within 1 m below surface) were huge compared to other terrestrial ecosystems. As expected, soils of different vegetation types showed different texture with a higher percentage of sandy soils at the softwood sites, while loamy soils prevailed at hardwood sites. Total C stocks of vegetation types were significantly different, but reflect differences in woody plant biomass rather than in soil C stocks. Mature hardwood and cottonwood forests proved to have significantly higher total C stocks (474 and 403 t ha-1, respectively) than young reforestations (217 t ha-1) and meadows (212 t ha-1). The C pools of softwood forests (356 t ha-1) ranged between those of hardwood/cottonwood forests and of reforestations/meadows. Our study proves the relevance of floodplains as possible C sinks, which should be increasingly taken into account for river management. Furthermore, we conclude that plant-species distribution does not indicate the conditions of sedimentation and soil C sequestration over the time span of interest for the development of soil C stocks.



Key words: carbon stocks / organic carbon / Donau-Auen National Park / fluvial ecosystems / riparian forest

Accepted September 27, 2009

#### 1 Introduction

Vegetation and soils are the biggest pools of organic C (OC) stored in terrestrial ecosystems. About 2200 Gt of C are located in the earth's upper surface layer and are part of the global C cycle (*Robert*, 2006).

Floodplains with high in- and outputs of C-rich sediments may be particularly important and sensitive natural pools of OC. The potential of floodplains to accumulate or release C is strongly influenced by climate change or direct human impacts. The IPCC (2001) assumes that higher temperatures and drier conditions may lead to increased decomposition of organic matter (OM) and higher C emissions to the atmosphere. Changes in the use of wetlands, such as conversion to agricultural/forestry land or restoration can result in high losses or gains of C (IPCC, 2000). In addition, human impact on the flooding regime of wetlands or on the sediment concentration of flooding water may affect carbon sequestration of floodplains. The quantification of the C stocks of riparian ecosystems and the knowledge on C-distribution patterns seems to be of increasing relevance for river-system management.

Mitra et al. (2005) estimated that wetlands occupy 4%–6% of the earth's land area and store C in the range of 202–535 Gt. Annual C-accumulation rates of wetlands are assumed to be 10–20 times higher than rates of terrestrial ecosystems (van der Valk, 2006). Such high accumulation rates are probable due to large biomass production, input of C-enriched sediments and anaerobic conservation of biomass. However, C storage in fluvial ecosystems has scarcely been quantified as yet (Hofmann and Anders, 1996; Giese et al., 2000, 2003; Fierke and Kauffman, 2005; Hazlett et al., 2005).

Different studies tried to predict C stocks based on relevant environmental properties. *Bernoux* et al. (2006) define the input of OM (rates and quality), the transfer of organic and inorganic C (deposition and erosion), and the decomposition of soil OM through bacterial mineralization as main processes controlling C sequestration in ecosystems. *Zhang* et al. (2002) used a process-oriented biogeochemical model to simulate the C fluxes in wetland ecosystems. Their model indicates air temperature, water-outflow parameters, initial soil C content, and plant photosynthesis capacity as the main

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controlling factors. In addition, other authors have emphasized the relevance of above- and belowground net primary production through vegetation for soil C accumulation and soil development (Zak et al., 1990; Giese et al., 2000). Vegetation data as well as land-cover type and forest-stand age have successfully been used to deduce C dynamics in ecosystems which are not affected by flooding processes; but these methods have not been approved for floodplain areas yet (Turner et al., 2004). In riparian areas, vegetation may also be indicative of fluvial geomorphic processes and conditions such as frequency, duration and intensity of floods, sediment deposition and erosion, water availability, and landform stability (Hupp and Osterkamp, 1996; Gurnell et al., 2002; Järvelä, 2003; Tal et al., 2004; Gurnell et al., 2005). In addition, C accretion is known to depend on soil age with the highest rates for OM-pool allocation found during the first few decades of soil development (Zehetner et al., 2009).

According to the state of the art, vegetation may represent the size of C stocks as well as processes of C storage. Classification of vegetation types with remote-sensing data has been carried out for several decades, and where vegetation types show marked differences in soil C stocks, it may facilitate the estimation of C stocks and dynamics in ecosystems (*McBratney* et al., 2003; *Patenaude* et al., 2005; *Myeong* et al., 2006).

The main objective of the present study was to quantify C storage in vegetation and soils of the Donau-Auen National Park, Austria. Vegetation was classified into different vegetation types, and the C stocks at sites of various vegetation types were determined. In particular, we assessed the following study questions:

- (1) Are there any differences in C stocks between relevant vegetation types?
- (2) How much OC is stored along Danube's northern river banks within the Donau-Auen National Park, which are still connected to river dynamics?

#### 2 Material and methods

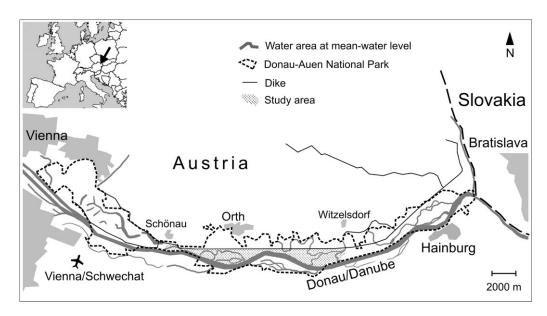
#### 2.1 Study area

The study was carried out in the Donau-Auen National Park, Austria (Fig. 1). The area is recognized by the IUCN as a Riverine Wetlands National Park, category II. With an area of > 9300 ha, the National Park protects the greatest wetland environment subjected to natural fluvial dynamics of such magnitude in Central Europe. For a distance of  $\approx$  36 km and an average width of 350 m, the Danube River flows through the National Park without any restraints caused by barrage.

Riparian hardwood and softwood forests cover 65% of the National Park's territory; meadows cover 15%, and  $\approx$  20% is under water. The habitat types represent a wide range of environmental conditions from the water body of the Danube River, oxbow lakes and side arms, gravel banks on islands and shores, steep riverbanks and riparian forests to meadows and xeric habitats.

Today Austria's Federal Law (Art. 15a, B-VG) has banned any commercial enterprise in the National Park, but anthropogenic effects from the past are still present. Regulation and river straightening were carried out in the 19th century in order to protect the area from flooding and improve navigation. The Marchfeld dike was built, cutting off large areas of floodplains from the natural dynamic of the Danube River. Forest utilization in broad sections of the riparian forests affected the original biocenosis.

Study plots were established at the N river bank of the Danube covering the dynamic parts S of the Marchfeld dike between the villages of Schönau (48°8′ N, 16°36′ E) and Witzelsdorf (48°7′ N, 16°48′ E). This area covers roughly 1310 ha and represents the typical riparian ecosystem of the National Park. Soils of the area are Haplic Fluvisols (calcaric) and Haplic Gleysols (calcaric). The climate is characterized by a mean temperature of 9.8°C and a mean precipitation of 533 mm (climate station: Schwechat, 48°07′ N, 16°34′ E,



**Figure 1:** The Donau-Auen National Park.

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184 m asl; Zentralanstalt für Meteorologie und Geodynamik, 2002). In the study area, the Danube River exhibits flow velocities between 2 and 2.5 m s $^{-1}$ , and a mean discharge of 1950 m $^3$  s $^{-1}$  with 1% and 99% quantiles of 850 m $^3$  s $^{-1}$  and 5000 m $^3$  s $^{-1}$ , respectively (Zehetner et al., 2009).

#### 2.2 Sampling design

Data collection was performed in a stratified randomized sampling design. In order to cover all relevant vegetation types within the Donau-Auen National Park, we divided the study area into polygons with more or less homogeneous vegetation via visual interpretation of aerial color–infrared (CIR-) photographs 76 of which were randomly selected. Within each of the 76 polygons, one  $10 \times 10 \text{ m}^2$  study plot was established. Vegetation was classified according to the dominant tree species and categorized as follows: (1) softwood forests, (2) cottonwood forests, (3) hardwood forests, (4) reforestations with hardwood species, (5) meadows and reeds. In addition, oxbow lakes and settlement area were mapped. Data were collected during field trips between June and August 2008. The vegetation categories were confirmed in the field in the majority of the polygons in February 2009.

#### 2.3 Soil analysis

Soil samples were extracted from the center of each study plot ranging from 0 to 100 cm in depth using an auger. We determined the thickness of the different soil horizons. Each soil horizon was analyzed separately for carbonate concentration and concentration of OC. In addition, texture of the different soil horizons was determined.

All samples that were analyzed for their organic- and inorganic-C concentrations were sieved (< 2 mm) and dried (104°C) before analysis. Total contents of C and N were determined from ground samples using a CN analyzer (Elementar). The carbonate-C content of the samples was determined using the same instrument after combustion of the soil samples at 530°C. According to Bisutti et al. (2007), all OC is removed at this temperature, while the C in carbonate is not affected by the combustion. The organic-C concentration of the sample was calculated from the difference between total and carbonate C. Considering the bulk density of the different horizons, we calculated the total amount of C stored within the soil up to a depth of 1 m. For the determination of bulk densities, we dug one soil profile per vegetation unit and sampled a defined volume of each soil horizon. We determined the weight of the soil sample after drying at 104°C. Preliminary investigations indicate that the variation of bulk densities of soils at a given depth within one vegetation unit is < 10% (data not shown).

#### 2.4 Vegetation analysis and biomass estimation

Within each 10  $\times$  10 m² study plot, we measured circumference at breast height as well as height of all stems > 15 cm in circumference and recorded the respective tree species. Tree height was determined trigonometrically. Based on these data, we calculated stem number per hectare, mean

**Table 1:** Biomass equations used for tree species found in the study area (abbreviations: AB = aboveground biomass, ABW = aboveground woody biomass, SV = stem volume, D = diameter at breast height, H = height).

Species	Equation	Source (Zianis et al., 2005)
Acer spec.	In <i>ABW</i> = -2.7606 + 2.5189 In <i>D</i>	Appendix A
Ailanthus altissima	$\ln ABW = -2.7606 + 2.5189 \ln D$	Appendix A We used equation for <i>Acer</i> spec.
Alnus incana	$AB = 0.0003 D^{2.42847}$	Appendix A
Carpinus betulus	$SV = 0.0002 D(2.2589 + 0.0014) H_{0.6029}$	Appendix C
Corylus avellana	$SV = -1.8683 + 0.2146 D^2 + 0.0128 D^2H + 0.0138 DH^2 - 0.0631 H^2$	Appendix C
Fraxinus excelsior	$\ln ABW = -2.4598 + 2.4882 \ln D$	Appendix A
Juglans regia	SV = 0.0002 D(2.2589 + 0.0014) H0.6029	Appendix C We used equation for Carpinus betulus
Populus spec.	$AB = 0.0519 D^{2.545}$	Appendix A We used equation for <i>Quercus robur</i>
Prunus padus	$SV = -0.0023 - 0.0012 D + 0.0001 D^2 - 7.8058 \cdot 10^{-6} D^3 + 3.3282 \cdot 10^{-4} H + 3.1526 \cdot 10^{-5} D^2H$	Appendix C We used equation for <i>Prunus avium</i>
Prunus serotina	$SV = -0.0023 - 0.0012 D + 0.0001 D^2 - 7.8058 \cdot 10^{-6} D^3 + 3.3282 \cdot 10^{-4} H + 3.1526 \cdot 10^{-5} D^2H$	Appendix C We used equation for <i>Prunus avium</i>
Pyrus communis	$\ln AB = -0.883 + 2.14 \ln D$	Appendix A We used equation for <i>Quercus robur</i>
Quercus robur	$\ln AB = -0.883 + 2.14 \ln D$	Appendix A
Salix alba	$SV = -1.8683 + 0.2146 D^2 + 0.0128 D^2H + 0.0138 H^2D - 0.0631 H^2$	Appendix C We used equation for Salix caprea
Tilia cordata	In <i>ABW</i> = -2.6788 + 2.4542 In <i>D</i>	Appendix A
Ulmus spec.	$SV = -0.0347 + 0.0043 D - 0.0001 D^2 - 1.7667 \cdot 10^{-6} D^3 + 0.0002 H + 3.8311 \cdot 10^{-5} D^2H$	Appendix C

separated from forested study plots. The final CA was carried out excluding plots on meadows and reeds in order to detect floristic differences between the respective forest types.

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tree height, and mean diameter at breast height (dbh). In addition, the cover of each vascular plant species was estimated visually. Species cover below 1% with several individuals per study plot was estimated as 0.5%. Single individuals were recorded as 0.01%. Tree, shrub, and herb layer were analyzed separately, and the total cover of each layer was also estimated.

Due to the unequally distributed number of samples per vegetation unit, differences of the stand and soil parameters between vegetation units were tested using a nonparametric Kruskal-Wallis test. As a post-hoc test, we used Bonferronicorrected pairwise Man-Witney-U tests.

Dry biomass of the tree component was calculated according to Zianis et al. (2005). The authors compiled empirical equations for the calculation of biomass based on diameter at breast height and tree height. We used equations for aboveground biomass or aboveground woody biomass (Tab. 1). Where only equations for stem volume were available, we multiplied the stem volume by 0.4451 to derive stem biomass according to Wulder et al. (2008). For shrubs, we used the equation for Corylus spec., uniformly.

To detect interactions of soil texture and vegetation units, a two-way contingency table was established which crossclassified the number of plots in each vegetation type and soil texture. In order to reduce cells with zero counts, we classified soil texture into the main categories of loam and sand according to the most dominant texture type along the soil profile. Log-linear models with and without the interaction between soil and vegetation were calculated and compared according to the likelihood ratio expressed by G2 (Quinn and Keough, 2003).

To assess large woody debris biomass, we measured length and mean circumference of all dead stems in each study plot with circumferences > 30 cm. We transformed volume to mass assuming a mean wood density of 0.6 t m<sup>-3</sup> as a rough estimate (Baritz and Strich, 2004; Chave et al., 2006).

All calculations were carried out with R version 2.8.0, applying the packages "vegan" and "cluster".

For the calculation of C stocks of living and dead biomass, we assumed a 50% C content for dry biomass, as is commonly conducted for C estimates (e.g., Giese et al., 2003).

#### 3 Results

#### 2.5 Statistical methods

#### 3.1 Ordination of vegetation

To confirm classification of vegetation units, an ordination of study plots was performed using Correspondence Analysis (CA) based on the cover of all species in each sample. For the initial CA, all plots located on meadows or in reeds were Vegetation analysis revealed 139 species of vascular plants with hardwood forests to be the most species-rich vegetation unit of our study area (Tab. 2). Ordination of study plots based on the cover of all species proved the classification of vegetation units according to the dominant tree species. In



× Tree species

Acer negundo

#### Cottonwood forests dominated by Populus alba

P. × canadensis

or Ulmus spec.

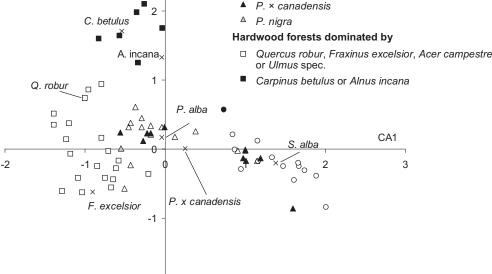


Figure 2: Correspondence Analysis of study plots and the most important tree species. First/second axis eigen values (percent inertia): 0.728/ 0.544 (8.2%/6.2%).

-2

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**Table 2:** Vegetation and soil parameters of different vegetation types. Means with standard errors (S.E.) in parentheses. Lowercase letters indicate significant differences regarding the post-hoc tests.

	Softwood forests	Cottonwood forests	Hardwood forests	Reforestations	Meadows and reeds
Number of study plots	14	26	21	6	9
Distance to main channel / m	419 (102) <sup>a</sup>	474 (88)a	617 (88) <sup>a</sup>	594 (137) <sup>a</sup>	447 (130)a
Distance to next channel / m	48 (5) <sup>a</sup>	248 (54) <sup>b</sup>	176 (23) <sup>b</sup>	133 (26) <sup>ab</sup>	199 (82)b
Species number / (100 m²)-1	13 (1) <sup>ab</sup>	13 (1) <sup>ab</sup>	16 (1) <sup>a</sup>	16 (3) <sup>ab</sup>	9 (2)b
Canopy cover / %	39 (4) <sup>a</sup>	41 (4) <sup>a</sup>	68 (3) <sup>b</sup>	58 (7) <sup>ab</sup>	0 (0)c
Cover of shrub layer / %	10 (4) <sup>ab</sup>	21 (4) <sup>a</sup>	25 (5)a	10 (4) <sup>a</sup>	0 (0)b
Cover of herb layer / %	74 (6)ac	62 (5)ab	48 (6)ab	37 (4)b	95 (4)°
Stem number / ha-1	740 (150) <sup>ab</sup>	480 (50)a	590 (80)ab	1530 (550)b	0 (0)c
Mean tree height / m	20 (2)ab	25 (1)a	23 (1)a	13 (2)b	_
Mean dbh / cm	51 (11.0)a	43 (3)a	41 (4)a	14 (2)b	_
Number of soil horizons	4.2 (0.5) <sup>a</sup>	4.2 (0.3)a	3.8 (0.2)a	3.2 (0.5)a	4.0 (0.3)a
Depth of Ah horizon / dm	1.7 (0.2) <sup>a</sup>	1.7 (0.2) <sup>a</sup>	1.5 (0.2)a	2.0 (0.4)a	1.6 (0.3)a
Cumulative depth of horizons with visible accumulation of organic matter / dm	5.5 (0.6) <sup>a</sup>	6.8 (0.3)ª	6.7 (0.4) <sup>a</sup>	5.7 (0.4) <sup>a</sup>	7.0 (0.7) <sup>a</sup>

the CA diagram, the sampling points clustered according to dominant tree species (Fig. 2). The first axis showed the known gradient from softwood to hardwood forests with cottonwood stands in intermediate positions. Along the second axis, all reforestations with *A. incana* and *C. betulus* were separated from other forest types (Fig. 2). The visually mapped vegetation units of the study area were thereby aggregated for comparison (Fig. 3).

#### 3.2 Specific properties of different vegetation types

Averaged distance to main channel showed a gradient from softwood to hardwood forests. The differences were, however, not significant (Tab. 2). In contrast, distance to next channel presented a correlation with vegetation units. Sampling plots of softwood vegetation prevailed in the proximity to next channel with an averaged distance of 48 m, while all other units except reforestations were significantly more distanced from any channel.

Hardwood-forest canopy cover was significantly higher than in other vegetation units, and the highest values for shrub cover were in hardwood forests. As a consequence, we observed the lowest cover of herbaceous plants in hardwood forests although this trend was not significant.

Hardwood reforestations were characterized by marked differences, in terms of stand parameters. Mean tree height and mean dbh were significantly lower, whereas stem number was higher than those of other forest types. By trend, stem number was higher and mean height lower in softwood forests than in cottonwood and hardwood forests. However, differences between these vegetation units were statistically not significant.

#### 3.3 Soils of vegetation types

According to the conceptual model of hardwood and softwood habitats, we expected different soil properties under hardwood and softwood vegetation. Under softwood vegetation, coarsely textured soils with low C content and a large number of soil horizons should represent the strong flooding dynamics of these sites. In contrast, hardwood sites should represent finely textured soils with high C content. Thick and uniform sedimentation horizons should indicate static flooding conditions.

However, our results proved these assumptions only by trend. Due to large variation within one vegetation type, differences of soil properties between different vegetation types were not significant. Counts of soil horizons were slightly higher in softwood and cottonwood stands than in mature and reforested hardwood stands. Correspondingly, organically enriched sedimentation horizons were less profound below softwoods than those below meadows, hardwood, and cottonwood stands (Tab. 2). Table 3 shows the distribution of sandy and loamy soils among different vegetation types. However, as for soil-profile characteristics, we could not statistically prove any interactions between vegetation and

Table 3: Number (and percentage) of study plots in different classes of soil texture and vegetation units.

	Softwood forests	Cottonwood forests	Hardwood forests	Reforestations	Meadows and reeds
Sand	7 (50%)	8 (31%)	6 (29%)	3 (50%)	4 (44%)
Loam	7 (50%)	18 (69%)	15 (71%)	3 (50%)	5 (56%)

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soil texture. On calculating generalized linear models, the reduced model without interactions could not be rejected in favor of the saturated model ( $G^2 = 2.72$ , df = 4, p = 0.606).

