Philipps Universität Marburg

Ecological effects of willow and poplar invasions at the Río Negro in northern Patagonia, Argentina

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“The river [the Río Negro] *is about two or three hundred yards wide, and is deep and rapid. The many islands, with their willow-trees, and the flat headlands, seen one behind the other on the northern boundary of the broad green valley, form, by the aid of a bright sun, a view almost picturesque.”*

Charles Darwin, *The Voyage of the Beagle*, 1839

List of papers

The main chapters of this thesis are based on two publications and two manuscripts with the following authorships and titles. These papers are referred to in the text by the Roman numerals I-IV.

I. **Thomas L.K.**, Tölle L., Ziegenhagen B. & Leyer I. (2012) Are vegetative reproduction capacities the cause of widespread invasion of Eurasian Salicaceae in Patagonian river landscapes? *PloS one* **7**, doi:10.1731/journal.pone.0050652.
II. **Thomas L.K.** & Leyer I. (2014) Age structure, growth performance and composition of native and invasive Salicaceae in Patagonia. *Plant Ecology* **215**, 1047–1056.
III. **Thomas L.K.**, Mosner E. & Leyer I. (in press) River dynamics and invasion: Distribution patterns of native and invasive woody vegetation at the Río Negro, Argentina. *Riparian Ecology and Conservation.*
IV. Fettweis K., Thies B., **Thomas L.K.** & Leyer I. (manuscript) Detection of land cover and vegetation change by invasive woody species spread at the Río Negro, Argentina.

For **paper I (Chapter 2)**, I assisted I. Leyer to develop the conceptual design of the study. I sampled cutting material along the Río Negro. Together with L. Tölle I conducted the greenhouse study and data analyses, and I had responsibility for the writing. For **paper II (Chapter 3)**, I created the general concept and the study design together with I. Leyer. I conducted all the field work and analyses, and I was responsible for the writing. For **paper III (Chapter 4)**, I developed the concept of the study in collaboration with I. Leyer. I worked out the study design, the statistical analyses and mainly wrote the manuscript. Regarding **paper IV (Chapter 5)**, I participated in developing the conceptual ideas, gave support during data collection in Argentina and commented on the manuscript.

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Preface

This thesis was carried out within the German-Argentinean cooperation project *"Predicting the effects of invasion processes of Eurasian floodplain willows in Patagonia"* funded by the German Research Foundation (DFG grant LE 1364/4-1). To our knowledge, it is the first study that deals with Salicaceae invasion and its consequences for a riparian ecosystem in Patagonia and for *Salix humboldtiana* Willd., the only native willow species in this region. Based mainly on field methods and modelling approaches I expected to gain insights into ecologically related questions concerning invasion processes. This basic research should provide a sound basis for understanding invasion of Salicaceae and for future management and conservation strategies. Population genetic approaches (i.e. hybridization and evolutionary processes) were conducted by the cooperation partner and compose a different thesis.

A general framework of this thesis is given in the Introduction (**Chapter 1**) illustrating the problem of invaded riparian ecosystems and, in particular, the invasion processes along the Río Negro in Northern Patagonia. The thesis is subdivided into four main topics which are represented each by a publication or manuscript. In **Chapter 2** the vegetative reproduction capacities of native and invasive Salicaceae were compared since the property to reproduce vegetatively plays a main role for the establishment and invasion success. In an experimental approach survival, growth performance and re-sprouting capacities were analysed for all relevant taxa taking soil moisture and soil composition gradients into account. In the following **Chapter 3** age structure analyses were carried out to clarify establishment processes. Measurements of growth performance in relation to the age of the trees of the four dominant Salicaceae taxa in mixed forest stands were conducted. Therewith can be assessed whether interspecific competition occur in older life stages in Salicaceae forests and, therefore, whether the native species can co-exist in the future. **Chapter 4** focuses on distribution patterns and ecological niches of the dominant native and invasive woody species along the Río Negro. Habitat distribution models were developed in order to relate abiotic factors (e.g. flood duration, average water level, flow strength, soil texture) to the presence and absence of different life stages of all target taxa. In the final paper (**Chapter 5**) land cover changes during the last decades focussing on the spread of invasive species were analysed. Therefore, remotely sensed Landsat satellite image data of the years 1986 and 2003 were compared in order to identify vegetation changes at the upper Río Negro valley. Finally, the main results of the chapters 2-5 are summarized and consequences for the conservation of *S. humboldtiana* are pointed out in **Chapter 6**.

Summary

Species of the Salicaceae family, particularly *Salix* species and their hybrids, are typical invaders of river systems throughout the world with severe consequences for native ecosystems. Along Patagonian streams, riparian softwood forests structured by dominant invasive Salicaceae are increasing significantly in abundance, area and species diversity. The region of the Río Negro in northern Patagonia has been invaded by several woody plant species with a dramatical increase in the recent decades. The most dominant taxa are willows of the *Salix alba* L. - *Salix fragilis* L. complex, a hybrid of this complex and *Salix babylonica* L. and *Populus* spp. Additionally, *Elaeagnus angustifolia* Willd. and *Tamarix* spp. have been spreading aggressively in recent years. This thesis provides information on the possible consequences of these invasions for *Salix humboldtiana* Willd., the only native woody species along the Río Negro, due to interspecific competition and invasion development.

Vegetative reproduction is especially for *S. fragilis* and its hybrids of great importance for the colonisation of new habitats and probably is the most important reason for their invasion success. In the first study (**Chapter 2**), a greenhouse experiment was conducted in order to assess the vegetative reproduction capacities of native and invasive Salicaceae (*S. humboldtiana*, *S.* × *rubens* Schrank, a *S. babylonica* hybrid and *Populus* spec.) under various soil composition and moisture gradients. The invasive willow hybrids showed better vegetative re-sprouting capacities and performed sinificantly better than *S. humboldtiana* and *Populus* spec. They developed more living cuttings as well as more and longer sprouts under all treatment combinations and they generated significantly higher above- and belowground biomass. After completely removing the shoot and root biomass of the cuttings re-sprouting capacities were evaluated in a second experiment. Again, the invasive willows had a superior re-sprouting ability and growth performance. These results demonstrate that invasive willows are able to establish more successfully by vegetative reproduction than the native willow and that they have a higher re-sprouting potential after disturbances. This ability in combination with the advantages of vegetative reproduction compared to generative reproduction seems

to be the key factor for invasive success and, simultaneously, a potential threat for *S. humboldtiana* through out-competition.

In a further approach (**Chapter 3**), age structure analyses were carried out which revealed that mixed adult forest stands are the results of joint establishment events with all Salicaceae taxa involved. The analysed 20 stands on islands had low mean ages <15 years. Investigation on growth performance (basal area, crown diameter, tree hight and crown base hight) of adult stands revealed that non-native willows and poplars are able to actively displace the native *S. humboldtiana* by competitive strength: A significant better growth performance with e.g. higher basal area and crown diameter was found for the non-native willows in adult life stage. Furthermore, adult *S. humboldtiana* was more frequent at the middle river stretch, while invasive willow hybrids showed an opposing pattern suggesting a downstream directed invasion process.

In order to evaluate the competition potential of invasive alien woody plants (Salicaceae, *Elaeagnus* and *Tamarix*) for *S. humboldtiana,* habitat distribution models for different life stages (seedling, juvenile, adult) were developed (**Chapter 4**). The objective was to identify ecological niches and relationships between species occurrence and different abiotic factors. Data on species (presence/ absence) and explanatory environmental variables were gathered in the field on 167 plots using a grid-based, stratified-randomized sampling design. The environmental variables flood duration, the amount of gravel and the location (upper or middle river valley) proved to be the best explanatory variables to describe the occurrence of the species. For all life stages, a strong niche overlap could be observed for *S. humboldtiana* and invasive taxa, particularly Salicaceae, with no remaining exclusive habitat for the native willow.

Additionally, investigations on vegetation change offer insights into invasion development (**Chapter 5**). Multispectral satellite images from the years 1986 and 2003 of the upper Río Negro were used to detect land cover changes. This study demonstrated that during this time period floodplain forests expanded considerably their distribution area by almost 150%, probably due to the explosive spread of non-native species.

The results of this thesis can be summarized as follows: Invasive willows have significant better vegetative reproduction and re-sprouting capacities, better growth performances in the adult life stage and exhibit a strong niche overlap with the native willow species. These findings indicate a high competition potential of invasive Salicaceae with, consequently, a possible out-competition and suppression of *S. humboldtiana*.