## 3.4 Carbon stocks of vegetation and soil among different vegetation types

Aboveground woody biomass reached the highest values in hardwood forests. However, we could not detect significant differences to cottonwood and softwood forests. Only hardwood reforestations had significantly lower biomass values than all other forest types. According to the C stocks of large woody debris, hardwood and softwood forests were significantly different (Tab. 4).

Soil C stocks did not show significant differences between the vegetation classes, but data show clear trends among vegetation types (Tab. 4). Depth of Ah horizons was lowest in hardwood forests. In contrast, Ah horizons of hardwood stands accumulated 48 t C ha<sup>-1</sup>, more than found in all other forest types (Tab. 4). Nevertheless, total C stocks of all horizons were highest in areas of meadows and reeds. The soils of hardwood and cottonwood forests as well as of hardwood reforestations were characterized by very similar C stocks, whereas total amount of soil C in softwood stands was clearly lower (Fig. 3).

According to a Kruskal-Wallis rank sum test, the sum of aboveground and belowground C stocks showed significant differences within the five determined vegetation classes (p < 0.001). Post-hoc tests differentiated mature hardwood and cottonwood stands from hardwood reforestations and meadows/reeds, whereas softwood forests did not exhibit significant differences to either.

Hence, according to total C stocks in soil and vegetation, we distinguished three main vegetation groups: (1) hardwood and cottonwood forests, (2) hardwood reforestations together with meadows and reeds, and (3) softwood stands which represented an intermediate position. Hardwood and cottonwood forests stored 435 t ha<sup>-1</sup> of total OC which was twice as much as in reforestations and meadows/reeds (214 t ha<sup>-1</sup>). Total stocks in softwood forests were 356 t ha<sup>-1</sup>.

#### 3.5 Total carbon stocks in the study area

We developed a map of vegetation-type distribution within our study area based on field observations and remote-sensing data (Fig. 4). The predicted distribution of vegetation types was proved by a second field survey and corrected where necessary. According to the surface area of each vegetation unit and its specific value of total C stocks per hectare (Tab. 5), we estimate a total C amount of 472,186 t to be accumulated in soil and vegetation for the complete study

**Table 4:** Carbon stocks of soil and vegetation of different vegetation types (Means with standard errors S.E. in parentheses, lowercase letters indicate significant differences regarding the post-hoc-tests).

	Softwood forests	Cottonwood forests	Hardwood forests	Reforestations	Meadows and reeds
Carbon stocks of Ah horizon / t ha-1	41 (10)a	46 (7)ª	48 (7)a	48 (8)a	58 (13) <sup>a</sup>
Carbon stocks of horizons with visible accumulation of organic matter / t $ha^{\!-1}$	113 (14)ª	136 (11) <sup>a</sup>	138 (10)ª	128 (24) <sup>a</sup>	154 (15)ª
Large woody debris / t ha-1	40 (13)a	22 (5) <sup>ab</sup>	7 (2)b	5 (4)abc	0 (0)c
Aboveground woody biomass / t ha-1	163 (26)a	199 (29) <sup>a</sup>	281 (59)a	35 (17) <sup>b</sup>	0 (0)c
Total C stocks of aboveground and belowground / t ha-1	356 (35)ab	403 (29)a	474 (61)a	217 (26)b	212 (21)b

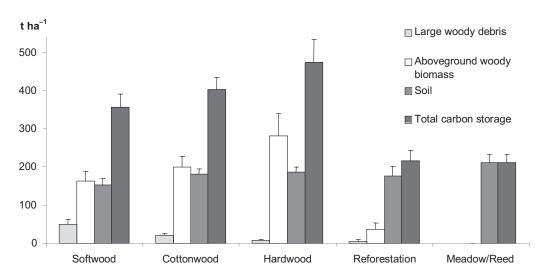


Figure 3: Distribution of C stocks in soil and vegetation of different vegetation types (bars represent standard error S.E.).

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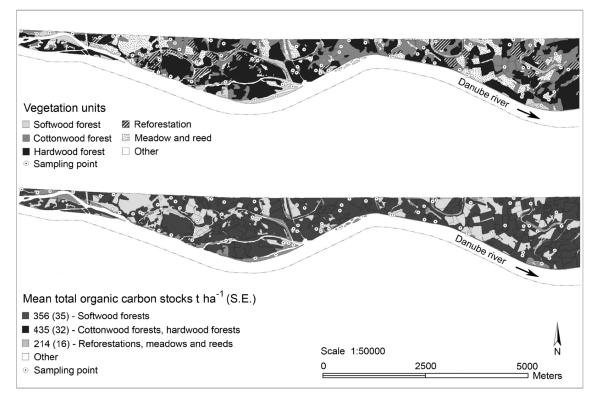


Figure 4: Survey of the study area, GIS-model of vegetation types and distribution of average C stocks.

area. With the exception of softwood forests, C pools are inconsistently scattered and no clear spatial patterns can be observed. The category with the highest value of C stocking (cottonwood and hardwood forests) covers almost 70% of the study area.

#### 4 Discussion

Our data confirm the relevance of the Danubian floodplains within the Donau-Auen National Park as C sink. Total C stocks in the study area ranged between 212 t ha<sup>-1</sup> for meadows and reeds and 474 t ha<sup>-1</sup> for hardwood forests. The values are comparable to those published for other temperate riparian ecosystems in Central Europe (*Hofmann* and *Anders*, 1996). The authors calculated 462 t ha<sup>-1</sup> total C for natural riparian forests and 216 t ha<sup>-1</sup> total C for agricultural land. Also our estimates on C stocks of aboveground bio-

mass are in agreement with literature. *Fierke* and *Kauffman* (2005) reported C stocks for aboveground biomass of mature cottonwood forests in the NW USA as high as 219 t ha<sup>-1</sup>. Compared to terrestrial forest ecosystems beyond floodplains, vegetation and soils in the National Park show OC stocks up to two times higher (*Hofmann* and *Anders*, 1996). Furthermore, it should be considered that we only determined C stocks up to 1 m in depth and that total stocks may be even higher. Our findings indicate clear differences to boreal river systems where upland and riparian forests exhibit consistently lower C storage and differences between both forest types were not significant (*Hazlett* et al., 2005). The latter may be attributed to missing river dynamics in the study on a boreal floodplain.

Soil C stocks measured in the central range of the Donau-Auen National Park are in line with values reported for the western limit of the park adjoining the Austrian capital

Table 5: Percentage cover and total C stocks of different vegetation units in the study area.

Vegetation units	Area / ha	Percentage of study area / %	Total carbon stocks per unit / t
Softwood forests	83.5	6	29,726
Cottonwood forests	562.1	43	226,526
Hardwood forests	319.5	24	151,443
Reforestations	85.0	6	18,445
Meadows and Reeds	217.2	17	46,046
Other	47.7	4	_
Total	1315.0	100	472,186

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(172–204 t ha-1; *Haubenberger* and *Weidinger*, 1990). However, spatial heterogeneity and temporal dynamics are known to influence the vegetation type and sedimentation regime. Following *Ellenberg* (1986), reeds, softwood, and hardwood forests are typical vegetation units in floodplains along gradients of inundation time and distance to river channel. We expected that vegetation type also indicates differences in soil and vegetation C stocks.

Our data provide evidence for the spatial proximity of softwood forests to river channels. Moreover, Correspondence Analysis clearly separated the different vegetation units along their ecologic amplitudes (Fig. 2). Due to different flooding tolerances of softwood and hardwood forests, the horizontal axis might be interpreted as a gradient of inundation duration. The intermediate position of all cottonwood stands (Populus alba, P. nigra, P. × canadensis) reflects the natural distribution range of this genus. However, the spreading along this gradient is expected to be a consequence of forest transformation from former hardwood and softwood stands to poplar afforestations—particularly with P. × canadensis—still reflected in the recent species composition. This is supported by the high percentage of cottonwood in the study area. The cessation of forestry operations and the promotion of native tree species in the area will lead to a succession toward natural forest types in the long run (Nationalpark Donau-Auen GmbH, 2007). The vertical axis of CA revealed a gradient of stand age from young plantations (Carpinus betulus, Alnus incana) to natural mature forests (Quercus robur, Fraxinus excelsior, P. nigra, P. alba, Salix alba). In spite of these results, vegetation types lack a clear spatial pattern in the study area (Fig. 4), which is presumably a consequence of heterogeneous flooding conditions and intensive geomorphic dynamics, which may have altered vegetation. In addition, numerous forestry operations in the past influenced the original forest composition.

We found significant differences among stand structure of vegetation types. Softwood and cottonwood forests exhibited a significant lower canopy cover than hardwood stands. In addition, hardwood reforestations had a lower herb cover and mean diameter than softwood stands along with a lower tree height and mean diameter than cottonwood and hardwood forests. Stem number in turn was higher than in cottonwood stands. In accordance, C pools of living woody biomass proved to be significantly lower in reforestations than in any other forest type. These findings emphasize the importance of successional stage and stand age to aboveground biomass as is equally shown for other floodplain ecosystems (*Giese* et al., 2003; *Fierke* and *Kauffman*, 2005; *Keeton* et al., 2007). Due to the fact that all other forest types are mature forests, their C pools were in a similar range.

Large woody debris contributed only little to overall C pools. Softwood forests showed significant higher stocks of large woody debris than hardwood forests. As softwood stands are most commonly adjacent to river channels, this pattern is presumably a consequence of increased transport and deposition of deadwood along the channel banks during flooding events.

Dynamic parts such as softwood forests are expected to show high numbers of shallower soil horizons with sand as the main grain fraction. Areas with rather static conditions, such as hardwood forests, usually display deeper horizons with higher contents of loam or clay (Jacobson et al., 2003). However, in our study, soil conditions did not differ significantly among vegetation types. In agreement with results from Mendonça Santos et al. (2000), the distribution of soil horizons within the floodplain is highly variable, depending on the soil microrelief and of buried back waters. Two reasons may explain the missing correlations between the analyzed soil properties and vegetation. (1) Recent vegetation may not represent the environmental conditions, which controlled C sedimentation over the large time span needed to build up the C stocks. According to the study of Fiebig et al. (2009), the substrate of the soils we analyzed deposited over > 500 y. Current distribution of vegetation types results as a consequence of historic shifts of the river course and corresponding environmental conditions and of forestry operations. (2) Species distribution is not sensitive enough to indicate differences in sedimentation regimes. In floodplains, continuous gradients from dynamic (ideal softwood) to static (ideal hardwood) conditions may exist (Cierjacks et al., submitted). In contrast, the concept of vegetation types used here is based on the concept of discrete changes of environmental properties. Stand properties may better indicate soil conditions as plant-species distribution.

The thickness of Ah horizons did not show pronounced differences among vegetation types. Depth of Ah horizons is mainly determined by the activity of soil organisms and may thus be independent from flooding conditions. However, hardwood forests exhibited the highest C stocks in the Ah horizon due to higher C concentrations than the soils of the other vegetation types. Finer sediments with higher C concentrations and a higher input of biomass from a closed canopy in hardwood forests may be the reason for this observation. The sedimentation of C-poor fractions and the repeated interruption of humus accumulation by water dynamics in turn might cause the low soil C stocks found in topsoil horizons of softwood forest sites.

Overall, total C stocks of soil and vegetation were significantly higher in hardwood and cottonwood forests than in reforestations and meadows/reeds with softwoods representing an intermediate position. This is a pattern mainly caused by aboveground biomass, whereas differences in soil C were not significant. However, soil C stocks are relatively high in all vegetation types, which indicates the soil's capacity to sequester C over a long time (soil-carbon-memory effect) and shows former ecologic conditions.

#### 5 Conclusion

In summary, our results substantiate the relevance of floodplains as C sinks in comparison to other terrestrial ecosystems. Although we found differences in vegetation C stocks between vegetation types as a consequence of stand age, we could not find clear correlations of vegetation type and soil conditions, which is presumably a consequence of the complexity of geomorphological processes in floodplains and 652 Cierjacks et al.

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even more of human influence on present tree-species composition. Therefore, it might be helpful to include spatial and geomorphological variables rather than potentially man-made vegetation types for modeling amounts and distribution of C stocks.

Our soil survey suggests two reasons for the importance of floodplains for C sequestration: The accumulation and covering of C-rich sediments (hardwood sites) might contribute to the sequestration of allochthonous OM, while the supply of C-poor sediments (softwood sites) might contribute to storage of autochthonous OM by the adsorption to the sediment surfaces. Mismanagement of riparian areas may convert present C pools into net C sources (*Busse* and *Gunkel*, 2002). Therefore, conservation strategies for floodplain ecosystems should, to a greater extent, incorporate the function of low-land floodplains as C sinks.

#### **Acknowledgments**

We are grateful to *Christian Baumgartner* and *Christian Fraissl* from the Donau-Auen National Park administration, who supported the work in the study area. *Nico Bätz, Sebastian d'Oleire-Oltmanns, Julie Donner, Kristin Fenske, Jospehine Hübener, Judith Kühn, Benedikt Litschko, Ingo Marziller, Susan Rohsmann, and Jan Schormann from Technische Universität Berlin helped during field work and data processing. We thank <i>Danny McCluskey* for checking our English. We also acknowledge the elaboration of Figure 1 by *Wilfrid Roloff.* This study was financed by *Deutsche Forschungsgesellschaft (DFG, LA 1398/4)* and *Technische Universität Berlin.* 

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C Round robin test on the soil disaggregation efficiency of ultrasound M. Graf-Rosenfellner, G. Kayser, G. Guggenberger, K. Kaiser, M. Kaiser, C.W. Müller, M. Schrumpf, T. Rennert, G. Welp and F. Lang

- unpublished manuscript -

#### C.1 Abstract

Application of ultrasound is widely used for the disaggregation of soil samples in suspension. Calorimetric calibration allows determining ultrasonic power and applied energy as a measure for disaggregating forces. Other properties of ultrasound devices (oscillation frequency and amplitude, sonotrode diameter) as well as procedure details (soil-water ratio, size, shape and volume of containers in which disaggregation is performed) may influence the disaggregation efficiency in addition to the applied energy. The study aimed testing whether the applied ultrasound energy is sufficient to define the disaggregation efficiency and the disaggregation response of soil samples. In a round robin test involving nine laboratories three reference soil samples were disaggregated by applying 30 J ml<sup>-1</sup> and 400 J ml<sup>-1</sup> in two separate experiments with ultrasonic devices and according to the procedure in daily operational use. Disaggregation results were determined by weighing masses of particle size class  $63 - 200 \,\mu m$  and  $200 - 2000 \,\mu m$  obtained by standardized submerged sieving and by analyzing particle size distribution <  $63 \,\mu m$  with dynamic imaging. Disaggregation efficiency was defined as the portion of the different size classes normalized to the portion of corresponding sizes classes obtained by standard soil texture analysis.

The ultrasound devices and protocols used by the participants varied widely. However, only about 6 % of the weighed masses in particle size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  were determined as outliers based on two different statistical methods for the identification of outliers. Differences in the size distribution of particles <  $63~\mu m$  between laboratories were even smaller (less than 3 % outliers out of 162~v alues).

Some ultrasound devices used by the participating laboratories producing more than two outliers in disaggregation results (four out of nine) differed from those of other labs regarding oscillation frequencies (24 or 30 kHz compared to 20 kHz), sonotrode diameters (10 and 14 mm compared to 13 mm) and ultrasound power (16 W compared to > 45 W). An influence of these parameters on disaggregation efficiency cannot be ruled out, and thus, need to be listed when reporting on sonication-based soil disaggregation. Finally, the overall small differences in disaggregation efficiency between the different laboratories show that disaggregation by application of ultrasound gives reasonably reproducible and comparable disaggregation results despite the use of different devices or procedures.

#### C.2 Introduction

The application of ultrasound to soil samples in suspension is a frequently used method for the disaggregation of soil aggregates since decades (*Edwards and Bremner, 1967; Watson, 1971; Watson and Parsons, 1974; Morra et al., 1991; Christensen, 1992; Field et al., 2006; Cerli et al. 2012, Kaiser et al., 2012; Grüneberg et al., 2013*). It is widely assumed that the aggregation power of this procedure is well defined by the ultrasonic energy, which can be calibrated calorimetrically (*North et al. 1972*). The application of a specific ultrasonic energy as obtained by this calibration should assure reproducibility of results of disaggregation experiments (*Schmidt et al., 1999*).

Ultrasound disaggregation is based on cavitation induced in a soil-water suspension by a probe, usually made of titanium (called sonotrode), that oscillates with a frequency of 20 kHz or higher (*Kaiser and Asefaw Berhe, 2014*). While the sonotrode is oscillating, pressure waves propagate from the tip into the suspension. In this wave field, areas of low and high pressure occur consecutively depending on frequency and amplitude of the oscillation. During phases of low pressure, steam bubbles are formed in the suspension, preferentially at cracks or crevices on surfaces of solids, such as soil aggregates and other soil constituents (*Suslick, 1988; Edmonds, 1981*). After a period of growth during negative pressure phases, these gaseous bubbles collapse in subsequent phase of high pressure phase upon reaching a critical size (*Suslick, 1989*). While collapsing, liquid micro-jets are formed which expose solids such as aggregates to a mechanical force and loose particle bindings (*Kaiser and Asefaw Berhe, 2014*).

With regard to these processes an influence of oscillation frequency or amplitude on the cavitational effect, and thus, on disaggregation efficiency seems likely (*Christensen*, 1992; *Amelung and Zech*, 1999; *Mayer et al.*, 2002; *Mentler et al.*, 2004). Also the geometry of the oscillating tip may influence disaggregation efficiency because the tip surface area determines of the structure of the pressure field in suspension (*Schomakers et al.*, 2011). Alongside factors related to the ultrasonic device differences in procedures of disaggregation experiments may affect disaggregation efficiency, namely the soil-water ratio but also the size, shape and material of containers in which the disaggregation is performed (*Watson 1971, Christensen, 1992*).

With regard to the diversity of ultrasonic devices used for disaggregation experiments it seems questionable whether the basic concept of ultrasonic energy as determined by calorimetrical calibration is a proxy for disaggregation. *Schmidt et al. (1999)* worked out differences between ultrasonic devices with regard to the efficiency of transformation of electrical power to cavitational power as determined by calorimetrical calibration: They showed that the ratio between power displayed by the ultrasonic devices during operation (electrical power) and calorimetrically determined power may differ between devices of different manufacturers. They also showed that

this ratio differed also between identical models used in different laboratories. These differences in transformation efficiency can be explained by different properties of devices used but also by differences in procedures for disaggregation or calorimetrical determination of power if identical devices were used by different operators (*Schmidt et al.*, 1999).

In a recent publication, *Pöplau and Don (2014)* suggest that reproducible results of ultrasound dispersion can only be assured if ultrasonic power is defined in addition to ultrasonic energy. Hence, the aim of the round robin test presented here is to test if the applied ultrasound energy is sufficient to define the disaggregation efficiency of ultrasound application and to predict the disaggregation response of soil samples. To achieve this the participants were asked to disaggregate three different soil samples by applying two predefined energies (30 and 400 J ml<sup>-1</sup>) after calorimetrical calibration of ultrasonic power according to their own procedure with their own devices as used in regular laboratory operation. After this, the disaggregation efficiency was analyzed comparing particle size distributions and relating the results to the procedure used.

#### C.3 Material and methods

#### C.3.1 Reference material

Three different soil samples were selected for the disaggregation experiments of the round robin test. The samples differed in properties known to control the stability of soil aggregates: clay and silt content, content of organic and inorganic carbon. Finally, soil samples were from A horizons of a Calcaric Eutric Leptosol (soil sample id #1) and from a Haplic Chernozem (soil sample id #2) as well as a Bt horizon of a Haplic Luvisol (soil sample id #3) (soil classification according to *IUSS Working Group WRB, 2015*). All soil samples were air-dried and sieved < 2 mm prior analysis. A mechanical sample splitter was used for preparing representative sub-samples.

Selected properties of the soil samples are listed in Table C-1. The soil pH was measured in H<sub>2</sub>O in suspension at a soil:water ratio of 1:2.5, according to *DIN 19684-1 (1997)*. The total carbon contents of the soil samples were determined using an elemental analyzer (Vario EL Cube, Elementar Analysensysteme GmbH, Hanau, Germany) on grounded and water-free samples. The content of organic carbon (OC) was calculated as the difference between total carbon and the carbon in the samples after combustion at 550°C according to *Bisutti et al. (2004)*. Inorganic carbon (IC) was determined by gas-volumetric measurement of CO<sub>2</sub> evolved upon treatment with H<sub>3</sub>PO<sub>4</sub> using a "Wösthoff-Apparatus" (Wösthoff GmbH, Germany). Soil texture was determined according to *DIN ISO 11277 (2002)* in a combined sieving/pipette procedure after removal of organic material and complete chemical dispersion in 0.5 M NaP<sub>2</sub>O<sub>7</sub>.

Table C-1: Properties of reference soils samples used in round robin test (IC is inorganic carbon, OC is organic carbon).

Soil type/ horizon		рН	IC	ОС	Clay	Silt
(IUSS Working Group, 2015)	ID	(H <sub>2</sub> O)	[%]	[g kg <sup>-1</sup> ]	[%]	[%]
Calcaric Eutric Leptosol / A	#1	7.5	2.55	159	39.6	53.3
Haplic Chernozem / A	#2	8.0	0.08	20.4	28.0	68.5
Haplic Luvisol / Bt	#3	7.6	0	2.2	24.2	73.0

#### C.3.2 Round robin test procedure

Reference soil samples #1, #2 and #3 were filled in 100 ml PE bottles without air-filled headspace above the solid material in order to avoid unwanted disruption of aggregates by mechanical forces occurring during transport due to movement of material in the bottle. Supplementary material needed for conducting the experiments (identical plastic bowls for submerged sieving procedure and plastic bottles for sample transport and storage) was provided and sent with reference materials to nine participating laboratories **A** to **I**.

Soil-water suspensions of the reference soils were prepared at each laboratory by weighing 5 g of soil and adding 100 ml of deionized water (soil-water ratio of 1:20). The suspension was swayed gently to remove air entrapped between particles while avoiding unwanted disaggregation. Then, the suspension was left for 30 min for equilibration. The partners in the participating laboratories were asked to conduct disaggregation according to their own procedures. Details on procedure and used equipment possibly affecting disaggregation efficiency (see first column of Table C-2) were documented in a questionnaire. Energies used for application were identical and predefined for all participants (30 J ml<sup>-1</sup> and 400 J ml<sup>-1</sup>). In addition, the ultrasonic power determined by calorimetric calibration for disaggregation was demanded to be > 45 W, according to recommendation by *Pöplau* and *Don* (2014).

After disaggregation, the particle size fractions < 63  $\mu$ m, 63 – 200  $\mu$ m and 200 – 2000  $\mu$ m were obtained using a submerged sieving procedure. In preliminary experiments, submerged sieving was found to have the least disaggregating effect compared to other wet-sieving techniques (data not shown). A detailed description of the sieving procedure was provided to minimize disaggregation during sieving: A 200  $\mu$ m mesh-size sieve on top of a 63  $\mu$ m mesh-size sieve was placed in a standardized plastic bowl filled with 5 I of deionized water. The 200  $\mu$ m mesh of the upper sieve was submerged 2 cm beneath the water-surface during the entire sieving procedure.

Table C-2: Overview to properties of ultrasonic devices used and disaggregation procedures used applied participating laboratories in the round robin test. (\*) calculated from ultrasonic power and sonotrode surface; (\*\*) Results from enquiries at the device manufacturers; (1) Used for application of 30 J ml<sup>-1</sup>; (2) Used for application of 400 J ml<sup>-1</sup>; PE = Polyethylene; PC = Polycarbonate

Laboratory abbr.	A	В	C	D	Ε	F	G	Н	J
Manufacturer and Device designation	Sonics Vibra Cell VCX 500	Bandelin Sonopuls HD 2200	Hielscher UP200S	Branson S250D	Branson Sonifier 250	Branson W250D	Bandelin Sonopuls MD 2200	Sartorius Labsonic M	Branson Digital Sonifier 450D
Sonication power [W] [J s <sup>-1</sup> ]	48	63.61	56.9	59.33	48.98	45.68 <sup>(1)</sup> / 73.04 <sup>(2)</sup>	57.1	16.03	60.61
Diameter of sonotrode [mm]	13	13	14	13	13	13	13	10	13
Output control setting	60%	50%	75%	50%	Level 7	60% <sup>(1)</sup> / 90% <sup>(2)</sup>	70%	100%	72%
Oscillation frequency [kHz] <sup>(**)</sup>	20	20	24	20	20	20	20	30	30
Amplitude [μm] <sup>(**)</sup>	68	77	88	117	104	90 <sup>(1)</sup> / 131 <sup>(2)</sup>	107	125	106
Intensity of ultrasound [W cm <sup>-2</sup> ] <sup>(*)</sup>	36.16	47.92	36.96	44.7	36.9	34.41 <sup>(1)</sup> / 55.03 <sup>(2)</sup>	43.02	20.4	45.66
Immersion depth [mm]	15	15	20	10	15	15	15	15	15
Duration of use of sonotrode	3 months	30 h	80 h	40 h	11 months	17 h	since 08.12.2014	new	3 months

Use of degassed water?	no	no	no	no	no	no	no	no	no
Cooling of suspension during sonication?	yes	yes	no	no	yes	yes	yes	yes	yes
Container used for disaggregation	250 ml bottle (PE), 64*120 mm	500 ml bottle (Duran© glass), 86*181 mm	no data available	250 ml beaker (Duran© glass), 60*100 mm	200 ml bottle (PE), 59*114 mm	250 ml beaker (glass), 70*100 mm	no data available	250 ml bottle (PC), 61.8*127.6 mm	no data available

After release of air entrapped below the sieves the disaggregated suspension was poured on the 200  $\mu$ m sieve. The two sieves were pressed together and moved manually up and down for 5 minutes with the amplitude of 2 cm. One cycle was conducted in 4 seconds (total of 75 cycles in 5 minutes).

After sieving, particles retained on the sieves were collected, dried and weighed. The sub-samples which passed the 63  $\mu$ m sieve were sent as suspension to the coordinating institute. Possible dispersion during parcel transport of suspensions was tested by shaking suspensions on a reciprocal shaker for 5 days in preliminary trials. Comparing particle size distributions before and after shaking indicates that the size distribution of particles and aggregates < 63  $\mu$ m was not changing significantly during parcel transport (data not shown).

In addition, the reproducibility of the described sieving method and sample treatments was tested in intra-laboratory experiments prior to the round robin test. These experiments were repeated multiple times in the coordinating laboratory using the same ultrasonic device, and variations in results with regard to masses in particle size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  were determined. Variations in results determined in this intra-laboratory test were used as a proxy for the bias caused by the method to obtain particle size fractions and not related to differences in ultrasound devices and procedures

#### C.3.3 Determining size distribution of particles < 63 µm

Size distribution of particles  $< 63 \mu m$  was determined using a digital dynamic image analyzing technique. Using the QICPIC®image analyzer with LIXELL® unit for application of samples in suspension (Sympatec GmbH, Clausthal-Zellerfeld, Germany) the size distribution of particles can be determined by analyzing particle images of a large number of pictures taken by an image sensor while the suspension is passing through a cuvette (see Figure C-1). In our experiment, 450 pictures per second were taken with an exposure time of 4 nanoseconds during a period of 30 seconds. The optical unit of the sensor (M4) used allows to measure particles ranging in size between  $6-682 \mu m$ . Thereby, a total number of more than 106 particles in each sample was detected which allows to determine particle size distributions with an error less than 1 % (ISO 14488:2007, 2011). Quality assurance was performed by analyzing a reference material (MICROSOIL® M8 silica powder, EUROQUARTZ GmbH, Dorsten, Germany) of a known particle size distribution before every sample. In our experiment, particle size distributions were expressed as the Feretmin value distribution which is defined as the smallest possible distance between two parallel tangents on opposing sides of the particle at an arbitrary angle (Merkus, 2009). It can be assumed that it is close to diameters determined by sieving. Particle size distribution was given using equidistant particle size classes (26 in the analyzed size range between 6 and 100 µm). For further characterization, and statistical

evaluation of particle size distributions, descriptive diameters  $D_{10}$ ,  $D_{50}$  and  $D_{90}$  were determined for each distribution. These parameters represent particle size thresholds where 10 % ( $D_{10}$ ), 50 % ( $D_{50}$ ) or 90 % ( $D_{90}$ ) of the all detected particles are smaller than the respective parameter. These parameters are known to describe particle size distributions sufficiently for statistical evaluation (*Merkus*, 2009).

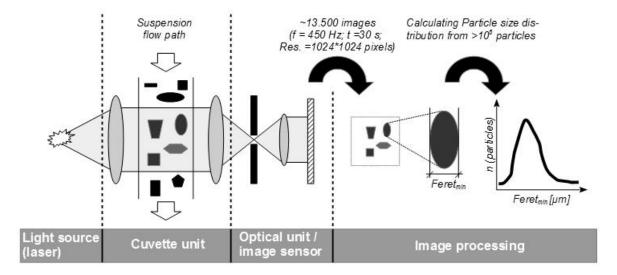


Figure C-1: Mode of operation of QICPIC® digital dynamic image analyzer

#### C.3.4 Evaluation of results and statistics

Masses in particle size fractions obtained by the participating laboratories were evaluated with regard to potential bias based on the distance from the mean value. Two methods were applied to identify outliers in particle masses in size fractions  $63 - 200 \,\mu\text{m}$  and  $200 - 2000 \,\mu\text{m}$  as well as in parameters describing size distribution of particles <  $63 \,\mu\text{m}$  (D<sub>10</sub>, D<sub>50</sub> and D<sub>90</sub>) for each participating laboratory. In accordance to *Wilcox (2010)* outliers were identified by comparing the respective value obtained in a laboratory ( $x_i$ ) with standard deviation ( $\sigma$ ) of mean value of all laboratories ( $\bar{x}$ ) if

$$x_i < \bar{x} - 2\sigma \lor x_i > \bar{x} + 2\sigma$$

In case of extreme outliers, it is possible that this method masks other outliers due to strong impact on standard deviation. Therefore, another method for outlier identification was used based on the comparison of single values with quartiles of the distribution. This method is less sensitive against masking of outliers by extreme values. According to this method a value  $(x_i)$  is identified as an outlier if

$$x_i < Q_1 - 1.5 \cdot IQR \lor x_i > Q_3 + 1.5 \cdot IQR$$

In this equation  $Q_1$  represent the lower quartile,  $Q_3$  the upper quartile, and IQR the interquartile range  $(Q_3 - Q_1)$ .

#### C.4 Results and discussion

# C.4.1 Aggregate stability of the soil samples and intra-laboratory reproducibility of soil disaggregation

Mean weights of size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  were determined in independent experiments carried out by the coordinating laboratory after dispersion of all reference soils with  $30~J~ml^{-1}$  and  $400~J~ml^{-1}$ , using the same ultrasonic device. Results of intra-laboratory tests on reproducibility of the described procedure, including particle masses of individual size fractions and standard deviation as a measure of variation of results, are provided in Table C-3.

Table C-3: Means of masses of particles in size fractions obtained after application of 30 J ml<sup>-1</sup> and 400 J ml<sup>-1</sup> in intra-laboratory experiments  $(\bar{x})$  including standard deviation  $(\sigma)$  and number of independent experiments (n).

Soil	US	Size	n	$\overline{x}$	σ
ID	Energy	fraction			
	[J ml <sup>-1</sup> ]	[µm]		[g]	[g]
#1	30	63 – 200	3	0.89	0.02
	_	200 – 2000	3	1.64	0.05
	400	63 – 200	4	0.24	0.02
		200 – 2000	4	0.81	0.12
#2	30	63 – 200	4	0.08	0.00
	_	200 – 2000	4	0.06	0.00
	400	63 – 200	4	0.07	0.01
		200 - 2000	4	0.05	0.01
#3	30	63 – 200	5	0.16	0.05
		200 – 2000	5	0.05	0.01
	400	63 – 200	3	0.10	0.01
		200 – 2000	3	0.03	0.00

The means of masses of particles in size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  reveal that only soil sample #1 was still aggregated after application of  $400~J~ml^{-1}$ : 21~% of material was found in size fractions >  $63~\mu m$  while standard soil texture analysis showed that only 8~% of particles in that size class. Soils samples #2 and #3 were found to be disaggregated to a large extent already after application of  $30~J~ml^{-1}$ . In these soil samples, masses in fractions >  $63~\mu m$  after application of  $30~J~ml^{-1}$  were similar to the contents of particles in respective size class as determined by standard texture analysis. However, decreasing masses in size fractions  $200-2000~\mu m$  after application of  $400~J~ml^{-1}$  compared to masses in the same fraction after application of  $30~J~ml^{-1}$  reveal that disaggregation was not complete after  $30~J~ml^{-1}$  even in soil samples #2 and #3.

A low standard deviation for weights of size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  in preliminary test ( $\sigma$  ranged between 0.00 to 0.12 g) reveal a satisfying reproducibility of results gained with the described procedure. With regard to the complexity of the sieving procedure, consisting of a number

of individual operations, the reproducibility of the results obtained within one laboratory seems to be sufficient for determining possible differences in disaggregation results induced by differing ultrasound devices or protocols.

C.4.2 Particle size distribution of samples disaggregated in frame of the round robin test

Variation of weights of size fractions 63 – 200 μm and 200 – 2000 μm determined after disaggregation of reference soil samples #1, #2 and #3 with 30 J ml<sup>-1</sup> in the participating laboratories were found to be larger than those in the intra-laboratory test (see Figure C-2). Most striking differences in variation were found for soil sample #1, for which standard deviation was 0.31 g for size fraction  $63 - 200 \, \mu m$  and  $0.46 \, g$  for size fraction  $200 - 2000 \, \mu m$  (intra-laboratory test:  $0.02 \, and$ 0.05 g, respectively). However, means of determined masses in these size classes did not differ from means determined in preliminary intra-laboratory experiments. These two observations were expected because different operators will increase the systematic error of the procedure but also provide first indication that results gained by different operators are comparable with regard to the mean weights in size fractions. Differences in disaggregation efficiency caused by the use of different ultrasound devices or procedures may contribute to the larger inter-laboratory variation of results than in the intra-laboratory test. Quality assessment of results determined in single laboratories revealed that 3 out of 54 determined masses of the size fractions  $63 - 200 \, \mu m$  and  $200 - 2000 \, \mu m$ were identified as outliers because of differing more than +/- 2\sigma from the mean weight of the respective fraction. Also based on interquartile range, 3 out of 54 determined masses were identified as outliers. Overall, less than 6% of determined weights of size fractions (6 in 108 tests) were found to be outliers according to both criteria after dispersion with 30 J ml<sup>-1</sup>.

Results of size fraction weights determined after application of 400 J ml $^{-1}$  by partners in the participating laboratories showed similar results with regard to variation and number of outliers (Figure C-3) as those after application of 30 J ml $^{-1}$ . Largest differences in standard deviations between the round-robin test and the intra-laboratory test were found for soil sample #1. Means of weights were also very similar with the exception of masses in particle size class 200 – 2000  $\mu$ m of soil sample #1 (intra-laboratory test: 0.81g (+/- 0.12g); round robin-test: 0.55 (+/- 0.14 g).

Less than 10% of determined values were found to be outliers according the two applied criteria, yet the IQR approach was more sensitive than the standard-deviation approach: Only 4 of the 54 of results were identified as outliers base on the +/-  $2\sigma$ -criteria; according to the IQR approach 6 out 54 values were classified as outliers.

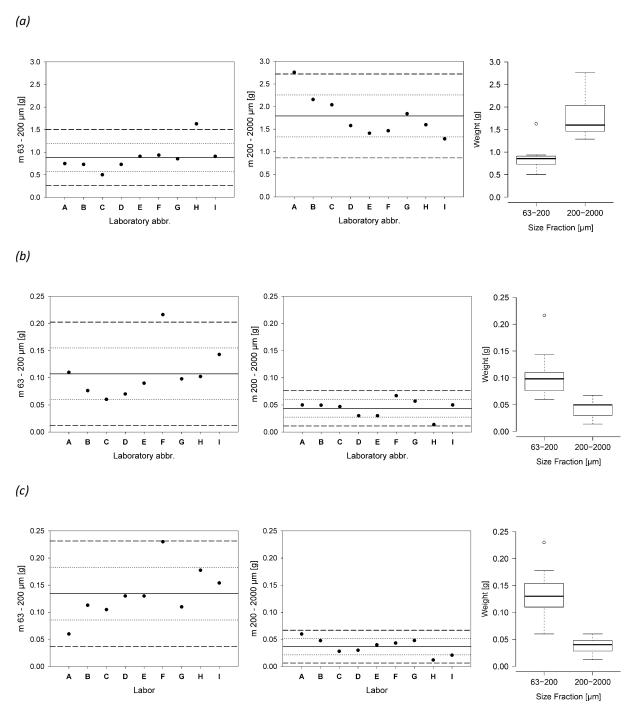


Figure C-2: Masses in particle size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  after application of  $30~J~ml^{-1}$  as obtained by the laboratories participating in the round robin test: soil sample #1 (a), soil sample #2 (b), soil sample #3 (c). Single values are given including criteria for determination of outliers based on standard deviation (solid line:  $\bar{x}$ ; dashed line: +/-  $2\sigma$ ; dotted line: +/-  $\sigma$ ). Box-whisker-plots provide range of IQR (size of the box), criteria for determination of outliers based on IQR (whiskers:  $\bar{x}+1.5\cdot IQR$  and  $\bar{x}-1.5\cdot IQR$ ), outliers detected according to this criteria (o) and median value (solid line).

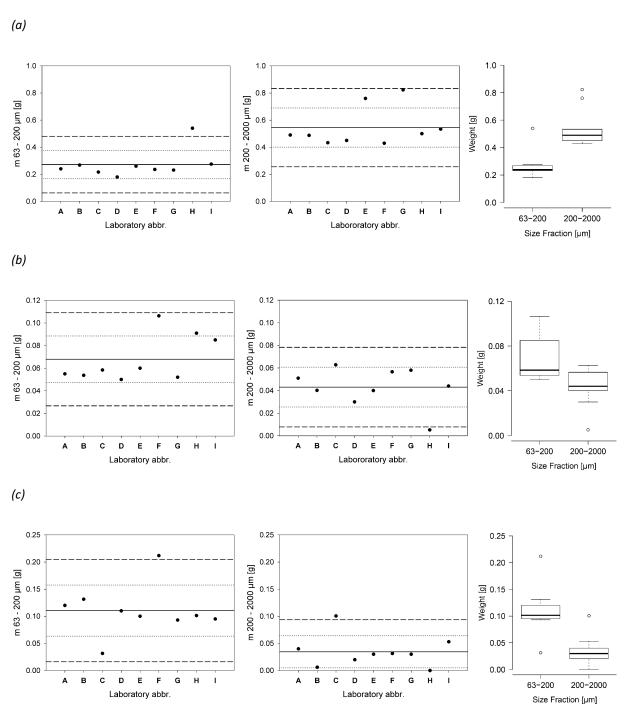


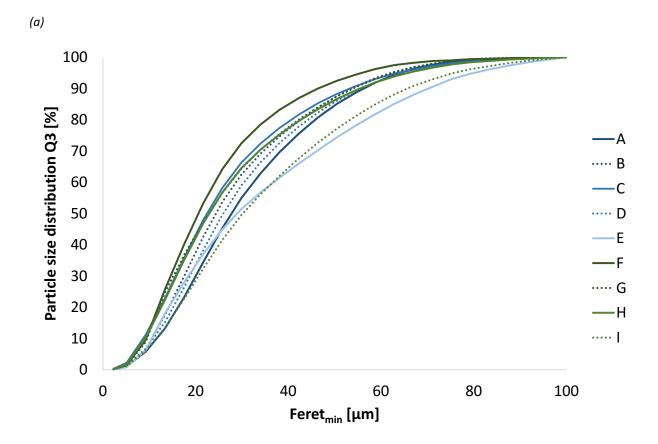
Figure C-3: Masses in particle size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  after application of  $400~J~ml^{-1}$  as obtained by the laboratories participating in the round robin test: soil sample #1 (a), soil sample #2 (b), soil sample #3 (c). Single values are given including criteria for determination of outliers based on standard deviation (solid line:  $\bar{x}$ ; dashed line: +/-  $2\sigma$ ; dotted line: +/-  $\sigma$ ). Box-whisker-plots provide range of IQR (size of the box), criteria for determination of outliers based on IQR (whiskers:  $\bar{x}+1.5 \cdot IQR$  and  $\bar{x}-1.5 \cdot IQR$ ), outliers detected according to this criteria (o) and median value (solid line).