Zusammenfassung

Salicaceen, insbesondere verschiedene *Salix*-Arten und ihre Hybride, sind weltweit typische Invasoren von Flusssystemen und können schwerwiegende Folgen für heimische Ökosysteme hervorrufen. An den Flüssen Patagoniens nehmen Weichholzauwälder, die von invasiven Salicaceen dominiert werden, an Häufigkeit, Fläche und Artenvielfalt deutlich zu. So beobachtet man entlang des Río Negro in Nordpatagonien eine Invasion verschiedener holziger Pflanzenarten, die in den letzten Jahrzehnten dramatisch angestiegen ist. Dominant hierbei sind die Weiden des *Salix alba* L. - *Salix fragilis* L. Komplex, ein Hybrid dieses Komplexes und *Salix babylonica* L. und *Populus* spp. Zusätzlich breiten sich in letzten den Jahren *Elaeagnus angustifolia* Willd. und *Tamarix* spp. aggressiv aus. Die vorliegende Doktorarbeit liefert Daten zu möglichen Auswirkungen dieser Invasion auf *Salix humboldtiana* Willd., die einzige heimische Baumart am Río Negro.

Vegetative Reproduktion spielt besonders für *Salix fragilis* und ihre Hybride eine wichtige Rolle bei der Besiedelung neuer Habitate und ist wohl der wichtigste Grund für ihre erfolgreiche Invasion. Diese Hypothese wurde in der ersten Untersuchungsreihe in einem Gewächshausversuch zur vegetativen Reproduktionsfähigkeit der heimischen Weidenart (*S. humboldtiana*) und der invasiven Salicaceen (*S.* × *rubens*, ein *S. babylonica* Hybrid und *Populus* spec.) unter verschiedenen Gradienten der Bodenbeschaffenheit und Feuchte getestet (**Chapter 2**). Beide invasive Weidenhybride zeigten eine bessere Wiederaustriebsfähigkeit und wuchsen signifikant besser als *S. humboldtiana* und *Populus* spec. Sie hatten mehr lebende Stecklinge sowie mehr und längere Triebe unter allen getesteten Bedingungen und bildeten mehr Biomasse (über- und unterirdisch) aus. Nach Entfernung der gebildeten Biomasse von den Stecklinge wurde in einem nachfolgenden Experiment die Wiederaustriebsfähigkeit dieser Stecklinge gemessen. Erneut hatten die Stecklinge der invasiven Weiden eine bessere Austriebsfähigkeit und Wachstumsperformance. Diese Ergebnisse zeigen, dass invasive Weiden eine erfolgreichere vegetative Reproduktion als die heimische Weidenart haben und ein höheres Potenzial für ein Wiederaustreiben nach Störungen besitzen. Diese Eigenschaften

scheinen ein zentraler Faktor für ihre erfolgreiche Invasion zu sein stellen gleichzeitig eine mögliche Bedrohung für *S. humboldtiana* durch Verdrängung dar.

In einer weiteren Untersuchung wurden Analysen der Altersstruktur und Wachstumsperformance in gemischten adulten Waldbeständen auf Flussinseln durchgeführt (**Chapter 3**). Die ausgewerteten 20 Bestände hatten ein relativ geringes, aber einheitliches Alter (im Mittel < 15 Jahre), was auf gemeinsame Etablierungsereignisse aller erfassten Arten weist. Die Analyse der Wachstumsperformance (Grundfläche, Baumhöhe, Kronendurchmesser, Kronenansatzhöhe) in adulten Beständen zeigte, dass invasive Weiden und Pappeln durch ihre Konkurrenzstärke in der Lage sind, die heimische *S. humboldtiana* aktiv zu verdrängen: Im adulten Stadium besaßen die invasiven Weiden beispielsweise deutlich größere Grundflächen und Kronendurchmesser. Adulte *S. humboldtiana* konnte häufiger am mittleren Flusslauf nachgewiesen werden, während die invasiven Weidenhybride am Flussoberlauf häufiger waren. Dies deutet auf einen flussabwärts gerichteten Invasionsprozess hin.

Um das Kokurrenzpotenzial von invasiven holzigen Arten (Salicaceen, *Elaeagnus, Tamarix*) gegenüber *S. humboldtiana* zu ermitteln, wurden Habitatmodelle für unterschiedliche Altersklassen (Keimlinge, junge und alte Vegetation) entwickelt (**Chapter 4**). Dabei sollten die ökologischen Nischen und Zusammenhänge zwischen Artvorkommen und abiotischen Bedingungen (z.B. Überflutungsdauer, durchschnittlicher Wasserstand, Strömungsstärke) identifiziert werden. Daten zu den Arten (Vorkommen/ Nichtvorkommen) und erklärenden Umweltvariablen wurden mit einem stratifiziert-randomisierten Stichprobendesign auf 167 Plots gesammelt. Die Umweltvariablen Überflutungsdauer, Kiesanteil und Ort (Ober- bzw. Mittellauf) konnten am besten das Vorkommen der Arten beschreiben. Für alle Altersklassen ergab sich eine starke Nischenüberlappung der invasiven Arten, insbesondere der nicht-heimischen Salicaceen, mit *S. humboldtiana*. Auf keinen Standorten konnte ausschließlich *S. humboldtiana* nachgewiesen werden.

Zusätzlich wurden multispektrale Satellitenbilder des Flussoberlaufes aus den Jahren 1986 und 2003 ausgewertet (**Chapter 5**). Daraus geht hervor, dass in dieser Zeitspanne Weichholzauwälder ihre Verbreitung um fast 150% ausdehnten, was höchstwahrscheinlich an einer explosionsartigen Verbreitung der nicht-heimischen Arten lag.

Die Ergebnisse dieser Doktorarbeit können wie folgt zusammengefasst werden: Invasive Weiden haben eine signifikant bessere vegetative Reproduktion und Wiederaustriebsfähigkeit und eine bessere Performance im adulten Stadium als die heimische Weidenart sowie eine starke Nischenüberlappung mit dieser. Diese Ergebnisse verdeutlichen das hohe Konkurrenzpotenzial von invasiven Salicaceen und eine daraus resultierende mögliche Unterdrückung und Verdrängung von *S. humboldtiana*.

Chapter 1 – Introduction

1.1 Riparian ecosystems and invasion

Floodplain ecosystems and their habitats are characterized by a high biological potential, dynamic processes, productivity and species richness. Multiple natural disturbances create a shifting habitat mosaic with a structural diversity from the river head to the delta with different landscape elements (Naimann and Décamps 1997; Ward et al 2002). Hydro-geomorphic processes with frequently occurring destructive floods create, maintain and degrade riparian habitats (Steiger et al 2005) and provoke a permanent rearrangement of the landscape. These hydrological and fluvial developments determine plant distribution patterns (Hupp and Osterkamp 1996; Richardson et al 2007; Stromberg et al 2010). Opportunities for plant establishment, i.e. bare sediments, mostly occur after floods by erosion and sedimentation processes, and the following flood regime determines whether seedlings may establish and survive (Bendix and Hupp 2000).

These characteristics make riparian ecosystems especially prone to biological invasion of non-native species. Main reasons for this high susceptibility to the establishment of non-native plants are the environmental heterogeneity across river corridors (Ward et al 2002), the natural disturbances and the hydrological connectivity (Ward et al 2002) which serves as dispersal vector for invasive plants (Renöfält et al 2005; Leyer 2006). Besides natural characteristics that make riparian ecosystems vulnerable to exotic plant invasion, also river regulation, causing a reduction in peak flow and considerable changes in hydrology, may push these processes (Naiman and Décamps 1997; Catford et al 2011). The diversity and abundance of alien plants in river floodplains has increased significantly throughout the world (Richardson et al 2007) representing a serious problem for conservation biologists. Indeed, riparian corridors are among those habitats, which are very susceptible to alien plant invasion and they have been invaded by exotic species more than other habitats (Planty-Tabacchi et al 1996; Stohlgren et al 1998; Stohlgren et al 1999). The introduction of exotic plants that naturalize in a large-scale manner is an important human-induced threat to riparian zones with a predicted continuance in the future (Tockner and Stanford 2002). In this context, the definition of 'naturalized' used in this thesis follows Pyšek et al (2004) and refers to '*alien plants that sustain self-replacing populations for at least 10 years without direct intervention by people (or in spite of human intervention) by recruitment from seed or ramets (tillers, tubers, bulbs, fragments, etc.) capable of independent growth*'. When producing reproductive offspring and spreading over a large area, they are invasive (Pyšek et al 2004).