Our results show that there is only a small bias within masses of particles in size fractions obtained by the participating laboratories. The low numbers of outliers detected and similar mean weights of size fractions obtained in intra-laboratory tests and round-robin test indicates that results of disaggregation experiments conducted by application of ultrasound is largely comparable despite different ultrasound devices or procedures. Means of coefficient of variation of results for masses in size fractions obtained by different laboratories was much higher ( $C_v = 38 \%$ ) than in intra-laboratory tests ( $C_v = 12 \%$ ) showing that variation can be relatively large.

Size distributions of particles < 63 µm (see Figure C-4, Figure C-5, and Figure C-6) determined in the round robin test showed that variation within that particle size class was smaller than for the size classes > 63 µm (see Table C-4). Largest variations were found for soil sample #1 (coefficients of variation: 9.8 – 14.0 %). Variations were much lower for soil samples #2 and #3 (coefficients of variation: 0.9 – 5.5 %). This observation may be explained by the higher stability of aggregates in soil #1, which make that soil sample most sensitive to possible differences in ultrasound treatment. Among parameters D<sub>10</sub>, D<sub>50</sub> and D<sub>90</sub> only one outlier was detected according to standard deviation (n = 162):  $D_{90}$  determined after dispersion with 30 J ml<sup>-1</sup> of soil #1 by laboratory  $\boldsymbol{E}$  was slightly above the limit using standard deviation ( $x_{D90,E} = 70.2 \,\mu\text{m} > 70.1 \,\mu\text{m}$ ). The application of the more sensitive outlier criteria (IQR) identified seven outliers out of 162 values. However, less than 3 % of all determined D<sub>10</sub>, D<sub>50</sub>, and D<sub>90</sub> values were identified as outliers indicating that bias found for size distribution of particle < 63  $\mu$ m is even smaller than for masses in particle size fraction > 63  $\mu$ m. Differences in disaggregation of fractions > 63  $\mu m$  do not always change the size distribution of particles < 63 µm. One possible explanation is that there is no preferential release of particles of a certain size upon disaggregation. Thus, differences in disaggregation efficiency reveal more homogenous changes in size distribution of particles < 63 µm. Anyway, when comparing size distributions of particles < 63 µm with masses of particle size classes > 63 µm parameters D<sub>10</sub>, D<sub>50</sub>, and D<sub>90</sub> do not provide information on the absolute masses in certain size classes. It is possible that these parameters are therefore less sensitive to possible differences.

Table C-4: Parameters  $D_{10}$ ,  $D_{50}$  and  $D_{90}$  describing size distribution of particles < 63  $\mu$ m after disaggregation with 30 J ml<sup>-1</sup> and 400 J ml<sup>-1</sup> of three reference soil samples (#1-#3) obtained by partners in the participating laboratories. Means of all results ( $\bar{x}$ ) as well as coefficient of variation ( $C_v$ ) and thresholds for determination of outliers using two criteria are listed. Outliers detected using one or two of the criteria are given in italic and bold numbers.

			Laborato	ory abbr.													
Soil ID	US Energy	Parameter	А	В	С	D	E	F	G	Н	1	$\underline{x}$	ð	$\overline{x} - 2\sigma$	$\overline{x} + 2\sigma$	$Q_1 - 1.5$ $\cdot IQR$	$Q_3 + 1.5$ $\cdot IQR$
	[J ml <sup>-1</sup> ]						- [μm]					[µm]	[%]	[µm]	[µm]	[µm]	[µm]
#1	30	D <sub>90</sub>	56.3	53.7	53.3	55.6	70.2 <sup>(+)</sup>	46.4 <sup>(-)</sup>	54.0	55.5	<i>65.7</i> <sup>(+)</sup>	56.7	11.8	43,4	70,1	49,8	60,2
		D <sub>50</sub>	27.8	24.4	22.5	26.0	29.0	20.6	22.9	23.0	30.2	25.1	12.3	19,0	31,3	15,5	35,2
		D <sub>10</sub>	11.6	10.5	8.7	10.8	10.6	9.3	9.5	9.1	11.4	10.2	9.8	8,2	12,2	7,1	13,0
	400	D <sub>90</sub>	53.0	41.8	35.9	45.3	46.3	44.1	37.5	55.6	51.9	45.7	14.1	32,8	58,6	26,5	67,1
		D <sub>50</sub>	23.9	16.9	16.2	16.7	18.9	18.2	15.5	20.9	21.0	18.7	14.0	13,4	23,9	10,4	27,1
		D <sub>10</sub>	9.2	7.0	6.3	6.2	8.5	7.9	7.3	8.1	7.9	7.6	12.3	5,7	9,5	5,5	9,6
#2	30	D <sub>90</sub>	38.1	37.9	34.6	37.5	35.5	35.4	34.9	38.0	35.7	36.4	3.7	33,7	39,1	31,8	41,6
		D <sub>50</sub>	18.5	18.8	16.9	18.7	17.3	17.4	16.6	19.2	18.5	18.0	4.9	16,2	19,8	15,3	20,8
		D <sub>10</sub>	8.1	8.8	7.6	8.3	7.9	7.9	7.7	8.3	7.2	8.0	5.4	7,1	8,8	6,8	9,2
	400	D <sub>90</sub>	37.2	36.9	34.6	37.1	36.0	36.8	34.6	36.2	37.2	36.3	2.7	34,3	38,3	34,3	38,8
		D <sub>50</sub>	18.0	18.4	17.3	19.8	17.6	18.3	17.1	19.0	20.3	18.4	5.5	16,4	20,5	15,5	21,0
		D <sub>10</sub>	7.3	7.3	<b>7.0</b> <sup>(-)</sup>	7.6 <sup>(+)</sup>	7.2	7.4	7.3	7.4	7.7 <sup>(+)</sup>	7.3	2.5	7,0	7,7	7,1	7,5
#3	30	D <sub>90</sub>	41.4	42.2	42.1	42.1	41.9	41.4	40.7	41.7	41.2	41.6	1.1	40,7	42,5	40,3	43,1
		D <sub>50</sub>	20.7	21.8	22.6	22.4	22.2	21.4	20.5	22.6	22.5	21.8	3.5	20,3	23,4	19,8	24,1
		D <sub>10</sub>	7.6	7.7	8.1	8.0	7.9	7.7	7.7	7.9	7.7	7.8	2.0	7,5	8,1	7,3	8,3
	400	D <sub>90</sub>	42.8	43.7	43.5	43.2	43.2	43.3	42.4	42.7	43.1	43.1	0.9	42,3	43,9	41,1	44,0
		D <sub>50</sub>	24.4 <sup>(-)</sup>	25.5	25.2	25.7	25.4	25.6	24.8	25.2	25.4	25.2	1.6	24,4	26,0	24,7	26,0
		D <sub>10</sub>	9.7	10.3	10.0	10.8	10.2	10.7	9.9	10.4	10.1	10.2	3.2	9,6	10,9	9,5	10,9



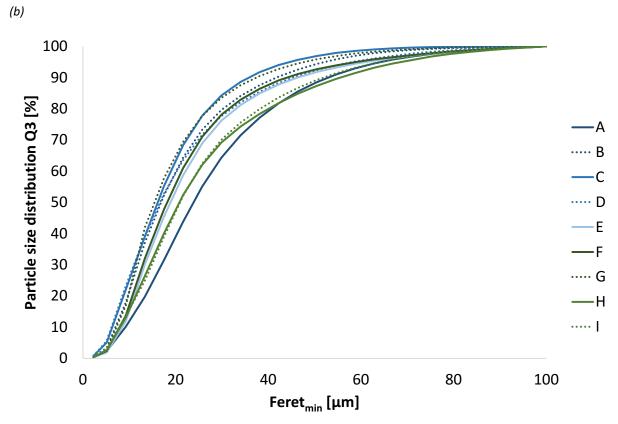


Figure C-4: Size distribution of particles < 63  $\mu$ m determined on soil sample #1 after disaggregation with 30 J ml<sup>-1</sup> (a) and 400 J ml<sup>-1</sup> (b) by laboratories (**A** to **I**) participating in the round robin test.

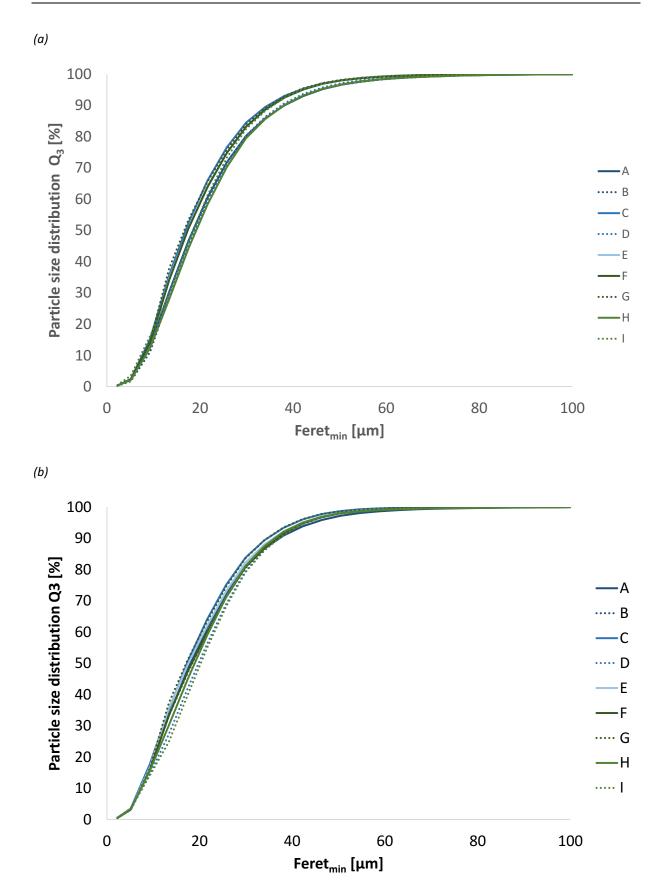
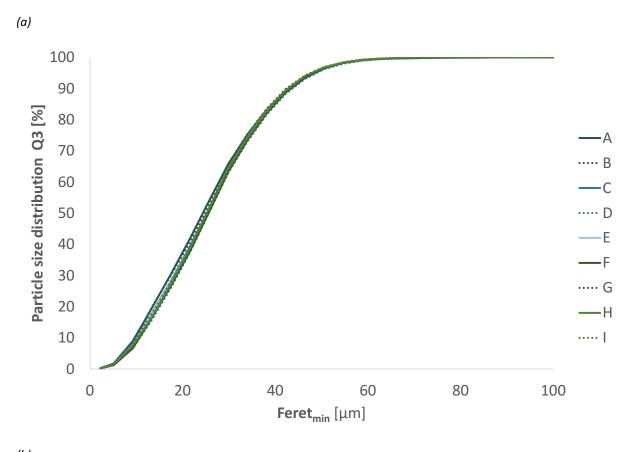


Figure C-5: Size distribution of particles < 63  $\mu$ m determined on soil sample #2 after disaggregation with 30 J ml<sup>-1</sup> (a) and 400 J ml<sup>-1</sup> (b) by laboratories (**A** to **I**) participating in the round robin test.



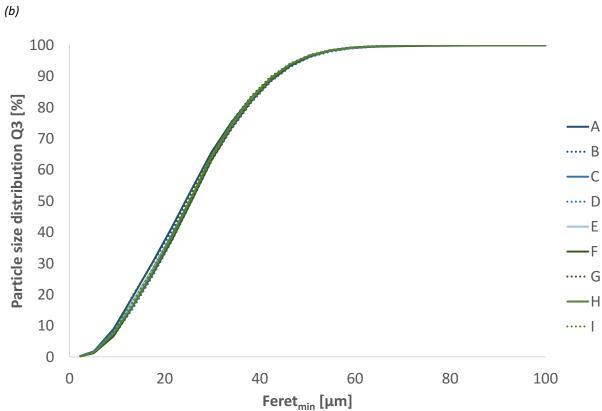


Figure C-6: Size distribution of particles < 63  $\mu$ m determined on soil sample #3 after disaggregation with 30 J ml<sup>-1</sup> (a) and 400 J ml<sup>-1</sup> (b) by laboratories (**A** to **I**) participating in the round robin test.

# C.4.3 Do ultrasonic device properties and applied procedures influence disaggregation efficiency?

Even though the total number of outliers was low, it differed with respect to the number of outliers gained in single laboratories. Table C-2 provides an overview on device properties and procedures applied for ultrasonic dispersion by the partners in the nine participating laboratories (*A* to *I*) (results from questionnaire and data obtained from manufacturers of devices). The ultrasonic devices used in the round robin test were by different manufactures and a single model was not used in more than one lab. Consequently, parameters such as diameter of the sonotrode (10 to 14 mm) or amplitude (68.4 to 131.2 μm) differ between laboratories. The time sonotrodes (which need to be replaced regularly because cavitation also damages the sonotrode itself) were used before experiments for round robin test varied highly. Two laboratories (*C* and *H*) used devices oscillating in a frequency different from 20 kHz (23 and 30 kHz, respectively). The ultrasonic power (and output control needed to obtain this power) used for dispersion was in the range between 16 and 73 W. One laboratory (*H*) did not achieve the demanded ultrasonic power (> 45 W) with the device used. Nonetheless, we decided to include the results obtained by this laboratory into the round robin test to see if the expected influence of low ultrasonic power on dispersion is detectable.

The results showed that applied procedures for disaggregation experiments also differ among the participating laboratories. Immersion depth of the sonotrode was 15 mm in most cases, laboratories C and D immerse the sonotrode 20 and 10 mm into the suspension, respectively. Containers differing in volume, shape and material were used for disaggregation in participating laboratories. Laboratory F applied the energy for dispersion using different ultrasonic powers whereas all the other labs carried out dispersion with 30 J ml<sup>-1</sup> and 400 J ml<sup>-1</sup> with the same ultrasonic power. None of the participating laboratories used degassed water for the sample suspension, two participants (C and D) did not cool the suspension during application of ultrasound.

Comparing used ultrasound devices and applied experiment protocols of laboratories with two or less outliers (no outlier: B; one outlier: D and G; two outliers: A and I) similarities were found. The ultrasonic devices used by these laboratories have the same oscillation frequency (20 kHz), sonotrode diameter (13 mm) and used the same immersion depth (15 mm). In contrast, the laboratories with more than two outliers differ with regard to these parameters. Laboratory H (total of six outliers found) applied the energy with low ultrasonic power which was already expected to influence disaggregation efficiency (Pöplau and Don, 2014). In addition, a sonotrode with a diameter of 10 mm and oscillating with a frequency of 30 kHz is used in that laboratory. The device used in laboratory C (three outliers detected) differed in oscillation frequency (24 kHz) from the devices used by the laboratories with two or less outliers. In contrast, the ultrasonic device used by laboratory F (six outliers detected) is comparable to low-outlier laboratories. Yet, the applied procedure of

laboratory **F** differs from all other laboratories because different ultrasonic powers were used for applying the two predefined energies. Both ultrasonic powers were higher than the demanded threshold of 45 W and there is no obvious explanation how this different procedure effects disaggregation efficiency and causes outliers in particle masses of size fractions. Similar to that, no obvious difference in device or disaggregation procedure applied in laboratory **E** (three outliers detected) is obvious.

Summarizing the presented results, the influence of differences in device properties or applied disaggregation procedure was very little. The slightly larger number of outliers detected within the results obtained in laboratories which used ultrasonic devices or procedures differing with regard to oscillation frequency and sonotrode diameter from laboratories with less than two outliers may indicate that an influence of these parameters on disaggregation efficiency cannot be ruled out.

# C.5 Conclusion

The presented study indicates that bias for disaggregation efficiency due to different ultrasonic devices and procedures applied is little. Only few outliers were identified for masses of particle size fractions  $63-200~\mu m$  and  $200-2000~\mu m$  and even less for size distribution of particles <  $63~\mu m$ . We were able to show that results of disaggregation experiments obtained in different laboratories are comparable to a large extent, despite different devices and varying procedures. However, the determined higher variation of results has implications for identification of differences in disaggregation results obtained when using different devices or procedures. Two factors were identified, which may impact disaggregation efficiency in addition to applied ultrasonic energy and power: oscillation frequency and sonotrode diameter. We suggest conducting further studies to quantify the influence of these properties on disaggregation results or simply standardizing parameters.

Unfortunately, a lack of reference material with a defined grade of aggregation and stability of aggregates does not allow any assumption about the true disaggregation results. Only the fact that devices using the same oscillation frequency and sonotrode diameter disaggregate soil samples to same extent does indicate that results obtained by using these machines are closer to the true values compared to results gained by using ultrasonic devices with other properties. Future research need to be carried by performing comparative experiments while varying a suspicious parameter and keeping the other conditions constant. Regardless of this, detailed description of the ultrasound device used for disaggregation and the procedure seems mandatory for ensuring comparability of disaggregation methods and obtained results. As a first step, reference material used for the presented study is available for repeating the described experiments with other ultrasonic devices or disaggregation procedures and can be requested from the corresponding author.

# C.6 Acknowledgments

We would like to thank the staff of all participating institutions for the collaboration.

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D Soil formation and its implications for the stabilization of soil organic matter in the riparian zone

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Published in:

Catena (2016), Vol. 139, 9 – 18.

DOI: 10.1016/j.catena.2015.11.010

http://www.sciencedirect.com/science/article/pii/S0341816215301594

## D.1 Abstract

Riparian woodlands consist of different landscape units characterized by different hydroecomorphological site conditions that are reflected in the distribution of soils and tree species. These conditions are determined by flooding frequency and duration, distance to river channels, elevation and water flow velocity. The influence of these environmental drivers on the stabilization of soil organic matter (SOM) has as yet not been investigated. Hence, the aim of our study is to link soil formation and its drivers with stabilizing processes of SOM in riparian floodplain forests. We investigated soils and sediments at two sites in the ash-maple-elm-oak alluvial forest zone (AMEO sites) and two sites in the willow-poplar alluvial forest zone (WiP sites) within the riparian zone of the Danube near Vienna (Austria). Sediments and soils were characterized based on texture, contents of organic carbon (OC), nitrogen, Fe oxides, and soil pH. Density fractionation was used to separate OC fractions in terms of stabilization process and resulting OM turnover time: the free light fraction (fast turnover), the light fraction occluded in aggregates (intermediate turnover) and the heavy fraction of OM associated tightly to mineral surfaces (slow turnover).

At both sites, soil and sediment properties reflect the hydroecomorphological site conditions for formation of the landscape units in the riparian zone: Soils at AMEO sites develop during constant deposition of fine-textured sediment while water flow velocity is low. Progressing soil development causes a continuous decrease in OC content with increasing soil depth, mainly from fractions with fast and intermediate turnover. As a consequence, the heavy fraction clearly dominates with around 90% of OC. Temporally variable flooding conditions with occurring turbulences found at WiP sites result in a discontinuous change of soil properties with increasing soil depth. Former topsoil horizons buried by huge amounts of sediments seem to keep the OC fractionation typical for topsoil horizons with extraordinarily high amounts of light fraction OM (free and occluded) representing 20 – 40% of total OC. The presented results confirm that sedimentation and soil formation are simultaneous processes at AMEO sites. At WiP sites both processes seem uncoupled with alternate phases of sedimentation and soil formation. Thus, the frequent burial of topsoil material formed at WiP sites seems to enable the conservation of unstable organic matter fractions at this part of active floodplains.

# D.2 Introduction

Soil formation in the riparian zone is determined by the delivery and erosion of sediments as well as alternating redox conditions in periodically inundated soils (*Naiman and Décamps, 1997; Bai et al., 2005; Rinklebe et al., 2007*). It is widely acknowledged that mineral floodplain soils contain huge stocks of organic carbon (OC) (*Batjes, 1996; Zehetner et al., 2009; Cierjacks et al., 2010; Ricker et al., 2013*) for two reasons (*Rinklebe et al., 2001*): (1) sediment entering the floodplain may contain significant amounts of allochthonous organic matter from terrestrial and riverine sources (*Pinay et al., 1992; Cabezas and Comín, 2010*), and (2) floodplain ecosystems are often characterized by high net primary production (*Tockner and Stanford, 2002*), which provides the soil with large amounts autochthonous organic matter. The resulting amount of OC stocks underline the significance of floodplain soils in the regional and global carbon cycle, and the need to understand the dynamics of soil organic matter (SOM) in these ecosystems (*Mitra et al., 2005; Rieger et al., 2014*).