Not only the river may modify the natural morphology of its channel, but also softwood floodplain forests themselves, which are a major biological and structural component of riparian zones since they act as ecosystem engineers (Barsoum 2002; Steiger et al 2005) driving hydro-geomorphological processes (reviewed by Gurnell 2014). Consequences of vegetation can be channel incision and narrowing (Osterkamp and Hupp 2010). Non-native plants may alter water levels if they use more water than native trees and, if invasion results in a larger area covered by plants (Hultine and Bush 2011), are thus in the position to modify their environment significantly (Corenblit et al 2014). A well-known example is the invasion of Salt cedar (*Tamarix* spp.) along North American watercourses which results in reduced ground water levels and water flows as well as in replacement of natural vegetation (Di Tomaso 1998).

Salicaceae species of riparian softwood forests are typical pioneer species and are adapted to the natural dynamics of rivers and to strong and frequent disturbances. In addition to a high growth rate, they have a short life span and high tolerance to flood. Eurasian willows are typical invaders of riparian ecosystems worldwide and will continue to change native communities in the coming decades. Their importance as woody plant invaders has already been recognized with e.g. *S. fragilis* L. belonging to those species that are very widespread and occur in six or more different regions (Richardson and Rejmánek 2011). Mainly planted for ornamental or functional use willows naturalized widely along rivers worldwide, e.g. in Australia (Cremer 2003), New Zealand (Glova and Sagar 1994), South Africa (Henderson 1991) and North America (Shafroth et al 1994) and can cause severe changes of the habitats they invade. In Australia, several willows are declared as 'Weeds of National Significance' (Cremer 2003; Adair et al 2006) since they invade sometimes riverbanks and wetlands explosively and replace native riparian vegetation. Further consequences of invasive willows are manifold:

they can alter fluvial dynamics and facilitate the development and growth of sand bars and islands (Moggridge and Gurnell 2009), divert streams, provoke erosions (Cremer 2003) and changes of water resources and light conditions have consequences e.g. for macroinvertebrates (Lester et al 1994) and bird assemblages (Holland-Clift et al 2011).

Effective reproduction and different reproduction strategies in combination with high growth rates may explain the invasion success of *Salix* taxa. Species' reproduction systems belong to the most important factors that determine if species can naturalize widely outside the native range (Baker 1974). A characteristic trait of *Salix* species is the ability to regenerate either sexually or asexually under a range of environmental conditions (review in Karrenberg et al 2002). The reproduction via vegetative fragments is regarded as one explanation for the success of invasive alien plants (Kolar and Lodge 2001). Accordingly, *S. fragilis* is so far the most extensive invasive willow in Australia due to its vegetative spread (Cremer 2003). In particular *S. fragilis* and *S.* × *rubens* Schrank have breaking points at the twig bases leading to brittle branches (Beismann et al 2000). Broken twigs fall into the water and can be carried downstream, where they are able to resprout on suitable habitats (Beismann et al 2000). Vegetative dispersal units (broken twigs) can tolerate broader environmental conditions than seedlings and usually have higher survival rates which could favour the invasive willow taxa (Asaeda et al 2011).

Several non-native willow and poplar species are cultivated in Argentina. Some of them naturalized widely and imply a potential threat to the native vegetation. In Patagonia, the naturalization of non-native willows and poplars have led to significant changes in riparian ecosystems over the last century. Especially *Salix* taxa, in particular *S.* × *rubens*, have built wide spread softwood forests along river margins and dominate several riparian communities. Along the Río Negro in Northern Patagonia, great changes due to invasive woody tree species could be observed, especially in the last decades, so that today, dense floodplain forests can be observed which are dominated by invasive Salicaceae taxa with, so far, unknown effects for *Salix humboldtiana* Willd., the only native tree species in this region. Compared to other Patagonian rivers, the invasion process has reached an advanced stage with involved evolutionary processes (Budde et al 2011). This is probably based on the fact, that human influence is more intense in the Río Negro region within the otherwise low populated

Patagonia, and several non-native species have been planted next to the river. This assumption is consistent with findings from Speziale and Ezcurra (2011) who showed that alien species richness is higher around cities in northwestern Patagonia. In order to contribute to a better understanding of these invasion processes, this thesis focuses on the development of Salicaceae invasion and competition among native and invasive species at the Río Negro.

1.2 Study area – the Río Negro

As study area, the largest Northern Patagonian river system, the Río Negro, was chosen since this free flowing river is characterized by natural dynamics (Fig. 1). Strong hydrological and geomorphological processes provide an ideal habitat for floodplain pioneer species and, thus, native as well as invasive Salicaceae occur frequently.

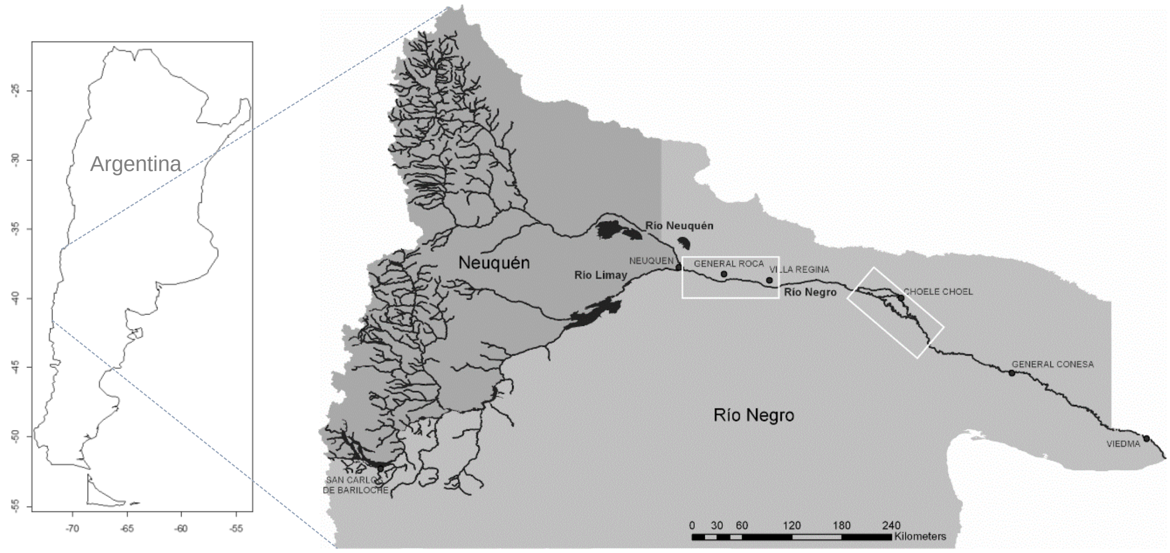

Figure 1 The Río Negro in northern Patagonia. All studies were conducted in the marked sections in the upper and the middle river stretch.

Two tributaries form the river near the city of Neuquén at 225 m a.s.l., the rivers Limay and Neuquén, which rise both in the Andean Mountains. The Río Limay is the main feeder river of the Río Negro with a mean discharge of 726 $m^3 s^{-1}$ (Cushing et al 2006). It originates from the glacial Lago Nahuel Huapi and is naturally regulated by oligotrophic lakes (Cushing et al. 2006). Since the 70s of the last century all together five dams have been built along the river for hydroelectric purposes. The smaller eutrophic Río Neuquén that originates in the north-west of the province Neuquén has a mean annual discharge of 296 $m^3 s^{-1}$. Due to unpredictable floods it was diverted into the Lago Pellegrini in 1914 (Cushing et al 2006). Since that time four additional dams have been built along this river.

Near the city of Neuquén, the confluence of the rivers Limay and Neuquén, the Río Negro flows approximately 630 km to the Atlantic Ocean crossing the Patagonian High Plateau. The river drains a catchment area of 65000 km^2 (Cushing et al 2006) and has a mean annual discharge of 900 $m^3 s^{-1}$ near the city of Neuquén (AIC, Autoridad Interjurisdiccional de las Cuencas de los ríos Limay, Neuquén y Negro) with pronounced water level fluctuations up to 2.52 m at the water gauge near Mainqué in the upper valley in the years 2006-2010 (DPA, Departamento Provincial de Aguas Río Negro) (Fig. 2). The natural flow regime is governed by snowmelt in the Mountains, but since the onset of dams at the Rivers Limay and Neuquén, flood disturbances are occasional and depend on the need of energy. The Negro River itself is not regulated by dams and still shows natural dynamics. Despite river bank stabilisation it flows in its natural shape slightly meandering and forming exposed vegetated islands (Fig. 3 c).

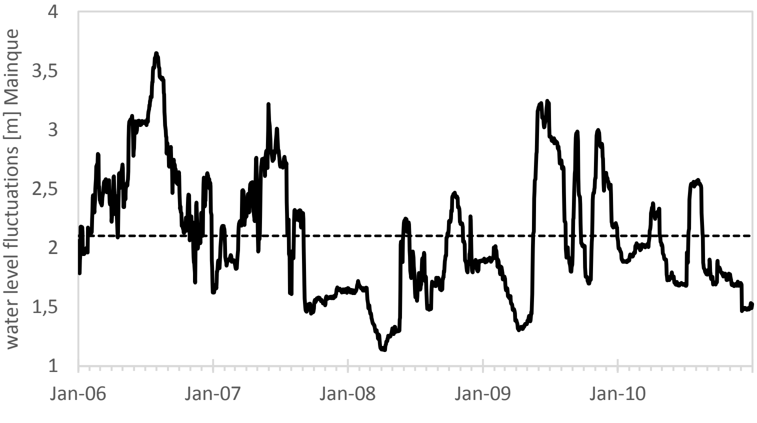

Figure 2 Hydrograph of daily water levels (2006-2010) of the Río Negro at the water gauge Mainque, near Villa Regina (DPA, Departamento Provincial de Aguas Río Negro). The dashed line displays the average value.

The area of the Rio Negro has a temperate semi-arid to cold arid climate (Klich 2000). The mean annual precipitation rate is less than 250 mm and the mean annual temperature is 14-20 °C (Gut 2008). The amount of rainfall decreases from the Andean Mountains to the Atlantic Ocean: At the origin of the Río Negro the mean annual precipitation is 500 mm and decreases to 100 to 200 mm towards the sea (Cushing et al 2006). The valley at the upper part of the river is up to 10 km wide and around 100 to 250 m deep. It is situated in the Patagonian tableland and surrounded by the dry shrubby Patagonian steppe.

Unlike the dry treeless Patagonian steppe, the valley offers great conditions for agriculture due to sufficient temperature and soil moisture (Naumann 1996) and has therefore a great ecological value. Like other riparian ecosystems (Tockner and Stanford 2002) the Río Negro is strongly threatened by changing land-use patterns: Riparian corridors are transformed to cropland or infrastructure development. Almost all available area is used for intensive agriculture. The upper river valley (Alto Valle) is the principle region of Argentina for apple and pear production. Hence, the valley is characterised by large fruit plantations, mostly at the northern side of the river, which range to the river shores (Fig. 3 b). It is obvious that a large amount of water is excessively withdrawn for intensive agriculture. Simultaneously, pesticides and effluents are discharged into the riparian ecosystem. Downstream at the middle river valley (Valle Medio) large plantations are less frequent and farming and cattle breeding become more important. In the Valle Medio, the river shape is characterised by a stronger meandering and branching. On account of the high anthropogenic disturbance near the river margin either by planting, argiculture or grazing all analyses of this investigations were conducted on islands in the active zone of the river with far less human influence.

The natural vegetation of the river valley is composed of *Salix humboldtiana* as the only native tree species and of the typical shrubby steppe vegetation adapted to semi-arid or arid conditions (e.g. *Larrea spp.*, *Prosopis spp.*, *Baccharis spp.*) (Gut 2008). However, great changes in species communities occurred during the last decades due to invasive species, and gallery forests, dominated by invasive Salicaceae, occupy large areas (Fig. 3 a). The valley has been invaded by alien woody species like *Salix* spp., *Populus* spp. *Elaeagnus angustifolia* and *Tamarix* spp., and several herbaceous species that may alter the ecosystem profoundly.

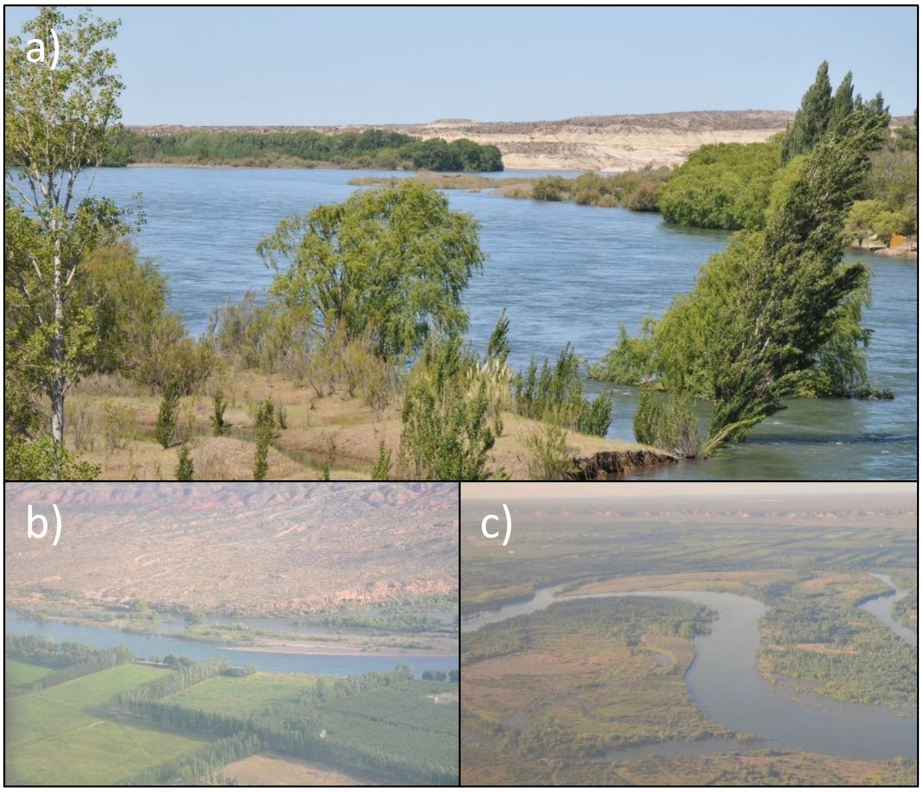

Figure 3 Woody vegetation along the Río Negro: a) mixed Salicaceae forests and dynamic processes, i.e. erosion and uprooting during flood events; b) the southern shore of the upper reach is characterized by the uprising high plateau, the northern shore by fruit plantations with enclosing planted poplar and/or willow rows which range to the river shore; c) example for the meandering and islands in the curse of the river.

1.3 Woody vegetation along the Río Negro

Salix humboldtiana Willd. is the only native floodplain tree species that occurs along the Río Negro, as well as the only native willow species in South America. It is native to southern Mexico and Central and South America. *S. humboldtiana* occurs along river margins in warm temperate and subtropical climates and is intolerant to frosts (Isebrands and Richardson 2014) and shade (Parolin et al 2002). In its distribution center in subtropical parts of South America, *S. humboldtiana* is an evergreen tree that can form monospecific stands. It is known to be the first colonizing pioneer tree species of newly formed sediment banks along river margins (Liotta 2001; Parolin et al 2002; Casco et al 2010).

The southernmost occurrence of *S. humboldtiana* is at the river Chubut in the Santa Cruz province, about 500 km south of the study area. It is not ascertainable to which extent *S. humboldtiana* occurred along the river Negro in the past. It was probably frequent at the river banks of the rivers Limay and Negro forming floodplain forests (Hauman et al 1947). Although *S. humboldtiana* is still a frequently occurring species along the Río Negro (Fig. 4 a,b), floodplain forests are today dominated by invasive species (Fig 4 c) with negative effects for the native species. In other parts of Argentina, e.g. the Pampean grassland, *S. humboldtiana* became scarce due to the colonization of exotic tree species (Zalba and Villamil 2002).

S. alba L., *S. fragilis* L. and their hybrid *S.* × *rubens* Schrank (Fig. 4 f) are typical invaders in riparian ecosystems, and willows of this complex naturalised widely along Patagonian river systems. Contrary to other literature referring only to *S. fragilis* in Patagonia (e.g. Correa 1984; Serra et al 2013), Budde et al. (2011) could only identify individuals of the hybrid *S.* × *rubens*. Salix species are dioecious with male and female individuals. In the North Patagonian lake region, Budde et al. (2011) reported an expanded distribution of a single female clone of the *S. alba-S. fragilis* complex suggesting a successful invasion only by vegetative reproduction. These findings correspond with other studies that also assume a successful invasion of single clones. In Australia, only male *S. fragilis* occur (Cremer 2003), whereas only females are known to grow in South Africa (Henderson 1991). Accordingly, female *S.* × *rubens* trees were almost exclusively found in Colorado, USA (Shafroth et al 1994). Although mainly female trees of the *S. alba-S. fragilis* complex occur along the Río Negro, few male trees exist (pers. observation) and several genotypes could be detected (Budde et al 2011).