Accretion and stocks of OC seem to respond to different drivers in riparian forests as input of OC is related to sediment quantity whereas long-term OC stocks rely on stabilization processes (*Rieger et al., 2014*). Consequently, the OM dynamics in floodplain soils are not only a matter of the input of organic matter but also stabilization of SOM against mineralization (*Bernoux et al., 2006; Bernal and Mitsch, 2008*). However, few publications (*e.g. Zehetner et al., 2009*) consider stabilization processes of SOM in riparian ecosystems. Quantification of processes of SOM stabilization in different landscape units formed by differences in hydroecomorphological drivers of soil formation are yet not existent.

Hydroecomorphological drivers. According to Naiman and Décamps (1997) riparian zones are the most diverse and dynamic biophysical habitats on earth. A complex interplay of flooding, geomorphology and vegetation leads to various landscape units with specific characteristics in the riparian zone (Harris, 1987; Clerici et al., 2011; Gurnell, 2014). Following a conceptual model introduced by Piégay and Schumm (2003), the river hydrosystem can be divided into three main spatial gradients (lateral, vertical and longitudinal). In this model, the main drivers for differences along the gradients are water flow velocity, sediment load and inundation time and frequency (Bendix and Hupp, 2000; Busse and Gunkel, 2001; Busse and Gunkel, 2002; Bornette et al. 2008, Du Laing 2009). Along these gradients, sediment changes with regard to quality and yield (Asselmann and Middelkoop, 1995; He and Willing, 1998; Lindbo and Richardson, 2001): Areas close to the main river channel and low altitude above sea and mean water level receive high amounts of coarse sediments due to increased water flow velocity during flooding. This implies the formation of sandy soils with a high number of distinguishable soil horizons caused by different sedimentation events (Cierjacks et al., 2011). High frequency of flooding (Rinklebe et al., 2007) may further induce the formation of redoximorphic features in these soils. In areas with higher elevation distant to the main

river channel, decreased water flow velocity during flooding results in the sedimentation of fine material and in the formation of loamy soils.

Furthermore, tree species composition of alluvial forests shows pronounced differences among these landscape units, which are results of diverging demands of tree species on sediment and soil quality and plant-specific sensitivity against inundation (*Rieger et al., 2013*). Hence, tree species distribution may be used as an indicator to identify different landscape units (*Wisskirchen, 1995; Cierjacks et al., 2010; Suchenwirth et al., 2012*): tree species with high tolerance against inundation and wood with a low specific density and high flexibility such as *Salix* spp. and *Populus* spp. form the willow-poplar zone (WiP zone) that prevails along the main river channel and side branches at low-lying sites. In contrast, species that are more sensitive against inundation and have a higher wood density (*Acer spp., Fraxinus excelsior, Ulmus spp., Quercus robur*) grow more distant to the main channel or side branches at high-lying sites and form the ash-maple-elm-oak zone (AMEO zone).

These differences in sources and quality of autochthonous OM, soil forming factors and sediment or soil properties along the lateral and vertical gradient are known to influence OM dynamics in riparian forests (*Rinklebe et al., 2001; Cierjacks et al., 2011; Rieger et al., 2013, 2014*). However, it remains an open question how such differences are also related to stabilization processes of SOM.

Stabilization of SOM. In general, stabilization of SOM is governed by three different mechanisms: chemical stabilization by adsorption of SOM to mineral surfaces, spatial inaccessibility of SOM against mineralizing microorganisms by occlusion into soil aggregates, encapsulation within a macromolecule matrix or hydrophobic environments and preservation due to chemical recalcitrance of SOM or its compounds (Christensen, 1996; Sollins et al., 1996; Knicker and Hatcher, 1997; von Lützow et al., 2006; Jastrow et al., 2007). Recent findings indicate stabilization of SOM is a result of physicochemical and biological conditions in the surrounding (micro and macro) environment that reduce the probability of SOM decomposition (Schmidt et al., 2011). In particular, the pivotal role of soil structure and aggregation for SOM stabilization is underlined through previous results on the relevance of physical SOM stabilization due to spatial inaccessibility (Sollins et al., 1996). There is also scientific evidence that similar processes may be found in young soils in the riparian zone (Guenat et al., 1999; Bullinger-Weber et al., 2007).

Several pools of SOM with different levels of resistance against mineralization can be determined using various fractionation approaches based upon physical or chemical properties of SOM (von Lützow et al., 2007). One of these approaches is density fractionation of SOM, which is suitable to differentiate particulate OM (POM) with low density and soil constituents with higher densities such as soil minerals with OM associated to their surfaces (Golchin et al., 1994; Gregorich and Beare, 2008). The light fraction is present in soil in two states (Christensen, 1992) and can be attributed to either the fast turnover pool if it is not or only loosely attached to other soil constituents (Baisden et

al., 2002; Poirier et al., 2005; John et al., 2005) or the intermediate pool with a wide range of turnover rates if it is occluded in soil aggregates (Jastrow et al., 2007; von Lützow et al., 2007). The OM stabilized on mineral surfaces contributes mainly to the slow turnover pool of SOM.

Hypotheses. Mechanistic links between the formation of soils in different landscape units within riparian floodplains and the development of the different pools of organic matter are hardly considered. Thus, the overall aim of our study is to quantify processes of SOM stabilization in different landscape units in the context of environmental drivers of soil formation in the riparian zone of the Danube. Yet, by combining both our knowledge on floodplain soil formation and stabilization of SOM, hypotheses on the distribution of organic matter in different pools in riparian zones can be deduced with aggregate formation representing one of the most important links between soil formation and SOM stabilization (Guenat et al., 1999; Bullinger-Weber et al., 2007). In particular, we assessed the following hypotheses: (1) Sediment delivered to the floodplain consists of unstructured parent material and flocs of particles that are formed in-situ during the transport in the river (Nicholas and Walling, 1996; Droppo, 2001). Soil-borne aggregates derived from eroded top soils undergo disaggregation during transport in the river (Woodward and Walling, 2007; Grangeon et al., 2014). As a result of this separation of formerly occluded POM, OM in sediments is mainly present as free POM and OM associated to mineral surfaces. (2) Soil structure formation is among the most relevant soil forming processes in floodplains (Guenat et al., 1999; Bullinger-Weber et al., 2007). Accordingly, an increasing amount of OM is stabilized in soil aggregates with increasing soil age (Jastrow, 1996). It is known that aggregates built of coarse material are less stable than aggregates consisting of fine material (Kaiser et al., 2012). Consequently, aggregation and OM stabilization by occlusion is of lower relevance in coarse soils at WiP sites compared to fine soils at AMEO sites. (3) Stabilization of organic matter on mineral surfaces most likely depends on the available surface area, which is negatively correlated with particle size. Consequently, there will be a higher amount of OM stabilized on mineral surfaces in fine soils in the AMEO forest zone compared to coarse soils in the WiP forest zone. (4) The continuous delivery of sediments implies that sub soil material has undergone longer time of soil development compared to top soil material at the same site (Lair et al., 2009a). The activity of meso- and macrofauna is decreased in subsoil compared with material in top soil (Fontaine et al., 2007). In combination with a lack of delivery of fresh POM to subsoil, we hypothesize that the amount of OM in free or occluded states decreases with increasing soil depth. The content of OM associated to mineral surfaces is only slightly affected by progressing soil development and does not decrease with increasing soil depth because of the long turnover time of OC in this fraction.

Our study is based on the characterization of soils at four riparian sites with natural inundation regimes in two main alluvial forest types distinguished by indicator tree species: two sites at low

elevation close to the main river channel (WiP sites), and another two sites more distant to the main river channel at higher elevation (AMEO sites). In addition to soils, freshly deposited sediments were sampled at the four sites. Sediments can be regarded as the parent material for soil development and allow us to investigate site dependent soil forming processes.

## D.3 Material and methods

## D.3.1 Soil and sediment sampling

Soils were sampled at four different sites situated within the active floodplain of the Danube in the 'Nationalpark Donau-Auen' (east of Vienna, Austria). The study area is one of last remaining floodplains of the Danube in central Europe that is still subject to fluvial dynamics and shows extensive riparian forest vegetation. However, *Lair et al.* (2009b) figured out that the flooding regime in this area is far away from being called "natural" or undisturbed due to human interferences in the river catchment. Nevertheless, since the establishment of the national park in 1996, human activities have been minimized (*Bundesgesetzblatt, 1997*) and the riparian ecosystem has developed almost without further disturbance. The International Union for Conservation of Nature (IUCN) classified the national park as a Riverine Wetlands National Park, Category II.

The continental climate of the park has a mean annual temperature of 9.8 °C and a mean annual precipitation of 533 mm (closest climate station Schwechat, 48°7′ N; 16°34 E, ASL: 184 müA, *Zentralanstalt für Meteorologie und Geodynamik, 2002*). According to Lair et al. (2009a) the water discharge of the Danube varies between 850 and 5000 m³ s<sup>-1</sup> (1% and 99%-quantiles of mean discharge) within the area of the national park.

The chosen sampling sites represent the main landscape units with WiP and AMEO forest types indicating different soil properties (see Table D-1). They represent extremes of a gradient in hydroecomorphological site conditions which was developed by *Cierjacks et al. (2010, 2011)* for the same area using a survey of 76 sites in an area of roughly 1310 ha. According to the conceptual model for the formation of these habitats, alluvial forest zones dominated by willow and poplar are associated with coarsely textured soils and low OC contents (WiP sites) whereas zones with ash, maple, elm and oak as the dominant tree species are predominantly found on loamy textured soils with higher OC contents (AMEO sites, *Cierjacks et al., 2010*). Consequently, sites WiP 1 and WiP 2 are situated much closer to main river channel and to side branches at lower elevation than sites AMEO 1 and AMEO 2.

Soils were sampled by soil horizon according to *Ad-hoc Arbeitsgruppe Boden (2005)* in autumn 2008. All samples were air-dried (max. 30°C) after sampling. Metal soil cylinders with a diameter of 5.5 cm and a height of 4 cm were used for sampling soil with a defined volume for the determination of bulk density (BD). Bulk density was calculated by dividing dry mass of the soil by the known volume of the cylinders.

Artificial grass mats (area 40·60 cm², Astro-Turf ©) were used as sediment traps to gain sediment material deposited during the year after soil sampling according to *Steiger et al. (2003)*.

Table D-1: Overview sampling sites

Site name (according to alluvial forest zonation)	Coordinates in grid zone 33 U (UTM)	Soil classification (IUSS Working Group WRB, 2014)	Distance to main river channel [m]	Altitude above sea level (above mean water level) [m]	Dominant tree species
WiP-1	624186 m E 5331664 m N	Calcaric Fluvisol (episiltic)	100	151 (6)	Populus alba, Populus × canadensis
WiP-2	622458 m E 5332156 m N	Calcaric Fluvisol (endoloamic, episiltic)	104	154 (10)	Salix alba
AMEO-1	632539 m E 5332623 m N	Calcaric Fluvisol (siltic)	1150	164 (20)	Ulmus spec., Fraxinus excelsior, Quercus robur
AMEO-2	626614 m E 5332180 m N	Calcaric Fluvisol (siltic)	1000	166 (22)	Acer campestre, Fraxinus excelsior

At each site, two sediment traps were affixed to the soil surface using 15 cm steel nails with a maximum distance of 10 m to the soil pit at the same elevation. After one year of exposure, the sediment traps were collected. The sediment gained was regarded as the parent material for soil formation and had not undergone any soil forming process at the sites.

We were not able to recover one trap at site AMEO-1 and both traps at site WiP-2. With the low amount of material on the remaining trap at site AMEO-1 only a limited number of analyses could be carried out. For chemical characterization of sediment at site WiP-2, we sampled sediment material by hand using a trowel from a thin layer (0.5 cm) of the soil surface directly after a sedimentation event. We sampled only material that was obviously freshly deposited and showed no plant succession. Due to trap loss at WiP-2, we could still determine various sediment properties for this site but not the rate of sedimentation.

# D.3.2 Soil and sediment analyses

Bulk soil pH was measured following the procedure given in *DIN 19684 (1997)* in deionized water (EC  $< 0.06 \ \mu S \ cm^{-1}$ ) with a soil:solution ratio of 1:2.5.

Carbon and nitrogen contents of soils and sediments were determined using an elemental analyzer (Vario EL III Elemental Analyzer, Elementar ®). Carbon content (Ctot) and nitrogen (Ntot) were determined in milled samples dried at 105°C. Aliquots of milled samples were combusted at 550°C to remove organic carbon. We assume that the carbon content of samples combusted at 550°C represents exclusively inorganic carbon (IC) in the form of calcite, which is chemically stable up to this temperature (Bisutti et al., 2004). Consequently, organic carbon (OC) content can be calculated as the difference C<sub>tot</sub> – IC. Bulk densities and horizon thicknesses were taken into account when calculating OC stocks of single soil horizons. If thickness of soil horizon was below 5.5 cm (diameter of metal soil ring), the mean BD of the adjacent soil horizons above and below was used for calculation of OC stocks. The OC stocks for full soil profiles were calculated to a depth of 97 cm (which was the shallowest investigated depth of all analyzed soil profile) as a sum of OC stocks in each horizon. Soil texture was measured following the method described in DIN/ISO 11277 (2002) by sieving (particle diameter > 63  $\mu$ m) and sedimentation (< 63  $\mu$ m). Iron bound to non- or poorly crystalline oxides and hydroxides was extracted using ammonium oxalate solution according to the procedure given by Schwertmann (1964). Following Mehra and Jackson (1960), dithionite-carbonate-citrate solution (DCB) was used to extract FedCB from non-crystalline and crystalline oxides and hydroxides. Iron concentrations in both solutions were determined using an atomic absorption spectrophotometer (Perkin Elmer) to calculate soil content of Feox (oxalate extractable Fe) and FeDCB (Fe extractable with dithionite-citrate-bicarbonate solution).

# D.3.3 Density fractionation of organic matter

Density fractionation of organic matter is suitable to gain fractions of SOM contributing to OC pools characterized by degree of resistance against mineralization. The method we used was conducted following a slightly modified procedure according to *Golchin et al. (1994), Grünwald et al. (2006) and Don et al. (2009)*. We used sodium polytungstate (SPT, Na<sub>6</sub>[H<sub>2</sub>W<sub>12</sub>O<sub>40</sub>]) solution (TC Tungsten Compounds, Grub am Forst/Germany) with a density ( $\rho$ ) of 1.6 g cm<sup>-3</sup> (+/- 0.02 g cm<sup>-3</sup>) to separate the light fraction (LF) with low density ( $\rho$ <sub>LF</sub> < 1.6 g cm<sup>-3</sup>) from solids with higher densities. Sodium polytungstate solution is the preferred high-density solution because it is less toxic compared to alternative solutions (*Plewinsky and Kamps, 1984; Christensen, 1992*) and a density of 1.6 g cm<sup>-3</sup> can be easily achieved (*Six et al., 1999*). The chosen density of 1.6 g cm<sup>-3</sup> is suitable for separation of light

fractions from minerals with higher densities (heavy fractions, HF), even if these contain up to 20% OM (*Christensen, 1992; Golchin et al., 1994; Cerli et al., 2012*).

25 g of air-dried and sieved (< 2 mm) soil or sediment material were placed in 200 ml centrifugation vessels along with 100 ml of SPT solution. The vessel containing the soil-SPT suspension was swayed gently to achieve complete mixing with minimal input of mechanical forces to avoid disintegration of water-stable aggregates. Finally, another 25 ml of SPT solution were added to wash residual solid particles off the vessel walls into the suspension and to adjust a solid-solution ratio of 1:5. After 1 h, the suspension was centrifuged at 2600 RCF x g for 26 minutes to separate the free, light fraction (f-LF,  $\rho_{f-LF}$  < 1.6 g cm<sup>-3</sup>) from solids with  $\rho$  > 1.6 g cm<sup>-3</sup>. These denser solids consist of loose mineral particles and mineral-organic particle associations forming soil aggregates. Soil aggregates contain occluded LF (o-LF,  $\rho_{o-LF}$  < 1.6 g cm<sup>-3</sup>) but the whole aggregate body shows a higher density than 1.6 g cm<sup>-3</sup> due to a larger portion of mineral constituents compared to o-LF. According to Stokes' law (which assumes ball-shaped particles), the mode of centrifugation used assures the sedimentation of particles with  $\rho > 1.6$  g cm<sup>-3</sup> and a diameter > 0.78  $\mu$ m, and turned out to be suitable for satisfying separation in preliminary experiments (data not shown). The floating f-LF material was aspirated from the suspension surface together with most of the SPT solution, while denser material ( $\rho$  > 1.6 g cm<sup>-3</sup>) remained in the centrifugation vessel. Gained f-LF material was filtered on C-free glass fiber filters (particle retention  $> 1.5 \mu m$ ), and the SPT solution was reused for further fractionation steps of the same samples. The f-LF on the filter was washed thoroughly using deionized water until the remaining SPT was removed (conductivity of filtrate < 50 µS cm<sup>-1</sup>). The SPT-free f-LF was rinsed from the filter into PE bottles, frozen and dried using lyophilization. Finally, dry masses of washed f-LF were determined. Organic carbon and N content were measured in milled material using an elemental analyzer (Vario EL III Elemental Analyzer, Elementar ®). If sample mass was too low for milling (< 20 µg), the entire sample was analyzed for OC and N. The measured total carbon content of f-LF represents OC content of the samples because carbonates are not present in this fraction (Kreyling et al., 2013) due to a density of carbonate minerals greater than 1.6 g cm<sup>-3</sup>. The used SPT solution was added to the remaining solids (after checking the density of the solution using an aerometer) in the centrifugation vessel. Before separation of o-LF material (ρ<sub>o-LF</sub> < 1.6 g cm<sup>-3</sup>), soil aggregates were disrupted by mechanical forces occurring in a cavitation field generated by a titanium probe vibrating with ultrasound frequency in the suspension. A "Branson W-250 Sonifier" (© Branson Ultrasonics Cooperation, Danbury CT/USA) with a cylindrical probe ( $\emptyset = 13$  mm) was used for sonication. The vibration frequency was 20 kHz with a peak-to-peak amplitude of approximately 100  $\mu$ m (output control set '7'). The output power was determined calorimetrically with 54.0 W (+/- 1.2 W) (North et al., 1976) and is well above the threshold for reproducibility of ultrasonic dispersion recommended by *Poeplau and Don (2014)*.

We applied a total energy of 400 J ml $^{-1}$  to the suspension. All material with  $\rho$  < 1.6 g cm $^{-3}$  released from disrupted aggregates under these conditions were classified as o LF. After sonication, o LF was separated from the remaining material with  $\rho$  > 1.6 g cm $^{-3}$ , filtered, washed, dried and measured following the same procedure as described above for f LF processing. To remove SPT solution from the remaining HF material ( $\rho_{HF}$  > 1.6 g cm $^{-3}$ ) the liquid was aspirated and the remaining HF material was washed with deionized water until electric conductivity comparable to soil (< 200  $\mu$ S cm $^{-1}$ ) was measured in the suspension. The HF material was ground and Ctot and Ntot were determined using an elemental analyzer (Vario EL III Elemental Analyzer, Elementar  $^{\circ}$ ). Organic carbon content was calculated following the procedure described for bulk soils.

# D.4 Results and discussion

D.4.1 Soil and sediment properties in different landscape units within the riparian zone

The sampled soil profiles were all classified as Calcaric Fluvisol (see Table D-1) according to *IUSS*Working Group WRB (2014). An overview of properties of developed soils and fresh sediment lying above these soils is given in Table D-2.