The second dominant invasive *Salix* taxon along the Río Negro is a hybrid of the *Salix alba-S. fragilis* complex and S*alix babylonica* L. (hence called *S. babylonica* hybrid, Fig. 4 d,f). *S. babylonica* is native in East Asia, but it has been introduced to many countries as an ornamental tree. For the *S. babylonica* hybrid, too, mainly female trees could be observed in the study area, but male trees could be found occasionally. Adult trees of the *S. babylonica* hybrid can be easily distinguished from *S.* × *rubens* by the “weeping habitat” of the trees and the previous development of leaves of the *S. babylonica* hybrid during vegetation period (Fig. 4 f).

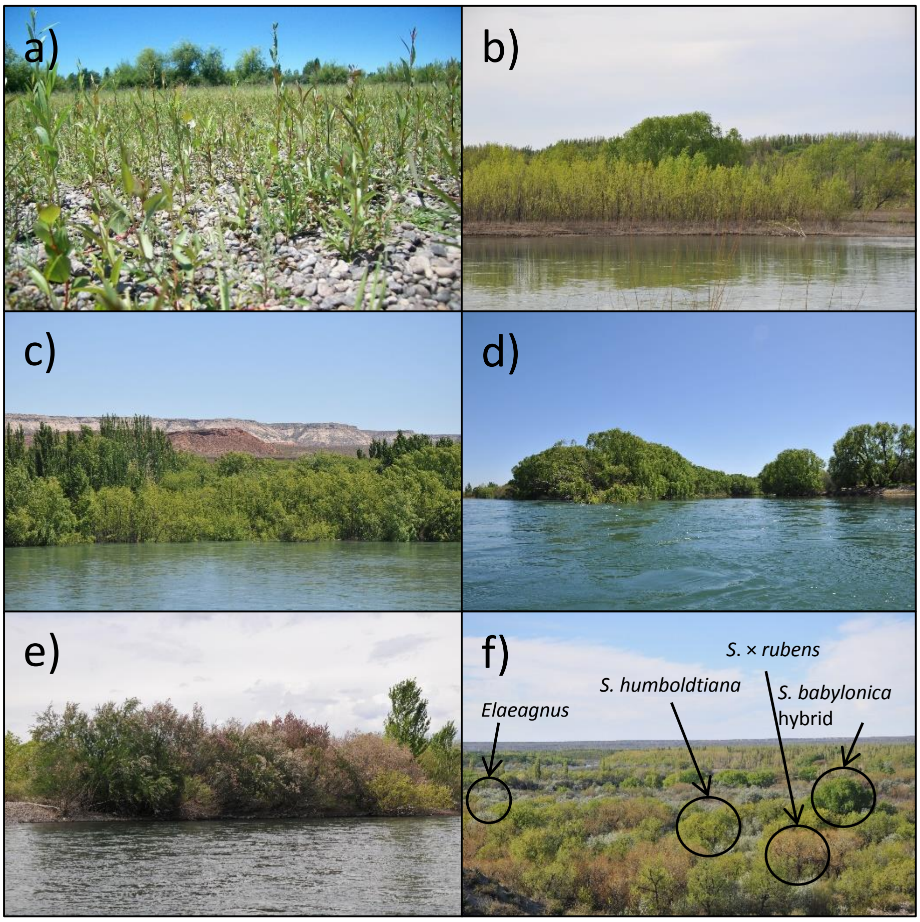

Figure 4 Different life stages and species compositions of softwood forests at the Río Negro: a) Salicaceae seedlings, mainly from *S. humboldtiana* interspersed with invasive willows and poplars; b) one-year old willow gallery, in this case dominated by *S. humboldtiana*; c) adult softwood forest composed of different species; d) island with adult trees mainly composed by the *S. babylonica* hybrid; e) *Tamarix* spp.; f) adult floodplain forest next to the river on former pasture. The marked *Salix* taxa can be easily distinguished early in the growing season: the *S. babylonica* hybrid with foliation and its weeping habitat, *S.* × *rubens* without leaves and *S. humboldtiana* with its light green crowns.

The exact date of the first appearance of non-native willows in Patagonia is unknown. Their introduction probably started in the late 19th or early 20th century by European settlers. Willows and poplars were planted widely for the protection of river banks, as windbreaks and wood source (Naumann 1996; Peri and Bloomberg 2002) and most likely for ornamental reasons. In the Parana River Delta, approximately 900 km linear distance northwards, willows and poplars are extensively cultivated, including hybrids of *S. babylonica*, *S. alba* and *S. matsudana* Koidz. (Borodowski and Suárez 2004; Garau et al 2008). In these plantations several clones of different species and hybrids exist. Among them are different hybrids with *S. babylonica*, having been cultivated since 1953, with a hybrid between *S. babylonica* and *S. alba* (with male and female clones) achieving the best productive results (Borodowski and Suárez 2004).

While the invasion along Patagonian rivers probably started with vegetative reproduction (Budde et al 2011), evolutionary and hybridization processes are possible and seem to become more important as it is the case in Australia where some species and hybrids now also spread by seeds (Cremer 2003). Willows are known to frequently hybridize (Argus 1974; Thiebault 1998; Adair et al 2006) and hybridizations between *S. humboldtiana* and *S. babylonica* as well as willows of the *S. alba-S. fragilis* complex are possible (Hunziker 1992; Borodowski and Suárez 2004). The use of both sexual and asexual regeneration strategies and the occurrence of several new hybrids will probably push the invasion of alien willow hybrids in the future.

Table 1 shows a roughly documentation of flowering time and seed release of the three dominant willow taxa during two growing seasons along the Río Negro. The flowering times differ between the taxa with the *Salix alba-S. fragilis* complex beginning with flowering at the end of August. Although differences in the flowering time between species exist and may vary between years, they partly overlap for all taxa making cross pollinations possible.

Table 1 Mean time of flowering and seed release (average of 2009/2010 and 2010/2011, field observations).

	September	October	November	December	January	February
S. humboldtiana	oooooooooooooooooo					
			xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx			
S. babylonica hybrid	ooooooooooo					
		xxxxxxxxxxxxxxxxxxxx				
S. × *rubens*	ooooooooo					
		xxxxxxxxxxxxxxxxxx				

o = peak of flowering
x = peak of seed release

S. humboldtiana has a balanced sex ratio and produces large amounts of viable seeds in contrast to the invasive species (Fig. 4 a). The sex ratio of non-native willows is restricted which is indicated by the observed low number of seeds developed. In a small field study, in 400 capsules (with 8 trees, 10 catkins and 5 capsules per catkins involved) in total 3569 seeds were counted. The actual number of seeds per capsule ranged from 3 to 19. In contrast, only 268 seeds for the *S. babylonica* hybrid and 319 seeds for *S. × rubens* could be counted. For both these willows, the number of seeds per capsule ranged from 0 to 5. Although the invasive willows produce no or only few seeds in capsules so far, it could be observed that these seeds are viable. Interestingly, great differences exist for the time of seed release. While the few seeds of the invasive willows are all shed till the end of November, *S. humboldtiana* does not begin before end of November, and seed release lasts till the end of February (Table 2).

Table 2 Counted seeds of the three target willow taxa within 8 trees each with 10 catkins with 5 capsules. Displayed are the minimum and maximum of seeds per tree (i.e. 5 capsules in 10 catkins), as well as the mean and total number of all trees.

	Salix humboldtiana	*S. × rubens*	*S. babylonica* hybrid
min nr. of seeds	336	0	0
max nr. of seeds	526	116	149
mean nr. of seeds	446.1	39.9	34.5
total nr. of seeds	**3569**	**319**	**268**

Besides invasive Eurasian floodplain willows other woody species are powerful invaders in the Rio Negro valley including *Populus*, *Tamarix* and *Elaeagnus*. Like willows, poplars have been introduced to Patagonia by European settlers. *P. nigra* L., *P. deltoides* Bartram ex Marsh and their hybrid *P. × canadensis* Moench (Ares et al 2002; Diaz et al 2003) belong to forest building dominant taxa. Tamarisk species (*Tamarix* spp.) und Russian olive (*Elaeagnus angustifolia* Willd.) are spreading aggressively in the Río Negro valley and can alter riparian ecosystems profoundly, too (Fig. 4 e,f). Three *Tamarix* species, native to Eurasia and Africa, are reported to occur along the Río Negro: *T. gallica* L., *T. ramosissima* Ledeb., *T. chinensis* Lour. and probably their hybrids, too (Natale et al 2008; Natale et al 2010). Riparian landscapes in western USA have been changed greatly due to *Tamarix* invasion. As a consequence, restoration programs have been developed that remove this alien plant (Stromberg et al 2009). *Elaeagnus angustifolia*, native to southern Europe and central and eastern Asia, notably expanded in the middle Río Negro valley since circa 1980 (Klich 2000). *Elaeagnus* most frequently occurs on former pastures, forming dense stands on the outside of the active zone of the river. In adult Salicaceae forests *Tamarix* and *Elaeagnus* both usually occur as understory vegetation. The Russian olive, e.g., is also invading and altering semi-arid riparian ecosystems in the USA (Katz and Shafroth 2003; DeCant 2008; Mineau et al 2011).

The purposes of my thesis are: (1) to provide insights into the ecological developments behind native and non-native *Salix* recruitment and vegetation patterns, (2) to assess whether interspecific competition occurs, (3) to identify ecological niches and niche-overlaps of the main woody species, and (4) to discuss whether *S. humboldtiana* is negatively affected by the ongoing invasion process.

Chapter 2 – Paper I

Are vegetative reproduction capacities the cause of widespread invasion of Eurasian Salicaceae in Patagonian river landscapes?

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Abstract

In recent decades, invasive willows and poplars (Salicaceae) have built dense floodplain forests along most of the rivers in Patagonia, Argentina. These invasion processes may affect *Salix humboldtiana* as the only native floodplain tree species in this region. It is assumed, that the property to reproduce vegetatively can play an important role in the establishment of invasive species in their new range. Thus, in order to contribute to a better understanding of willow and poplar invasions in riparian systems and to assess the potential impacts on *S. humboldtiana* the vegetative reproduction capacities of native and invasive Salicaceae were analysed. In a greenhouse experiment, we studied cutting survival and growth performance of the three most dominant invasive Salicaceae of the Patagonian Río Negro region (two *Salix* hybrids and *Populus* spec.), as well as *S. humboldtiana,* taking into account three different moisture and two different soil conditions. In a subsequent experiment, the shoot and root biomass of cuttings from the former experiment were removed and the bare cuttings were replanted to test their ability to re-sprout. The two invasive willow hybrids performed much better than *S. humboldtiana* and *Populus* spec. under all treatment combinations and tended to re-sprout more successfully after repeated biomass loss. Taking into account the ecology of vegetative and generative recruits of floodplain willows, the results indicate that the more vigorous vegetative reproduction capacity can be a crucial property for the success of invasive willow hybrids in Patagonia being a potential threat for *S. humboldtiana*.

Introduction

The introduction of exotic species is one of the most serious causes of man-made changes in ecosystems and grew with increasing human migration and expanding trade (Mack et al 2000). When they naturalize and expand their range, introduced non-native species are called invasive (Richardson et al 2000) and can threaten ecosystems, habitats or species with their establishment and spread. Floodplains are known to be very sensitive to plant invasion because of regular natural as well as human-induced disturbances (Stohlgren et al 1999; Brown and Peet 2003). The hydrological connectivity of river corridors facilitates the dispersal of introduced invasive organisms (Renöfält et al 2005) and the bare-ground sites arising after flood events are ideal for the establishment of invasive pioneer species (Tabacchi et al 2005). In this context, willows and poplars are very successful in occupying new riparian habitats. In the southern hemisphere, introduced willows have widely naturalized along river margins including South Africa, Australia, New Zealand and South America (Correa 1984; Henderson 1991; Glova and Sagar 1994; Cremer 2003). In Australia, some invasive *Salix* species were declared as ‘weeds of national significance’ with e.g. *S. cinerea, S. babylonica* and *S. fragilis* spreading aggressively (Kennedy et al 2003; Cremer 2003; Adair et al 2006). Salicaceae are known to alter fluvial dynamics and to facilitate the development and growth of sand bars and islands (Moggridge and Gurnell 2009) and thus have traits that could alter the ecosystem profoundly if they are invasive. Other possible consequences of invasive willows are the displacement of native vegetation resulting in a loss in biodiversity, the obstruction and diversion of streams and consequent erosion (Cremer 2003). Reduction in the quantity and quality of water and changes in light conditions have consequences for e.g. macroinvertebrates (Lester et al 1994) and bird assemblages (Holland-Clift et al 2011).

Floodplain willows reproduce sexually as well as asexually. Sexual reproduction occurs through the release of a large number of minute, light seeds, which are readily dispersed by wind and water (Moggridge and Gurnell 2009). Seedling survival and establishment is controlled by various factors such as the presence of bare-ground sites and sufficient soil moisture. They do not tolerate burial by sedimentation nor extended submersion by flooding during the growing season (Karrenberg et al 2002). Therefore, in unfavourable years, sexual reproduction can be a rare event (Hughes et al 2001). The ability to reproduce vegetatively is frequently linked to species with a high risk of becoming invasive (Kolar and Lodge 2001). Vegetative reproduction provides an alternative regeneration strategy that is less sensitive in its environmental requirements due to carbohydrate reserves and pre-formed root and shoot primordia (Schier and Campbell 1976). Generally, whenever fragments of branches fall into the water and are swept away by the river, they are able to re-sprout as soon as they are deposited on suitable habitats (Beismann et al 2000). Since regeneration from seeds needs favourable conditions in a very short time period, vegetative fragments play the major role for *Salix* distribution after flood events (Asaeda et al 2011). Indeed, some studies already demonstrated the importance of vegetative reproduction for the invasive spread of willows (e.g. Shafroth et al 1994; Li et al 2005; Lin et al 2009; Budde et al 2011).

In Northern Patagonia, invasive willows and poplars of Eurasian origin have built floodplain forests in almost all riparian landscapes. Mainly willows originating from Europe dominate the Patagonian rivers (Naumann 1996; Budde et al 2011). Probably introduced to South America by European settlers in the 19th or 20th century, their distribution area has increased significantly in the last two decades (Budde et al 2011). One of the largest rivers in this region is the Río Negro, where dense gallery forests occur which are mainly composed of two invasive willow hybrids as well as poplar hybrids. The only native tree species in this area, ***Salix humboldtiana*,** is distributed throughout the warm regions of South America (Liotta 2001) but has become relictual in some places due to competition with invasive species (Zalba and Villamil 2002). The question arises as to whether *S. humboldtiana* can keep up with the invasive Salicaceae or whether it has been affected or will be affected by the still ongoing invasion process.

The aim of the study was to assess whether and how vegetative reproduction as one part of the life cycle could play a prominent role for the spread and establishment of invasive willows in Patagonian river landscapes and therefore contribute to a better understanding of *Salix* invasions in riparian systems.

Consequently, the following questions were addressed in two greenhouse experiments:

1. Do the invasive Salicaceae perform better than the native one regarding survival and growth performance of cuttings taking into account soil moisture and soil composition gradients which correspond to field conditions?
2. Do invasive Salicaceae possess better re-sprouting capacities and perform better than *S. humboldtiana* after repeated shoot and root removal?

Methods

Study Species and Sampling Location

The natural vegetation of the Río Negro floodplain is composed of *Salix humboldtiana* as the only native tree species and shrubby vegetation (e.g. *Larrea spp., Prosopis spp., Baccharis spp.*) (Gut 2008). Introduced tree and shrub species propagate intensively, mainly willows (*Salix spp.),* poplars (*Populus spp.),* salt cedar *(Tamarix spp.)* and Russian olive (*Elaeagnus sp*.). The Río Negro is located in the shrubby Monte steppe in northern Patagonia, Argentina. The climate is arid with a mean annual precipitation of less than 250 mm and a mean annual temperature of 14–20°C (Gut 2008). The Río Negro is formed by the confluence of the Limay River and the Neuquen River and discharges into the Atlantic Ocean. It is about 600 km long and has a mean annual discharge of about 900 m^3/s with pronounced water level fluctuations (DPA, Departamento Provincial de Aguas Río Negro). Both feeder rivers have several dams for energy generation purposes while the Río Negro itself is not regulated by dams and is characterised by pronounced erosion and sedimentation processes. However, unseasonal flooding events due to the dams in the feeder rivers influence the Río Negro as well.