The sedimentation rates during our sampling period (one year) at the study sites differed profoundly: At site AMEO 1, where only one out of two installed sediment traps was recovered, the sedimentation rate was 0.3 kg m<sup>-2</sup> a<sup>-1</sup> whereas it was found to be ten times higher at site AMEO 2 (2.6 and 3.9 kg m<sup>-2</sup> a<sup>-1</sup>, respectively). At the WiP sites two extremes were observed: for site WiP 1 the sedimentation rate was higher by orders of magnitude (88.9 and 67.9 kg m<sup>-2</sup> a<sup>-1</sup>, respectively) than the lowest sedimentation rate determined at AMEO sites. In contrast, flooding washed away sediment traps at site WiP 2 due to strong erosion. All sampled soils and sediments showed slightly alkaline pH values (range 7.19 – 8.60) and were rich in IC due to the calcareous parent material in the river catchment. No strong differences in pH or IC content could be observed between WiP and AMEO sites. The OC contents of sediments delivered to the sites WiP 1 and WiP 2 were lower than those delivered to the AMEO sites. OC content and bulk density (BD) of soil samples changed continuously with increasing soil depth at sites AMEO 1 and AMEO 2. Comparable overall trends were determined in soils at site WiP 1 and WiP 2, but changes were rather discontinuous. The largest OC stock of sampled profiles calculated as the sum of OC stocks in each horizon was found at AMEO 1 (183 t ha<sup>-1</sup>). In contrast, AMEO 2 had lower stocks (126 t ha<sup>-1</sup>) comparable to the sites WiP 1 and WiP 2 (124 t ha<sup>-1</sup> and 122 t ha<sup>-1</sup>, respectively).

Table D-2: Chemical characteristics of soil horizons and fresh sediments lying above the soil and soil bulk densities at the study sites (contents of OC and IC are given in percent of dry mass of soil or sediment).

Site	Sediment/	Bulk	pН	ОС	ОС	IC	$N_{tot}$	OC/N <sub>tot</sub>	Feox	Fe <sub>DCB</sub>	Fe <sub>ox</sub> /
	Soil depth [cm]	density [g cm <sup>-3</sup> ]	(H₂O)	content [%]	stock [t ha <sup>-1</sup> ]	content [%]	[g kg <sup>-1</sup> ]		[g kg <sup>-1</sup> ]	[g kg <sup>-1</sup> ]	Fe <sub>DCB</sub>
WiP-1	Sediment 1	-	8.1	0.57	-	3.27	0.64	9	1.36	2.91	0.47
	Sediment 2	-	8.0	0.66	-	2.64	0.77	9	1.72	3.63	0.47
	0 – 8	0.80	7.8	2.52	16.2	3.27	2.10	12	2.38	4.33	0.55
	8 – 37	1.11	8.0	0.92	29.5	2.64	0.60	15	2.48	3.40	0.73
	37 – 41	n.d.	7.9	3.84	n.d.	2.48	0.99	14	3.01	4.67	0.64
	41 – 54	1.16	8.0	0.45	6.7	2.84	0.76	6	2.24	3.52	0.64
	54 – 65	1.04	7.9	1.58	18.1	2.89	1.54	10	2.85	4.35	0.65
	65 – 71	1.25	8.0	0.79	5.9	2.74	0.81	10	2.56	4.35	0.59
	71 – 92	1.23	7.9	1.43	36.9	3.01	1.38	10	3.06	4.73	0.65
	92 – 98	1.27	8.1	0.66	5.0	3.05	0.64	10	1.85	2.89	0.64
WiP-2	Sediment 1	-	7.7	1.38	-	2.59	0.99	14	1.93	3.91	0.49
	Sediment 2	-	8.1	0.89	-	2.72	0.66	13	1.82	3.80	0.48
	0 – 4	n.d.	7.4	1.58	n.d.	3.17	1.40	11	2.29	5.07	0.45
	4 – 16	1.07	7.2	2.42	30.9	3.28	1.97	12	1.96	4.82	0.41
	16 – 28	1.30	7.3	0.83	12.9	2.55	0.72	11	1.81	3.86	0.47
	28 – 33	1.10	7.3	1.03	5.7	2.79	1.01	10	2.17	5.08	0.43
	33 – 39	1.23	7.4	0.50	3.7	2.57	0.43	12	1.67	3.62	0.46
	39 – 44	1.09	7.4	1.07	5.9	2.74	0.90	12	1.63	3.15	0.51
	44 – 64	1.16	7.4	1.02	23.7	2.57	0.84	12	2.05	4.34	0.47
	64 – 66	n.d.	7.4	1.87	n.d.	2.75	1.51	12	2.07	4.85	0.43
	66 – 87	1.10	7.6	0.70	16.1	2.88	0.58	12	1.68	4.77	0.35
	87 – 110	1.09	7.6	1.10	27.5	2.51	0.92	12	1.92	5.04	0.38

AMEO-1	Sediment 1	-	7.5	6.92	-	3.38	5.35	13	n.d.	n.d.	n.d.
	0 – 14	0.81	7.6	4.68	53.3	3.16	5.33	9	2.28	5.86	0.39
	14 – 30	1.06	7.9	2.23	37.6	3.41	2.79	8	2.35	6.19	0.38
	30 – 48	1.25	8.1	1.75	39.5	3.39	2.08	8	2.34	6.12	0.38
	48 – 97	1.35	8.3	0.79	52.2	3.94	0.73	11	1.41	4.68	0.30
AMEO-2	Sediment 1	-	7.7	2.21	-	3.77	2.45	10	3.03	7.40	0.41
	Sediment 2	-	7.7	2.27	-	3.77	2.44	10	3.03	7.36	0.41
	0 – 13	1.14	7.8	2.80	41.4	3.35	2.84	10	2.70	7.34	0.37
	13 – 70	1.26	8.4	0.86	62.1	3.65	0.98	9	2.47	6.38	0.39
	70 – 80	1.44	8.6	0.22	3.1	3.27	0.22	10	1.10	3.83	0.29
	80 – 105	1.49	8.5	0.75	27.9	3.82	0.81	9	2.38	7.32	0.32

As shown in Figure D-1, soils at WiP sites were characterized by a wide range of soil textures whereas soils of sites AMEO 1 and AMEO 2 consisted of finer material with only minor variation throughout the soil profiles (except one horizon with coarser material at site AMEO 1 in 70-80+ cm depth ). The determined textures of sampled sediments did not differ from the average soil texture at each respective site.

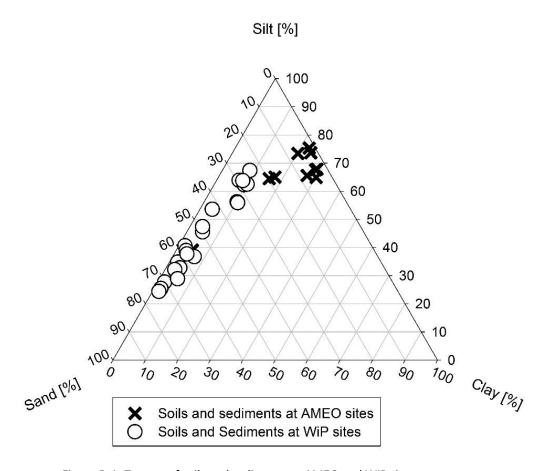


Figure D-1: Texture of soils and sediments at AMEO and WIP sites.

Along with finer soil and sediment texture,  $Fe_{DCB}$  contents are clearly higher in soils at AMEO sites (mean of all sampled soils at AMEO sites is  $6.0 \text{ g kg}^{-1}$ ) compared to soils at WiP sites (mean of all sampled soils at WiP sites =  $4.3 \text{ g kg}^{-1}$ ). The mean ratio of  $Fe_{ox}$  to  $Fe_{DCB}$  ("weathering index") is slightly higher in all soils at both WiP sites (mean = 0.52) than in the soils at AMEO sites (mean = 0.35) indicating a lower crystallinity of pedogenic Fe-oxides at the WiP sites. Furthermore, a decreasing  $Fe_{ox}/Fe_{DCB}$  ratio and higher crystallinity was observed with increasing soil depth at all investigated sites.

Calculated OC stocks were larger at AMEO sites than at WiP sites, and comparable to data published by *Cierjacks et al. (2010)* and *Rieger et al. (2014)* from the same location. In accordance with the presented model for formation of landscape units along the lateral and vertical gradient in a river hydrosystem, parent material at WiP sites is much coarser than at AMEO forest zones. A high water flow velocity during flooding at WiP sites allows only coarse particles to settle whereas a lower water flow velocity at AMEO sites permits deposition of finer particles. Discontinuous change of soil properties along the soil depth at WiP sites (such as content of sand and silt or OC content) and the higher number of distinguishable soil horizons (n = 8 and n = 10 at WiP sites compared to n = 4 at AMEO sites) are presumably a result of temporally variable flooding conditions (*De Vivo et al., 2001; Coppola et al., 2010*). In addition, the loss of sediment traps installed at site WiP 2 due to erosion along with the high sedimentation rate at WiP 1 further exhibits the high spatial and temporal variability of flooding conditions within the WiP alluvial forest zone. In contrast, the fewer number of soil horizons at the AMEO sites indicate continuous formation through the deposition of rather low sediment loads in comparison to sites close to the main river channel (*Asselmann and Middelkoop, 1995*). The low sediment input results in fewer distinguishable soil horizons.

The crystallinity of Fe-oxides determined in soils formed at WiP and AMEO sites are in agreement with results presented by *Lair et al. (2009a)* for soils sampled in the same investigation area. The combination of these data with results obtained from different dating methods show the Fe<sub>ox</sub>/Fe<sub>DCB</sub> ratio is a reliable indicator for soil maturity (*Lair et al. 2009a*) if the soils are not influenced by groundwater (*Shaheen and Rinklebe, 2014*). In accordance, our data emphasize that sampled soils in the AMEO alluvial forest zone were subjected to further soil development compared to soils in the WiP zone. The same dynamic applies to soils sampled in deeper soil layers with more advanced soil development compared to top soil material. Additionally, slightly lower OC/N<sub>tot</sub> ratios determined in bulk soils at AMEO sites (mean = 9.2) compared to WiP sites (mean = 11.0) imply further decomposition along with progressed soil development. This is also supported by estimated soil ages in 1 m depth, which can be calculated using the measured net annual sedimentation rates. At the AMEO sites, 4000 a (AMEO 1) and 500 a (AMEO 2) would be needed to deliver parent material for the formation of 1 m of soil. In contrast, the sedimentation rates determined at WIP 1 reveal material

in lowest horizon of the soil profile was delivered less than 20 years ago. These age estimates are in line with soil ages for subsoil at a depth of 60 cm presented by *Lair et al. (2009b)* who calculated ages of < 42 a for soil in the WiP zone and 1284 a in the AMEO zone.

D.4.2 Linking soil formation in landscape units and the free light-fraction of the soil organic matter

Distribution of OC obtained with density fractionation (fractions f-LF, o-LF and HF) is shown in Table D-3 (OC contents in each fraction) and (percentage of recovered OC found per fraction). The calculated mass recovery rate was 98.2 % (mean of all fractionated samples). A satisfying reproducibility of results of density fractionation is revealed by low standard deviations of measurements.

The contents of OC in f-LF of fresh sediment varied two orders of magnitude (0.39 - 16.51 g kg<sup>-1</sup>) yet no significant difference between WiP and AMEO sites could be detected. This can be attributed to the low density of POM, which constitutes most of the OM in f-LF: It is known that large portions of POM consisting of plant debris or roots have a very low density, often lower than 1.0 g cm<sup>-3</sup> (Liao et al., 2006), and are consequently floating on water surfaces. Due to its low density, POM cannot settle during flooding events and distribution of free POM is obviously not related to other sediment properties. In addition, other explanations why free POM can or cannot be found in sediments regardless of hydroecomorphological site conditions should be considered. For instance, it is possible that local depressions in the surface topography induce the formation of parafluvial ponds after flooding that are no longer connected to the river system (Karaus et al., 2005). In such ponds, water can only infiltrate into the soil or evaporate. After the water is gone, the POM that was floating on the water surface is deposited on the sediment and sites with former parafluvial ponds consequently are enriched with OC in f-LF compared to sites without parafluvial ponds formed after the flooding events. As the formation of ponds can occur everywhere in the floodplain regardless of landscape unit, this explains why the amount of OM in the f-LF in sediments shows no tendency when landscape units are compared. In addition, autochthonous input of litter or fine roots (Rieger et al., 2013) at the site or transport by wind between sedimentation and sampling should be considered as a source of f-LF in our sediment traps (Langhans et al., 2013) which may increase OC in f-LF in the sediments regardless sedimentation conditions.

Table D-3: Means and standard deviation (SD) of OC contents in density fractions of sediment and soil samples. OC contents given in g OC in fraction per kg of dry soil or sediment.

Site	Depth	f-LF (SD)	o-LF (SD)	HF (SD)
	[cm]	[g kg <sup>-1</sup> ]	[g kg <sup>-1</sup> ]	[g kg <sup>-1</sup> ]
WiP-1	Sediment 1	0.71 (0.12)	0.75 (0.13)	5.16 (0.74)
	Sediment 2	0.77 (0.13)	1.56 (0.06)	7.33 (0.03)
	0 – 8	1.37 (0.05)	6.52 (0.14)	11.89 (0.60)
	8 – 37	1.39 (0.27)	2.97 (0.03)	4.48 (0.05)
	37 – 41	0.36 (0.03)	2.34 (0.11)	7.15 (0.55)
	41 – 54	0.54 (0.06)	1.97 (0.06)	5.74 (0.28)
	54 – 65	0.43 (0.01)	3.98 (0.23)	10.06 (0.62)
	65 – 71	0.35 (0.11)	1.85 (0.01)	7.30 (0.14)
	71 – 92	0.34 (0.04)	2.40 (0.03)	9.62 (0.38)
	92 – 98	0.33 (0.15)	1.12 (0.13)	4.84 (0.50)
WiP-2	Sediment 1	3.76 (0.26)	3.27 (0.38)	7.18 (0.00)
	Sediment 2	2.03 (0.13)	2.11 (0.36)	5.12 (0.13)
	0 – 4	1.52 (0.03)	3.70 (0.12)	9.73 (0.93)
	4 – 16	6.05 (0.21)	4.99 (0.52)	11.14 (0.72)
	16 – 28	1.39 (0.03)	1.43 (0.07)	4.54 (0.59)
	28 – 33	1.23 (0.16)	2.13 (0.06)	6.95 (0.74)
	33 – 39	2.27 (0.03)	1.89 (0.10)	5.36 (1.24)
	39 – 44	2.72 (0.01)	2.52 (0.09)	6.65 (0.48)
	44 – 64	2.34 (0.09)	2.49 (0.04)	7.11 (0.25)
	64 – 66	2.20 (0.05)	3.11 (1.15)	9.14 (1.01)
	66 – 87	0.59 (0.02)	1.06 (0.16)	4.14 (0.62)
	87 – 110	1.27 (0.11)	3.27 (0.09)	5.71 (0.21)
AMEO-1	Sediment 1	16.51 (0.83)	11.52 (0.42)	34.79 (2.16)
	0 – 14	1.92 (0.05)	4.40 (0.39)	27.01 (0.24)
	14 – 30	0.48 (0.01)	0.48 (0.06)	15.40 (0.02)
	30 – 48	0.29 (0.07)	0.56 (0.07)	11.17 (0.30)
	48 – 97	0.10 (0.03)	0.45 (0.01)	5.24 (0.30)
AMEO-2	Sediment 1	0.39 (0.13)	1.93 (0.17)	16.28 (0.16)
	Sediment 2	0.44 (0.20)	2.62 (0.16)	16.36 (0.12)
	0 – 13	0.65 (0.06)	2.31 (0.01)	20.77 (1.20)
	13 – 70	0.04 (0.03)	0.21 (0.03)	7.29 (0.64)
	70 – 80	0.06 (0.01)	0.08 (0.03)	2.03 (0.35)
	80 – 105	0.07 (0.02)	0.20 (0.02)	6.14 (1.59)

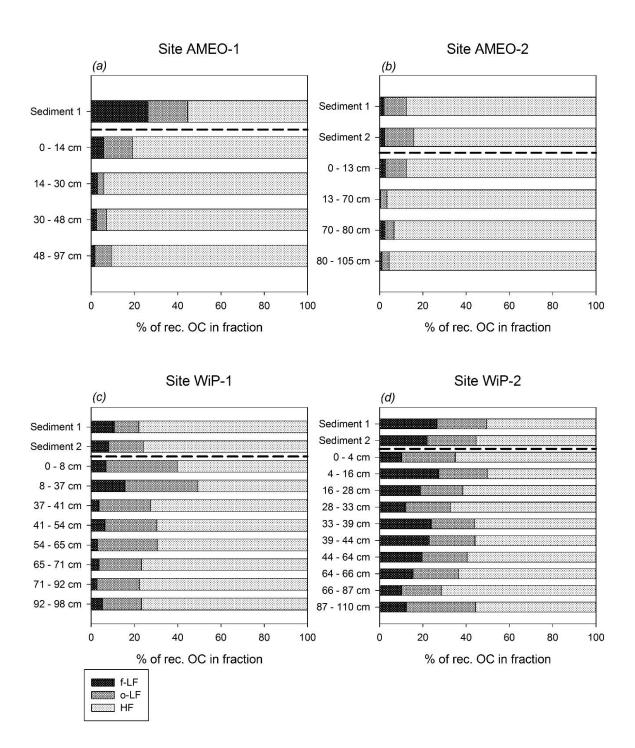


Figure D-2: Relative distribution of recovered OC among free light (f-LF), occluded light (o-LF) and heavy fractions (HF) in soil and sediments sampled at AMEO and WiP sites. Dashed line symbolizes soil surface.

We hypothesized that the highest portion of OC in f-LF is found in top soil horizons and decreases along with soil depth because of less bioturbation, lower rooting intensity and fast turnover of this fraction. The distribution pattern of OM in soils at the AMEO sites is in agreement with this assumption: the uppermost soil layers have the highest OC contents in form of f-LF that decrease with increasing soil depth. A different situation is found at the WiP sites where no decreasing trend in f-LF OC with soil depth could be observed; significant amounts of OC in f-LF are found throughout the profile. As already described, WiP sites are characterized by temporally variable flooding conditions, sedimentation and erosion rates. In our opinion, this situation is characteristic of soil development and C sequestration in WiP landscape units of the floodplain. When large amounts of sediments are deposited during single flooding events, as evident at site WiP 1, thick sediment layers can bury former top soils rich in OC and high f-LF OC (*Rinklebe, 2004; Gurwick et al., 2008; Blazejewski et al., 2009*), thereby conserving the properties of former topsoil horizons.

Stabilization of organic matter in soil aggregates as a process of soil formation? D.4.3 Our data showed that a mean of 18% of total recovered OC was found occluded in sediment aggregates at WiP sites and 14 % at AMEO sites. In the case of sites with a larger distance to the main river channel and a higher elevation (AMEO 1 and AMEO 2), sampled sediment material may partly consist of top soil material that was relocated with lateral flow within the same floodplain. This seems less probable at low-lying WiP sites near the main river channel because sediments there are brought along with floodwater that can be expected to enter the area for the first time. Crucially, the top soil material eroded in the river catchment and forming the sediments contains aggregates stable enough to sustain the mechanical stress during transport in the water flow - this contradicts our first hypothesis. An additional explanation for the origin of o-LF material is that mineral-organic flocs formed during transport in the river may contain POM (Droppo, 2001; Martilla and Kløve, 2014). With the methods applied it was not possible to determine to what extent the occluded OM derives from soil-borne aggregates or river-borne flocs. Aggregation of mineral particles in riparian soils is not only a result of in-situ soil formation: it might also be result of processes active in soils where the sediments are derived from or in river water. Although we could not observe pronounced differences in contents of OC occluded in aggregates (or river-bourne flocs) between sediments arriving at the different investigated sites, clear differences between site-specific soil samples could be revealed. In soils at the AMEO alluvial forest zone, OC in aggregates depleted with increasing soil depth compared to their respective sediment. This depletion was also observed for total OC. Regarding the relative distribution of each fraction, exclusively the portion of OC occluded in the uppermost top soil was comparable to the sediments. Deeper soil layers that had undergone soil formation for a longer time

than the top soil were further depleted of both total OC and o-LF compared to sediments and top

soil. This was already described by other authors (John et al., 2005; Grünewald et al., 2006) for a large number of soils. Still, different observations were made for the soils at sites WiP 1 and WiP 2: organic matter was enriched in top soil horizons of the soils compared to fresh sediments on top of the soils. With increasing soil depth a slight decreasing trend for total OC was also detected. Yet, this decrease was much less pronounced at the WiP sites than at the AMEO sites. Even in the lowest sampled soil horizon, the amount of OC in the o-LF was similar to that determined in the sediments. The relative distribution of recovered OC among fractions further supports the conclusion that occlusion is a relevant process for SOM stabilization in soils developed at the WiP sites and related flooding conditions, whereas it seems less relevant at the AMEO sites. Whereas 23.3 % (mean of all soil samples) of recovered OC was found occluded in aggregates at sites WiP 1 and WiP 2, much less was recovered in the AMEO site soils (6.1 %, mean of all soil samples). With regard to the coarser soil material at these sites, this is surprising and contradicts our second hypothesis: Aggregates in sandy soils are generally known to have lower mechanical stability than aggregates formed of finer material (Kaiser et al., 2012). Yet, other conditions besides soil texture may be favorable for aggregate formation at WiP sites. It is likely that low mechanical stability of aggregates cause fast aggregate breakdown, but immediate re-aggregation caused by the interplay of plant roots and earthworms throughout all soil layers (Bullinger-Weber et al., 2007; Fonte et al., 2012) may result in high portions of OC in o-LF as found at the WiP sites. Also the high number of wet-dry cycles can support fast reaggregation at the WiP sites. In addition, arbuscular mycorrhizal fungi, which occur in soils under willow-poplar stands in riparian forests (Beauchamp et al., 2006), are known to significantly contribute to stabilization of soil aggregates (Rillig and Murray, 2006). This has been reported for young and coarsely textured soil by Mardhiah et al. (2014) and may explain the high proportion of OC in o-LF found at the WiP sites. It is also possible that aggregates in subsoil horizons at WiP sites formed when these layers were situated closer to the surface. In this case, the aggregates would have sustained flooding and sediment burial, and are therefore still present in the subsoil (Blazejewski et al., 2005)

In addition, it must be considered that the amount of OC in the o LF is only one of various soil aggregate properties. During soil development, size and hierarchy of aggregates may change and therefore are not represented by the o LF fraction of SOM. Consequently, more studies are needed to link SOM fractionation to soil morphology and the stability of aggregates as a proxy for stabilization of SOM. Differences in litter production between AMEO and WiP sites are unlikely to explain the differences of OC present as POM at the respective sites. Biomass production was shown to be lower at WiP sites than at AMEO sites (*Cierjacks et al., 2010*). If this would have a consequence for the distribution of OC among density fractions we would expect a higher percentage of POM in

f-LF and o-LF at AMEO sites compared to WiP sites. In contrast, we observed higher percentage of POM at WiP sites than at AMEO sites.

## D.4.4 Stabilization of OM in heavy fraction

Our data showed the largest amount of OM stabilized on mineral surfaces in all soil and sediment samples. At AMEO sites, OC recovered in HF ranged between 84 and 97% of total (except for sediment at AMEO-1 with 55%). At WiP sites, the largest amounts of OC were determined as HF, but when compared to AMEO sites the relative portions of OC recovered in this fraction were much lower (50 to 76%). The observation that the majority of OC is bound to mineral surfaces and is consequently found in HF has already been reported for various soils (*Grünewald et al., 2006; Don et al., 2009; Grüneberg et al., 2013*). The overall higher portions of OC in HF in fine soils at AMEO sites compared to coarser soils at the WiP sites can be explained by the larger available particle surface area in fine soils and corroborate our third hypothesis. Figure D-3 shows the positive correlation between clay content and OC found in HF for all investigated soil and sediment samples. It is known that clay constitutes more than 99% of the total particle surface area in loamy soils (*Kretzschmar, 2010*) and is therefore a suitable measure for potential sorption surfaces. The high significance of the presented positive correlation (p level < 0.0001) backs the assumption that fine soils stabilize more OC through sorption on mineral surfaces than coarse soils.

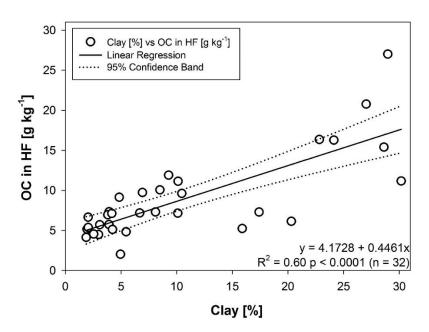


Figure D-3: Correlation between OC found in heavy fraction (HF) and clay content of respective soil or sediment sample (n = 32)

With regard to the AMEO sites, our results show that the relative portion of OC stabilized in HF is lowest in sediments and topsoil, and increases in subsoil layers (Figure D-2). The absolute amount of OC in the HF fraction continuously decreases from the uppermost soil horizon to the lowermost by a factor of 3.4 (AMEO 1) and 5.2 (AMEO 2). The decrease was found to be lower at both WiP sites (WiP 1: 2.4 and WiP 2: 1.7), and no continuous trend in the relative distribution of OC in HF with soil depth was found at the WiP sites.

Both findings for OC in HF at AMEO and WiP sites are supported by our results on weathering indices (soils at WiP sites are less weathered) and sedimentation rates (lower sedimentation rates at AMEO than at WiP sites) as covered in section D.4.1. Soils at AMEO sites have most probably undergone a longer and further soil development, and therefore show a lower weathering index and lower sedimentation rates. Consequently, OM in subsoil is further mineralized than in topsoil at AMEO sites and in all soil horizons at WiP sites. Progressed mineralization is indicated by decreased contents of total OC and OC in the HF. We have to admit that the assumption of progressed mineralization with increasing depth is not confirmed by depth distribution on OC/N<sub>tot</sub> ratios (see very similar values in all horizons at WiP and AMEO sites in Table D-2). Overall the OC/N<sub>tot</sub> ratios are rather narrow (close to 10) which might impair a further increase in OC/N<sub>tot</sub> ratio along with mineralization of OM.

Regardless, OC in HF is still relatively enriched in AMEO subsoil compared to AMEO topsoil and sediments due to longer turnover time of OM in HF than OM in f-LF or o-LF (fast and intermediate turnover).

Overall, OM in HF is mineralized to different extents in subsoil at AMEO and WiP sites, which contradicts our fourth hypothesis. Still, the basic assumption that mineralization of OM in HF is lower than of OM in f-LF or o-LF could be confirmed.

# D.5 Conclusion

In this study, the mechanisms of SOM stabilization in soils of WiP and AMEO zones of an active floodplain were analyzed. Our results confirm the assumption that soil properties within the riparian floodplain depend on local flooding conditions that determine soil properties and are represented by vegetation types. We confirm with our results that hydroecomorphological site conditions (indicated by the dynamics of frequency and duration of flooding and of water flow velocity) are important drivers of soil formation in active floodplains. At AMEO sites, the quantity and quality of delivered sediment as the parent material for soil formation does not change over time, and (slow) sedimentation occurs in parallel to progressing soil formation. In contrast, at the investigated WiP sites periods of soil formation are interrupted by flooding and subsequent extreme sedimentation or erosion due to present water turbulences and high flow velocity. As a consequence buried horizons occur at WiP sites, where properties of former topsoil horizons are preserved but are not observed at AMEO sites.

The difference in hydroecomorphological conditions are reflected by SOM stabilization processes dominating in the different riparian zones: Due to the burial process at WiP sites, a higher percentage of organic matter is stored as free particulate organic matter or within soil aggregates (f-LF + o-LF = 20-40%) than at AMEO sites (f-LF + o-LF < 10%). At the latter one only organic matter adsorbed to mineral surfaces resists progressing soil formation and degradation of organic matter. Thus, for the assessment of SOM stability at WiP-sites the stability and turnover of aggregates seems to be crucial.

# D.6 Acknowledgments

We would like to thank our colleagues at the Nationalpark Donau Auen for collaboration and ongoing support of our project, and Kenton Stutz for revising the language and style of the manuscript. This study was funded by the Deutsche Forschungsgemeinschaft DFG (grant number LA 1398/4).

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E Organic matter distribution in floodplains can be predicted using spatial and vegetation structure data

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Published in:

River Research and Applications (2011), Vol. 27(8), 1048 – 1057.

DOI: 10.1002/rra.1409

http://onlinelibrary.wiley.com/doi/10.1002/rra.1409/abstract

#### RIVER RESEARCH AND APPLICATIONS

River. Res. Applic. (2010)

Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rra.1409

# ORGANIC MATTER DISTRIBUTION IN FLOODPLAINS CAN BE PREDICTED USING SPATIAL AND VEGETATION STRUCTURE DATA

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#### **ABSTRACT**

Riparian forest ecosystems play a significant role in the storage of organic carbon. However, the knowledge on the spatial patterns of organic matter distribution which is crucial to the assessment of the C sequestration potential of riparian ecosystems is still lacking. The aim of our study was to identify predictors of organic matter distribution in floodplain soils and vegetation. We analysed the depth distribution of soil horizons to 1 m below the surface, calculated the organic C content and quantified living biomass and woody debris at 67 sampling plots in the Donau–Auen National Park (Austria) along principle spatial gradients (longitudinal, lateral and vertical to river direction). Multiple regression models were fitted using hierarchical partitioning of spatial information, which was supplemented by forest stand parameters as possible predictors of soil C. The concentration of organic C in the subsoil horizons increased significantly with distance to the main channel. In addition, the thickness of soil horizons enriched with organic matter increased downstream which probably indicates the effect of riverbed changes over the last two centuries. Model prediction of soil parameters was improved with the inclusion of vegetation structure variables which are a consequence of local river dynamics. Highly dynamic locations indicated by higher stem numbers, greater understory vegetation cover, lower mean stem diameter and lower canopy cover showed significantly lower concentrations of soil organic C and lower total organic C stocks. We conclude that spatial information and vegetation structure can indicate gradients of geomorphic floodplain dynamic, which is the main driver of organic matter storage. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: carbon sequestration; carbon stocks; Donau-Auen National Park; floodplains; riparian forest; river hydrosystem; organic matter; vegetation structure

Received 9 February 2010; Revised 25 March 2010; Accepted 7 April 2010

## INTRODUCTION

Despite playing an important role in the global carbon cycle, the source and sink functions of wetlands are not well understood (Mitra *et al.*, 2005). While most studies on carbon balance have focussed on peatlands (e.g. Lafleur *et al.*, 2003; Belyea and Malmer, 2004; Roulet *et al.*, 2007), non-peaty floodplain ecosystems have scarcely been considered.

Riparian forests show particularly high organic C stocks of both soil and vegetation compared to other terrestrial ecosystems (Cierjacks *et al.*, 2010; Hofmann and Anders, 1996; Giese *et al.*, 2000; Giese *et al.*, 2003; Fierke and Kauffman, 2005; Hazlett *et al.*, 2005; Shibata *et al.*, 2005). This has been attributed to the deposition of C-rich sediments during flooding (Pinay *et al.*, 1992) and to the high net primary production of riparian forests (Naiman and Décamps, 1997; Giese *et al.*, 2000; Clawson *et al.*, 2001; Gawne *et al.*, 2007). In particular, lowland floodplains are

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known to be autotrophic overall as C assimilation equals or even exceeds respiration (McTammany *et al.*, 2003, Shibata *et al.*, 2005). However, actual C stocks of floodplain ecosystems vary considerably depending on stand age, human disturbance, soil conditions and climate (e.g. Giese *et al.*, 2003; Chen *et al.*, 2005; Fierke and Kauffman, 2005; Hazlett *et al.*, 2005; Keeton *et al.*, 2007).

In contrast to soils not affected by flooding, soils within floodplains exhibit a pronounced spatial heterogeneity in terms of the horizontal and vertical distribution of horizons enriched with organic matter. Horizons may reach a cumulative thickness of more than 1 m. Apart from organic matter produced by riparian vegetation, the C content of floodplain soils is determined by organic and organomineral sediments. As such, a close correlation of sedimentation and C distribution in floodplains might be expected. The uneven delivery of large amounts of sediments along or near the river banks should result in a high number of soil horizons. In contrast, the low but continuous sedimentation (more gradual than bioturbation) at greater distances from the river creates soils with fewer horizons (Jacobson *et al.*, 2003) and finer-grained sediments

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with larger concentrations of organic matter. In addition, carbon accretion is know to depend on soil age with the highest rates for organic matter pool allocation found during the first few decades of soil development (Zehetner *et al.*, 2009).

Piégay and Schumm (2003) have divided the river hydrosystem into three main spatial gradients: (1) longitudinal gradient (upstream/downstream); (2) lateral gradient (distance to main or to next channel) and (3) vertical gradient (altitude above mean water level). Both sediment deposition and vegetation vary along these gradients due to differences in flow velocity, sediment load and inundation time (e.g. Friedman et al., 1996; Cooper et al., 1999; Bendix and Hupp, 2000; Busse and Gunkel, 2002; Bornette et al., 2008). Therefore, the hydrosystem approach might also be applied to the modelling of C distribution in floodplains. Although a few studies have incorporated elements of such an approach, none have done so comprehensively. Langhans et al. (2006), despite having developed a concept explaining C distribution along the lateral and vertical gradients, refrained from providing data allowing for carbon prediction. Other studies provide evidence of changing sediment traits along the longitudinal gradient that also affect C storage in sediments (Pinay et al., 1992; Petts et al., 2000).

In some cases, standing vegetation may indicate varying aspects of sedimentation conditions better than the abovementioned spatial gradients. For example, former geomorphologic processes, such as riverbed shifting or oxbow lake formation, can be indicated by current forest structure (Hupp and Bazemore, 1993; Bendix and Hupp, 2000). Differing intensities of flooding events over time and space in dynamic floodplain ecosystems result in a mosaic of simultaneously existing successional stages (Bendix and Hupp, 2000; Richards et al., 2002; Suzuki et al., 2002). The more general impact of spatial gradients on the deposition of sediments and standing biomass may therefore further be modified by local flooding conditions, which also vary over time (e.g. Latterell et al., 2006; Kondolf et al., 2007; Whited et al., 2007). As such, C pools also exhibit a close correlation with riparian forest development stages (Giese et al., 2000). In addition, pronounced differences in organic matter contents of forests and grassland soils compared to intensive cropland soils in floodplains have been described (Zehetner et al., 2009). However, possible relations between organic matter distribution and forest stand parameters in floodplains have not, to our knowledge, as yet been analysed.

Biomass and stand parameters, as well as position along main spatial gradients, can be analysed by remote sensing techniques (e.g. Mertes, 2002; Turner *et al.*, 2004; Whited *et al.*, 2007; Kooistra *et al.*, 2008). Thus, C pools in floodplain soils might be quantified with little ground data if these variables indicate processes of sedimentation and

erosion, as well as production and respiration of organic matter.

The aim of this study was to identify any correlations between the main spatial gradients of the hydrosystem and forest structure with soil and vegetation organic C stocks, taking the Donau–Auen National Park as a model system, which has been intensively studied (e.g. Lair *et al.*, 2009b; Zehetner *et al.*, 2009). In particular, we tested the following hypotheses:

- (1) C concentration, thickness and number of soil horizons as well as total C stocks in riparian soils and vegetation (living woody biomass and coarse woody debris) are related to the main spatial gradients of the hydrosystem (longitudinal, lateral and vertical).
- (2) The gradient-based prediction capacity of soil C stocks is heightened when adjusted for vegetation variables as indicators of local sedimentation conditions, former geomorphological processes and current autochthonous biomass production and ecosystem respiration.

## MATERIALS AND METHODS

Study area

The study area is located in the Donau-Auen National Park in the north-eastern plains of Austria. The National Park was established in 1997 and covers approximately 9300 ha. It is nearly completely covered by riparian forest, which along the main channel is subjected to natural flood dynamics. The area has been almost exclusively used for hunting over the centuries and some forests still show a more or less natural age structure influenced only by the flood regime. During the 1960s, stands of *Populus* × canadensis were planted and now cover about 40% of our study area (Cierjacks et al., 2010). Since gaining National Park status, forestry operations in the area have ceased, with exception of the control of invasive plant species (National Park Donau-Auen GmbH, unpublished manuscript). The area is considered to be the largest remaining riparian forest in central Europe, making it particularly suitable for the assessment of the study hypotheses.

We selected a dynamic part on the northern river bank south of the Marchfeld dike between the villages of Schönau (48°8'N, 16°36'E) and Witzelsdorf (48°7'N, 16°48'E), where soils are calcaric fluvisols with varying arrangements of sedimentation horizons, superficial and buried Ah horizons as well as carbon-poor C horizons within profiles to 1 m below the surface. Donau–Auen National Park is characterized by a typical temperate lowland climate with a mean temperature of 9.8°C and a mean precipitation of 533 mm (Climate station: Schwechat, 48°07'N, 16°34'E,

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184 m asl; Zentralanstalt für Meteorologie und Geodynamik, unpublished manuscript).

## Study design

To cover all relevant forest types, we divided the study area into patches with apparently homogeneous vegetation according to aerial colour-infrared (CIR-) photographs taken in 2007. Sixty seven patches were selected randomly. Only patches with forest vegetation, which covers about 79% of the study area (Cierjacks *et al.*, 2010), were considered as we intended to analyse the impact of forest stand structure on C stocks. Within each of the patches selected, we established one study plot of  $10 \, \text{m} \times 10 \, \text{m}$  for analyses of soil and stand structure. The position of each study plot was determined using Trimble Juno SC Handheld GPS (Trimble GmbH, Germany). Data collection was performed from June to August 2008.

## Soil analysis

Soil samples (0–100 cm in depth) were extracted from the centre of each study plot using an auger. We determined the vertical distribution of soil horizons regarding soil colour and texture. Afterwards, each soil horizon was sampled separately and carbonate concentration and the concentration of organic C were determined.

For the C analysis, all samples were sieved ( $<2 \,\mathrm{mm}$ ), dried (104°C) and ground. Total concentrations of C and N were determined using a CN analyser (Elementar, Analysensysteme GmbH, Germany). The carbonate C concentrations of the samples were determined using the same instrument after combustion of the soil samples at 530°C. According to Bisutti et al. (2007), all organic C is removed at this temperature, while the C in carbonate is not affected by combustion. The organic C concentration of the sample was calculated from the difference between total and carbonate C. We calculated the C stocks of the soil up to a depth of 1 m based on the C concentrations, the thickness and the bulk densities of each soil horizon. As it was not possible to determine the bulk density of the horizons at all 67 sampling sites, we estimated the bulk density of a given soil horizon based on four reference soil profiles in the study area: one in a softwood area, one in a hardwood area and two in the area covered by cottonwood. Within each reference profile, we determined the bulk densities of all soil horizons ranging from 0.8 g cm<sup>-3</sup> in Ah horizons to 1.6 g cm<sup>-3</sup> in loamy subsoil horizons. Based on the assumption that bulk density is mainly controlled by soil texture, soil depth and vegetation, we used the reference bulk densities of the corresponding depth, vegetation type and soil texture for each horizon in the sampling plots. Our assumption is supported by the fact that bulk densities of 20 loamy topsoil samples at

hardwood sites differed by less than 10% (results not shown). In addition, the bulk density values are in line with data reported from soil profiles at the Elbe river (Rupp, H., personal communication).

## Position in hydrosystem

The position of each study plot in relation to each of the three gradients of the hydrosystem was determined using ArcGIS version 9.2. Distance to main channel was determined using the 'NEAR' function which measured the nearest distance to a line along the northern river bank. The same line was used for the calculation of the longitudinal distance to the eastern border of the study area. Distance to the next channel was measured manually. Based on a digital terrain model (DTM) derived from laser scan data, we determined altitude above mean water level. We used water level data of the measuring point Wildungsmauer (48°06'N, 16°48'E; time period: 1961–1990) and calculated mean water level at a given location assuming a mean slope inclination of 0.4 m km<sup>-1</sup> (National park Donau-Auen GmbH, personal communication). Altitude above mean water level was determined through the difference of absolute altitude of a study plot and altitude of mean water level at the nearest connection to the main channel.

## Forest stand structure

In each study plot, we measured height and circumference at breast height of all trees >15 cm in circumference. Based on these data, we calculated stem number per ha, mean height and mean diameter. Living woody biomass was determined using the equations given in Zianis *et al.* (2005) as carried out by Cierjacks *et al.* (2010). Stem volume data were multiplied by 0.4451 to calculate dry biomass referring to Wulder *et al.* (2008). For all shrub species we used the equation for *Corylus avellana* (Zianis *et al.*, 2005).