For the greenhouse experiments, *S. humboldtiana* and the three most common invasive Salicaceae were chosen. These are *S.* × *rubens* (hybrid between *S. alba* and *S. fragilis*), a hybrid of *S. babylonica* and taxa of the *S. alba* - *S. fragilis* hybrid swarm including the parent taxa (hereafter referred to as *S. babylonica* hybrid), as well as *Populus* spec. which probably consists of different hybrids with *P. nigra var. italica* and other invasive *Populus* taxa involved as parents (e.g. *P. deltoides*, *P.* *× canadensis*). The determination of *S. humboldtiana* and *Populus* spec. could be carried out without difficulty due to characteristic leaf forms. Distinguishing features of the *S. babylonica* hybrid in comparison to *S. × rubens* are the earlier leaf development in spring as well as its hanging branches. In a preliminary study several samples were genetically determined using microsatellite markers sb243, sb194 (Barker et al 2003) and sb880 (King et al 2010) to relate the morphological characteristics of the hybrids to the taxonomical status (Mengel, C. pers. comm.). It could be shown, that especially the earlier leaf development of the *S. babylonica* hybrid could be used for determination purposes. For each target species, 30 cuttings were harvested from twigs from each of 10 randomly chosen adult individuals. As minimum distance between individuals of the same species 100 m were defined to avoid sampling shoots of the same mother tree. The diameter and length of the samples collected ranged between 5 and 15 mm and between 20 and 26 cm, respectively, according to literature recommendations (e.g. Chmelař and Meusel 1986). The twigs were cut in February 2010 at the Negro River near General Roca (39°06′ S, 67°37′ W) at the upper reach of the river and Luis Beltrán (39°15′ S, 65°45′ W) at the middle reach. The well-developed shoots with leaves were removed before the cuttings were wrapped in moist tissues and sent to Germany, where they were stored for two weeks in darkness at 4°C. Some cuttings across all taxa started to re-sprout slightly or develop roots during storage. Those sprouts and roots were cut off the day before the experiment started.

Experimental Design

Performance experiment

The cuttings of the four taxa involved were subject to three water and two soil treatments in a full factorial design. Two substrate combinations were chosen that imitate the natural conditions in Patagonia resulting from 164 analysed soil samples in the field. For fine-grained soil, a mixture of 40% sand, 50% loam and 10% humus was used and for coarse-grained soil, a mixture of 60% gravel, 30% sand, 5% loam and 5% humus. The soil were filled in perforated PVC boxes (30 cm * 20 cm * 5 cm) and these were inserted into trays to apply three water level treatments. These treatments covered a wide range of the large moisture gradient along which floodplain willows and poplars can grow. We included the treatments 'dry' (trays filled with water up to 1 cm after it had been dried out), 'moist' (water level permanently app. 2 cm under soil surface) and 'waterlogged' (cuttings permanently covered with water). Each factor combination was replicated five times for each taxon. Four bare cuttings of different individuals of the same taxon were laid horizontally in one box resulting in 30 boxes and 120 cuttings per taxon. It was ensured that four different individuals per taxon were chosen for each box by randomly selecting four cuttings from four of the ten 'mother' trees. Between 10 and 14 cuttings per individual were used in the experiment. Five response variables were measured: (i) number of living cuttings as a measure for cutting survival, (ii) number of sprouts, (iii) length of longest sprout emerging from a cutting as well as (iv) shoot and (v) root biomass. Cuttings were defined as dead when they did not develop sprouts until the end of the study. Except for the number of living cuttings which were noted as absolute numbers per box, analyses were conducted box wise as average value of each response variable of living cuttings per box. Therefore, the statistical replicates for all response variables were boxes, not the number of cuttings in each box. The multi-measured variables (i-iii) were taken five and four times within twelve weeks. After the last measurement on day 84, the shoot and root biomass of each cutting was harvested, oven dried in paper bags for one day at 90°C and weighed.

To avoid local effects, all boxes were located randomly in the greenhouse and shuffled every eight to eleven days. During the whole greenhouse experiment, temperature and light conditions remained constant at 15–20°C and 12 hours a day corresponding to the temperature at the Río Negro in spring. Cuttings were treated twice with nematodes (*Steinernema felitidiae*) since large numbers of fungus gnats appeared in the greenhouse. To avoid fungi infection (Ascomycetes), fungicides were applied every ten days (Universal-Pilzfrei M® and Duaxo Universal Pilz-frei®). Additionally, a biological broad-band insecticide (Spruzit®) was used once.

Biomass removal experiment

In a second experimental regime, the re-sprouting capacity after a further shoot and root biomass loss was tested in order to gain insight into the regeneration potential of the different taxa after multiple disturbances. For this purpose, cuttings of the four taxa subjected to the first experiment were used again after the shoot and root biomass harvest. Since an insufficient number of cuttings survived in the former ‘dry’ treatment, only cuttings of the moist and waterlogged water level treatments were included. Cuttings of the four taxa were chosen randomly from different individuals and each planted in a single box (275*88*65 mm) under standardised moisture and soil conditions (waterlogged; soil mixture of 30% gravel, 30% sand, 30% loam and 10% humus). Each factor combination of the previously applied treatments was replicated five times, summing up to a total of 20 cuttings per taxon. All boxes were located randomly and shuffled every seven days. The response variables (i) alive (ii) number of sprouts and (iii) length of longest sprout were measured once after 40 days.

Data Analysis

In the performance experiment, differences among taxa, soil moisture and soil composition concerning the five response variables were tested with three-way factorial ANOVAs followed by Tukey’s method of honestly significant differences.

To test the growth performance over time (number of sprouts, length of the longest sprout), general linear mixed models (GLMM) (Crawley 2007) were used with the repeated measures as the random factor.

Data analysis was carried out using the software package R (Version 2.12.2, R Development Core Team 2011). For the GLMM, the *lme* function in the software package *nlme* (Crawley 2007) was applied. Tukey’s all-pairwise comparisons were conducted in R with the *glht* function in the software package *multcomp* (Hothorn et al 2008).

In the biomass removal experiment, the re-sprouting capacity after shoot and root biomass removal was compared pairwise among taxa with binominal proportion tests (Crawley 2007). The effects of the taxa and the previously applied treatments (soil moisture and soil composition) on the response variables number of sprouts and length of longest sprout were tested with a three-way ANOVA followed by Tukey’s post-hoc test.

Constancy of variance and normality of errors were checked using diagnostic plots. If not fulfilled, the response variables were square root- or log-transformed (Crawley 2007).

Results

Performance Experiment

After 84 days, the *S. babylonica* hybrid (53.3%) and *S. × rubens* (57.5%), showed a higher survival rate than *S. humboldtiana* (29.2%) and *Populus* (38.3%). Species and moisture had profound effects on the number of living cuttings, the number of sprouts and the length of the longest sprout while the soil composition had lesser effects (Table 1). Generally, the growth performance increased for all taxa from dry to moist and waterlogged conditions, and for all treatments the invasive willows achieved the best results (Fig. 1). Independently of the taxa, a better performance could be noted in the fine than in the coarse-grained soil for some response variables (number of sprouts, length of longest sprout, shoot biomass; Table 1).

Table 1 Main effects of species, soil moisture and soil composition on the survival rate (living cuttings) and growth performance (n° of sprouts, longest sprouts, shoot biomass, root biomass) after 84 days (tree-way ANOVAs; response variables were square-root-transformed to improve the diagnostic plots) and general linear mixed models of the number of sprouts and the longest sprout over time. Values are F-values. Levels of significance (p) are denoted with *p<0.05, **p<0.01 and ***p<0.001.

	D.F.	**Living cuttings (ANOVA)**	**N° of sprouts (ANOVA)**	**Longest sprouts (ANOVA)**	**Shoot biomass (ANOVA)**	**Root biomass (ANOVA)**	**N° of sprouts (GLMM)**	**Longest sprout (GLMM)**
Species	3	10.42 ***	9.24***	21.39***	26.52***	37.80***	9.69***	10.64***
Moisture	2	93.0 ***	31.35***	20.48***	24.20***	22.84***	25.48***	52.75***
Soil	1	3.52	4.40*	5.38*	5.40*	0.15	4.32*	0.10
Species: Moisture	6	1.28	0.28	0.76	1.14	2.21*	1.46	7.19***
Species: Soil	3	0.56	0.90	0.74	1.18	0.40	0.37	1.58
Moisture: Soil	2	4.90 **	0.25	0.96	0.95	0.41	1.18	0.07
Species: Moisture: Soil	6	1.21	0.63	0.38	0.39	0.97	0.74	0.88
Residuals	96							