In addition, biomass of coarse woody debris >30 cm in circumference was calculated via average circumference and length of each stem present in the study plot. In contrast to other riparian ecosystems (Hyatt and Naiman, 2001), buried stems were scarce in the study area, presumably due to low sedimentation rates, and were therefore not considered for biomass estimation. Calculations were performed assuming a mean wood density of 0.6 t m<sup>-3</sup> as a rough estimate (Baritz and Strich, 2004; Chave *et al.*, 2006). In accordance with Giese *et al.* (2003), the biomass stocks were transformed to C stocks by multiplying by 0.5. The cover of canopy, shrub and herb layers were estimated visually. Biomass of herb layer and young shrubs was not considered due to the low overall contribution to

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above-ground biomass and a high seasonality of actual stocks.

#### **Statistics**

We analysed the impact of spatial position and vegetation parameters on number, C concentrations and cumulative thickness of Ah and C-enriched subsoil horizons as well as on C stocks of soil, living woody biomass and coarse woody debris by fitting multiple regression models. Firstly, the impact of distance along the longitudinal gradient, distance to the main and next channels as well as altitude above mean water level was analysed. Next, vegetation data (herb, shrub and tree cover as well as stem number and mean diameter), which are assumed to be a consequence of local river dynamics during the last decades, were incorporated into the models. Multicolinearity between variables was ruled out by excluding all combinations with Pearson-Bravais correlation coefficients >0.35. All data used were untransformed. We rejected stepwise variable selection techniques due to known statistical difficulties (Quinn and Keough, 2003) and used hierarchical partitioning for variable selection. Hierarchical partitioning uses all possible models and averages the improvement of model fit for each predictor variable, both independently and jointly, across all these models. The resulting predictors were incorporated into multiple regression models and model fit was calculated based on  $r^2$  and Akaike Information Criterion (AIC). All calculations were carried out with R version 2.8.1 and the hier.part package was used for hierarchical partitioning.

#### RESULTS

### C stocks along hydrosystem gradients

The study area is characterized by high C stocks totalling about 400 t/ha (Table I). Total soil organic C stocks increased significantly with distance to next channel and along the longitudinal gradient in an easterly (downstream) direction (Figure 1, Table II). The model was significant, but an  $r^2$  value of 0.14 indicates a low predictive power. The thickness of Cenriched subsoil horizons increased along the longitudinal gradient towards the eastern part of the study area, and the mean organic C concentration of these horizons increased with distance to the main and next channels. Both models were significant and show higher  $r^2$ -values than the total soil organic C model. The number of soil horizons decreased significantly with increasing distance to main channel. In contrast, models for the organic C concentrations of Ah horizons were only marginally significant. We detected a negative effect of altitude above mean water level on thickness of Ah horizons and organic C concentration, a negative effect of distance to main channel on thickness of Ah horizons and a positive impact of distance to next channel on C concentrations of Ah horizons. The findings indicated a complex pattern of C distribution in the hydrosystem, summarized in Figure 2 with all significant and marginally significant models.

Living woody biomass did not respond to spatial gradients of the hydrosystem, but biomass of coarse woody debris showed a significant negative correlation with altitude above mean water level (Table II).

Table I. Means (SE) of spatial information, vegetation structure, soil parameters and C stocks in the study area

Variable	Mean (SE)
Spatial information	
Distance to main channel (m)	518 (50)
Distance to next channel (m)	174 (24)
Altitude asl (m)	148 (0)
Altitude above mean water level (m)	2.5 (0.1)
Vegetation structure	
Cover of tree layer (%)	50.4 (2.5)
Cover of shrub layer (%)	19.0 (2.4)
Cover of herb layer (%)	58.1 (3.3)
Number of stems >15 cm in circumference per ha	661 (72)
Mean diameter at breast height of stems >15 cm in circumference (cm)	41.2 (3.0)
Soil parameters	
Horizon number of C-enriched horizons (0–1 m in depth)	4.0 (0.2)
Ah thickness (dm)	1.7 (0.1)
Ah C concentration (% weight)	3.0 (0.2)
Thickness of C-enriched subsoil horizons (dm)	6.4 (0.2)
C concentration of C-enriched subsoil horizons (% weight)	1.7 (0.1)
C stocks (t/ha)	
Soil (0–1 m in depth)	177 (7)
Living woody biomass (stems >15 cm in circumference)	202 (24)
Total coarse woody debris (stems >30 cm in circumference)	20 (4)

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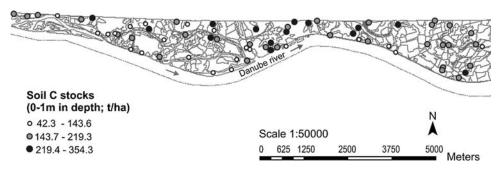


Figure 1. Classification of sampling points according to the respective soil C stocks

Vegetation variables as indicators for local river dynamics and C stocks

The inclusion of stand parameters improved the model fit for most of the carbon response variables (Table III). The number of soil horizons decreased significantly with increasing mean diameter and decreasing herb cover along with distance to main channel. The organic C concentration of Ah and C-enriched subsoil horizons was negatively correlated with herb cover. In addition, the C concentration of C-enriched subsoil horizons was also correlated with shrub and tree layer. In contrast, the thickness of soil horizons was not affected by vegetation parameters (Table III).

In combination with distance to next channel, organic C stocks in soil could be predicted by stem number as well as by the cover of the herb and tree layers. Locations characterized by high stem numbers and cover of herb layer and low canopy cover showed lower total soil organic C stocks than locations with low stem numbers and cover of herb layer (Figure 3). Model prediction was clearly higher  $(r^2 = 0.22)$  than that based on spatial gradients only.

Along with longitudinal distance and cover of tree layer, woody biomass C stocks were self-evidently correlated with stand's mean diameter. In contrast, C stocks of coarse woody debris decreased with cover of tree layer. The impact of altitude above mean water level was ruled out in this model.

Table II. Multiple regression models for predicting soil parameters, living woody biomass and coarse woody debris biomass according to the main gradients of the hydrosystem

			Model quality			
Variable	Coefficient	p	$r^2/\text{adj. } r^2$	p	AIC	
Number of soil horizons (0–1 m in depth)	0.004	0.000	0.1.1/0.10	0.000		
Distance to main channel (m)	-0.0013	0.002	0.14/0.13	0.002	230.9	
Thickness of Ah horizon (dm)						
Altitude above mean water level (m)	-0.2591	0.072	0.07/0.04	0.089	173.1	
Distance to main channel (m)	-0.0004	0.105				
Organic C concentration of Ah horizon (%	)					
Altitude above mean water level (m)	-0.3987	0.117	0.08/0.05	0.077	247.5	
Distance to next channel (m)	0.0018	0.067				
Total thickness of C-enriched subsoil horiz	ons (dm)					
Longitudinal distance (m)	-0.0003	< 0.001	0.34/0.33	< 0.001	247.4	
Organic C concentration of C-enriched sur	bsoil horizons (%)					
Distance to main channel (m)	0.0005	0.002	0.20/0.17	< 0.001	115.0	
Distance to next channel (m)	0.0005	0.171				
Total soil C stocks (0–1 m in depth; t/ha)						
Distance to next channel (m)	0.0763	0.034	0.14/0.12	0.007	731.7	
Longitudinal distance (m)	-0.0034	0.050				
Woody biomass C stock (stems >15 cm in	circumfaranca: t/ha)					
Longitudinal distance (m)	0.1592	0.182	0.03/0.01	0.182	1300.2	
. ,			0.03/0.01	0.102	1300.2	
Coarse woody debris C stocks (stems >30		· ·	0.05/0.05	0.022	100.5	
Altitude above mean water level (m)	-0.3463	0.032	0.07/0.05	0.032	189.5	

Longitudinal distances refer to distance to the eastern border of the study area.

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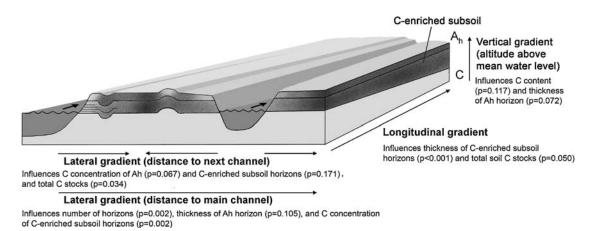


Figure 2. Summary of the observed gradients in thickness and C concentration of Ah and C-enriched subsoil horizons up to a depth of 1 m in the hydrosystem. *p*-values refer to factor significance in the linear regression models. C concentration in soil horizons is presented in the grey scale (darker grey indicates higher C concentration)

Table III. Multiple regression models for predicting soil parameters, living woody biomass and coarse woody debris C stocks according to the main gradients of the hydrosystem and vegetation structure

			Model quality			
Variable	Coefficient	p	$r^2$ /adj. $r^2$	p	AIC	
Number of soil horizons (0–1 m in depth)						
Distance to main channel (m)	-0.0009	0.027	0.29/0.26	< 0.001	221.9	
Cover of herb layer (%)	0.0167	0.010				
Mean diameter (cm)	-0.0221	0.001				
Thickness of Ah horizon (dm)						
Altitude above mean water level (m)	-0.2591	0.072	0.07/0.04	0.089	173.1	
Distance to main channel (m)	-0.0004	0.105				
Organic C concentration of Ah horizon (%	6)					
Distance to next channel (m)	0.0016	0.080	0.17/0.13	0.008	242.3	
Cover of herb layer (%)	-0.0176	0.010				
Altitude above mean water level (m)	-0.3957	0.104				
Total thickness of C-enriched subsoil horiz	zons (dm)					
Longitudinal distance (m)	-0.0003	< 0.001	0.36/0.34	< 0.001	247.4	
Organic C concentration of C-enriched su	bsoil horizons (%)					
Distance to main channel (m)	0.0004	0.019	0.39/0.35	< 0.001	101.0	
Cover of herb layer (%)	-0.0077	0.003				
Cover of shrub layer (%)	-0.0103	0.002				
Cover of tree layer (%)	0.0060	0.048				
Total soil C stocks (0–1 m in depth; t/ha)						
Stem number per ha	-2.6366	0.029	0.22/0.16	0.004	729.8	
Cover of herb layer (%)	-0.4982	0.049				
Cover of tree layer (%)	0.5624	0.097				
Distance to next channel (m)	0.0643	0.069				
Woody biomass C stock (stems >15 cm in	circumference; t/ha)					
Longitudinal distance (m)	0.2224	0.049	0.26/0.22	< 0.001	1268.0	
Cover of tree layer (%)	45.1936	0.049				
Mean diameter (cm)	75.6262	< 0.001				
Coarse woody debris C stocks (stems >30	cm in circumference;	t/ha)				
Cover of tree layer (%)	-0.020	< 0.001	0.16/0.15	< 0.001	180.4	

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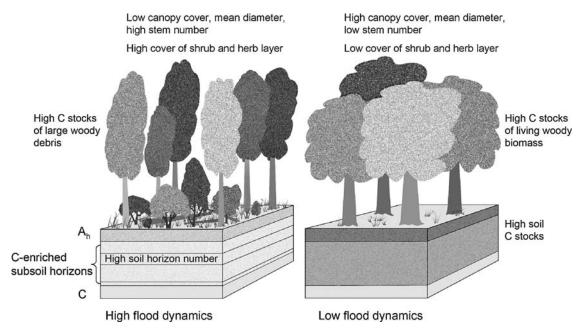


Figure 3. Soil C stock in relation to vegetation structure. The two sites represent the extremes of a gradient of fluvial dynamics (higher C concentration in the soil is represented by darker grey shading)

## **DISCUSSION**

C stocks along hydrosystem gradients

With about 180 t/ha for the superficial soil to a depth of 1 m, soil organic matter stocks proved to be clearly higher than those of other temperate forest ecosystems (Table I). A comparative study of Hofmann and Anders (1996) for different forest ecosystems in central Europe revealed that soil carbon stocks of non-riparian lowland forests ranged only between 50 and 150 t/ha.

C stocks varied along the main spatial gradients of the hydrosystem. The most pronounced patterns were detectable in the thickness and organic C concentrations of Cenriched subsoil horizons. Organic C concentration is higher in fine sediments (e.g. Pinay et al., 1995; Scott Bechtold and Naiman, 2006), which are deposited at low flow velocity. Consequently, C concentrations of Cenriched subsoil horizons increased with distance from the main and next channels. In the eastern part of our study area, however, soils showing large C-enriched subsoil horizons and total organic C stocks were located relatively close to the channel (Figure 1). This observation may imply the existence of a longitudinal gradient of organic matter sedimentation as described by Pinay et al. (1992); however, this may not be the case given the much shorter gradient in our study area. Alternatively, a shift of the Danube riverbed during the last three centuries, as identified by Hohensinner et al. (unpublished manuscript), may be the reason for the high C stocks close to the river in the eastern part of our study area. The Danube shifted to

the north during that time into less dynamic and C-enriched hardwood areas.

In contrast, Ah horizons were much less influenced by spatial gradients. The thickness of Ah horizons is known to be determined by the activity of soil biota, which shows a clear maximum in the upper 20 cm of the soil profile (Ekelund et al., 2001). We found marginally significant negative correlations with altitude above mean water level and with distance to main channel. This might be explained by increased litter accumulation and lower soil respiration in swales compared to elevations, which is supported by C concentration data, which also decreased with altitude. An increasing C concentration in Ah horizons with increasing distance to next channel, in turn, again supports the findings on C-enriched horizons. However, models showed a low predictive power. Spatial gradients in C concentrations of Ah horizons may be hidden by small-scale heterogeneity of biomass production and humus accumulation.

Patterns of total organic C stocks in soils resembled those of C-enriched subsoil horizons, which accounted for the major part of C stocks (Table I). Therefore, in parallel to thickness and C concentrations of C-enriched subsoil horizons, soil C storage was highest in the eastern part of the study area and at greater distances from the next channel.

Biomass prediction by means of spatial gradients did not produce significant models. Cooper *et al.* (1999), as well as Bendix and Hupp (2000), emphasized the impact of the lateral gradient on recruitment of riparian forests, which determines future stand structure. Models on ground water fluctuation and vegetation development in addition show the

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relevance of the vertical gradient for vegetation structure (Scott et al., 1999; Horton et al., 2001; Baird et al., 2005), although comprehensive studies are available mainly for herbaceous vegetation (Auble et al., 1994; Leyer, 2004). The lack of similar patterns in the study area might be attributed to a less pronounced river dynamic during the last decades due to river regulation activities (Lair et al., 2009a) along with a higher proportion of old forest stands. This is supported by the fact that vegetation units in the study area derived from species composition data did only weakly respond to spatial gradients (Cierjacks et al., 2010)—unlike in other river systems (e.g. Bendix and Hupp, 2000; Barker et al., 2002). In contrast, coarse woody debris was higher at low altitudes above mean water level. The latter shows that dead wood is not only more frequently deposited along channels but also accumulates in swales, which might be a consequence of increased tree mortality in the course of longer inundation times.

Vegetation variables as indicators for local river dynamics and C stocks

Floodplain vegetation structure is known to be predominantly influenced by flood dynamics (Bendix and Hupp, 2000; Cooper et al., 2003; Whited et al., 2007). Consequently, forest vegetation reflects local fluvial dynamic history, which overlaps main spatial gradients (Hupp and Bornette, 2003). The inclusion of vegetation structure as an indicator of local river dynamics in addition to the three hydrosystem axes into linear models led to improved model fit for the majority of carbon parameters. Locations with longer inundation time and higher flow velocity may be expected to show a higher risk of tree damage and mortality (visible as corrosion scars and horizontal stems; Hupp and Bornette, 2003). At dynamic locations, stand age is therefore lower, resulting in a stand structure with lower mean diameter and higher stem number (Everson and Boucher, 1998). In the course of increased die-back at these locations, canopy cover is reduced, which leads to increased cover of shrub and herb layer. Woody biomass is significantly lower in stands with low mean diameter, reflecting the importance of successional stage growth for biomass in riparian forests (Giese et al., 2003; Keeton et al., 2007). In contrast, coarse woody debris as an indicator both of local tree mortality and of proximity to water bodies connected to the main channel at high water levels increased significantly in stands with lower canopy cover. Model prediction remained low as deposition of coarse woody debris is influenced by various factors (Gurnell et al., 2002).

Along with vegetation, soil properties proved to be influenced by local conditions. We detected a significant positive impact of a low mean diameter and a high cover of herbaceous plants on number of horizons, which added to the effect of the lateral gradient. The C concentration of the Ah horizon decreased with cover of the herb layer. The model with altitude, distance to next channel and herb cover was significant, which might be explained by high local river meander dynamics and reduced activity of soil organisms, which have to colonize a site after inundation.

Locally dynamic sites with high stem number, open tree canopy and high herb cover showed significantly lower levels of soil C pools. Surprisingly, forest vegetation units of the Danube floodplain based on species composition did not show significant differences in soil C stocks (Cierjacks *et al.*, 2010). This might be attributed to the fact that particular tree species may persist a long time at a given site, even if fluvial geomorphology has changed, while structural parameters indicate the history of geomorphologic processes (Hupp and Bornette, 2003). Grassland, in contrast, exhibited clearly higher soil carbon stocks in the study area, whereas on arable land stocks were lower (Zehetner *et al.*, 2009; Cierjacks *et al.*, 2010).

In summary, our results are in agreement with the following conceptual model: Spatial gradients and forest stand structure are indicators for the geomorphic dynamics of a given site and reflect a gradient of fluvial dynamic within the study area. The extremes of this gradient are illustrated in Figure 3. Highly dynamic sites are characterized by low tree cover, high stem number and low distance from the main channel. These sites exhibit high dynamics of sedimentation conditions, which results in the formation of various but shallow soil horizons. Some of the horizons are coarsely textured and poor in organic matter, and buried Ah horizons indicate longer phases without larger sedimentation events. In contrast, sites with low river dynamics are indicated by high tree cover, low stem number and greater distance from the main channel. These sites show continuous but slow delivery of C-enriched fine-textured sediments. As a consequence, fewer, thicker horizons are found at less dynamic sites and C stocks are large.

All C pools in floodplains depend on natural flood dynamics. Both sedimentation and vegetation are known to be influenced by river engineering works (e.g. Merritt and Cooper, 2000; Elderd, 2003; Leyer, 2006; Hupp and Rinaldi, 2007). Consequently, C stocks in soils decrease as a consequence of dike construction (Haubenberger and Weidinger, unpublished manuscript). River bed incision results in decreasing groundwater levels and reduced flooding which may transform the C pools of riparian areas into net C sources (Busse and Gunkel, 2002). Therefore, conservation efforts such as dike perforation and river bed stabilization would lead to increased carbon sequestration of riparian areas. Furthermore, land-use changes will influence carbon dynamics in floodplains (Zehetner *et al.*, 2009). Hence, the importance of lowland

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floodplains as C sinks should be recognized by conservationists and landscape planners.

## **CONCLUSION**

We conclude that spatial information and vegetation data indicate gradients of geomorphic floodplain dynamic, which is the main driver of organic matter storage besides land use. The flood histories of different areas are evidenced by the varying quantity and quality of sedimentation and accumulation levels of organic C. The majority of the spatial information and stand parameters are detectable by remote sensing techniques (Mertes, 2002; Turner et al., 2004; Whited et al., 2007; Kooistra et al., 2008). Thus, indicators of recent fluvial dynamics, such as spatial gradients and forest stand data, are promising tools for modelling the C sequestration of floodplains. However, the models reported in this study still show low predictive power and further variables should be included to increase model quality. In addition, studies in other floodplains may improve the knowledge on carbon-related processes. In particular, high contents of inorganic C in soils might influence the amount of organic C in sediments due to differing stabilities of C complexes.

### ACKNOWLEDGEMENTS

We thank Christian Baumgartner and Christian Fraissl from the Donau–Auen National Park administration for their support during all phases of the project. Maren Babinsky, Nico Bätz, Sebastian d'Oleire-Oltmanns, Julie Donner, Kristin Fenske, Josephine Hübener, Fritz Kleinschroth, Judith Kühn, Benedikt Litschko, Arvid Markert, Ingo Marziller, Markus Menzel, Susan Rohsmann, Jan Schormann and Ulrike Ziechmann from the Technische Univerität Berlin helped during field work and data processing. Danny McCluskey kindly checked our English. The figures were prepared by Wilfried Roloff and one anonymous reviewer gave valuable comments on an earlier version of the paper. This study was financed by Deutsche Forschungsgesellschaft (DFG, grant number LA1398/4) and Technische Universität Berlin.

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