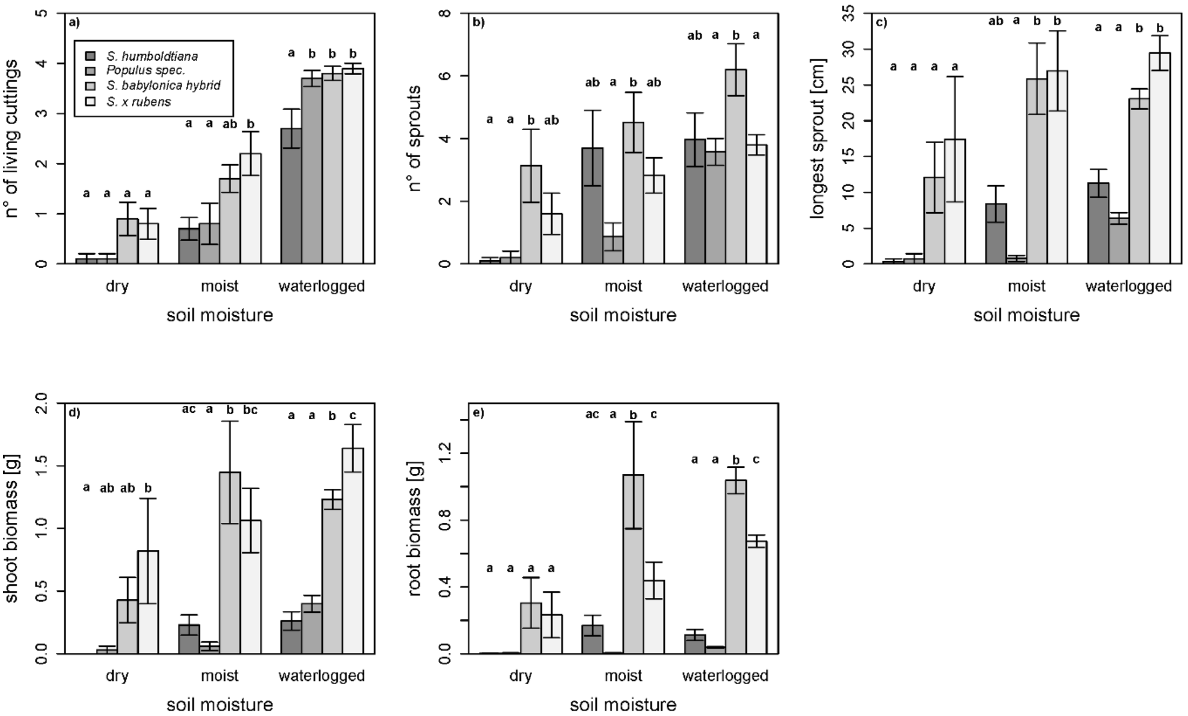

Figure 1 a-e Effects of species and soil moisture. Effects of species and soil moisture on a) number of living cuttings, b) number of sprouts, c) longest sprout, d) shoot biomass and e) root biomass (means ± SE) after 84 days. Different letters indicate Tukey’s HSD between the species (p<0.05).

Overall, the cutting survival tended to be lower for *S. humboldtiana* and *Populus* than for the two invasive willows with some significant results under moist and waterlogged conditions. Under dry conditions, almost all cuttings of *S. humboldtiana* and *Populus* died off (Fig. 1). For the number of living cuttings, an interaction between soil moisture and soil composition could be observed (Table 1) which was visible in the moist water treatment with a higher survival in the fine soil composition than in the coarse one. Regarding the number of sprouts, the *S. babylonica* hybrid performed best under all moisture conditions and, except under dry conditions, *S. humboldtiana* developed more or a similar number of sprouts in comparison to *Populus* and *S.* × *rubens* (Fig. 1). Additionally, in the waterlogged treatment, *S. humboldtiana* showed the most profound decline in the number of sprouts over time (Fig. 2a) while *Populus* had the fewest sprouts among all taxa (Fig. 2b). With respect to the lengths of the longest sprout, the two invasive willows performed much better than *S. humboldtiana* and *Populus* (Fig. 1) with a steep rise over time (Table 1, Fig. 2e–h). In accordance with these results, the two invasive willows performed considerably better than *S. humboldtiana* and *Populus* concerning shoot and root biomass production (Fig. 1 d,e).

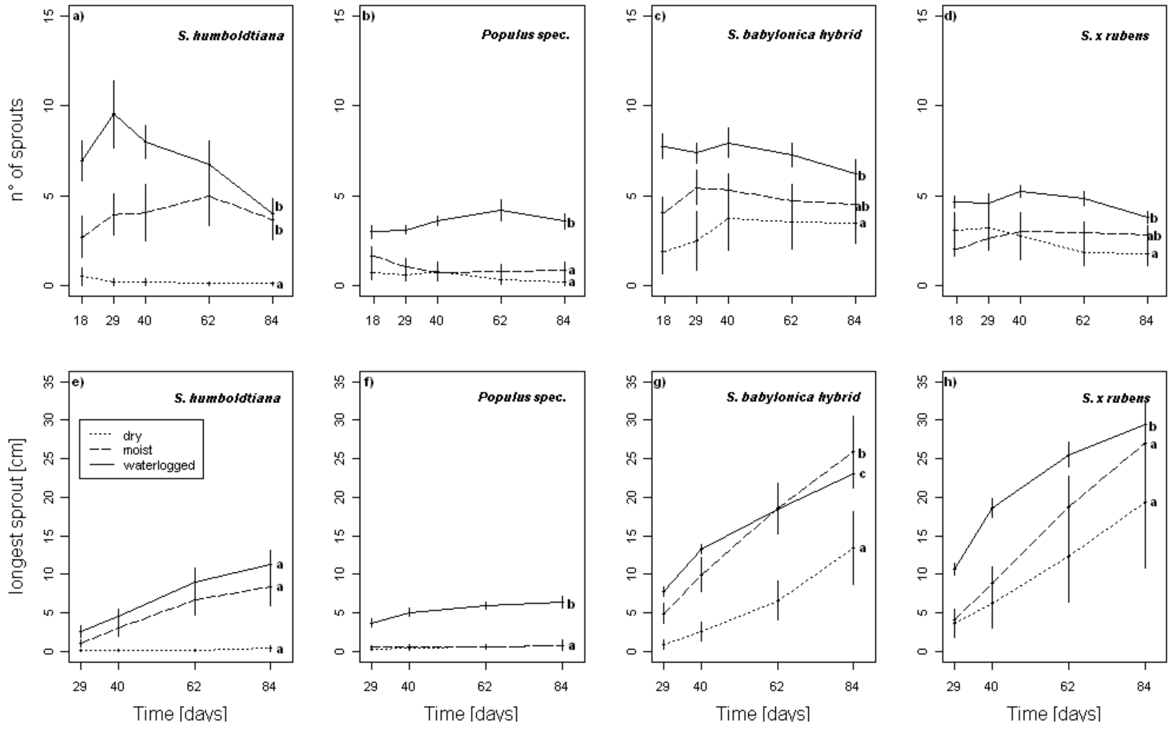

Figure 2 (a-h) Growth performance over time. Growth performance over time of the four species investigated under different soil moisture conditions. (a-d): effect on the number of sprouts (means±SE) after 18, 29, 40, 62 and 84 days; (e-h): effects on the longest sprout after 29, 40, 62, and 84 days. Different letters indicate differences ($p<0.05$) after Tukey's all-pairwise comparisons.

Biomass Removal Experiment

Re-sprouting proportions after further shoot and root removal tended to be higher in the two invasive willows (*S. babylonica* hybrid: 100%; *S. × rubens* 90%) in comparison to *S. humboldtiana* (75%) and *Populus* (70%), which was significant between *S. babylonica* hybrid and *Populus* ($X^2 = 4.90$; df = 1; $p<0.05$) and exhibited a clear trend in the case of *S. babylonica hybrid* and *S. humboldtiana* ($X^2 = 3.66$; df = 1, p = 0.056). The number of sprouts and longest sprout depended significantly on species and to a lesser extent, on the previously applied water treatment (Table 2). The *S. babylonica* hybrid produced the highest number of sprouts and differed significantly from *S.* × *rubens* and *Populus*. The two invasive willows had significantly longer sprouts than *S. humboldtiana* and *Populus* (Fig. 3). The former soil composition had slight effects on the number of sprouts with more sprouts emerging from cuttings which previously grew on fine grained soil (Table 2).

Table 2 Main effects of species, soil moisture and soil composition on the secondary re-sprouting capacities (number of sprouts and longest sprout) in the 2nd experiment after 40 days. Response variables were log-transformed to improve the diagnostic plots. Values are F-values of three-way ANOVAs. Levels of significance (p) are denoted with *p<0.05, **p<0.01 and ***p<0.001.

	D.F.	**N° of sprouts**	**Longest sprout**
Species	3	4.62**	9.82***
Moisture	1	6.48*	5.06*
Soil	1	4.71*	2.36
Spec: Moisture	3	1.18	1.44
Spec: Soil	3	1.03	0.89
Moisture: Soil	1	0.27	0.00
Spec: Moisture: Soil	3	2.35	0.89
Residuals	64		

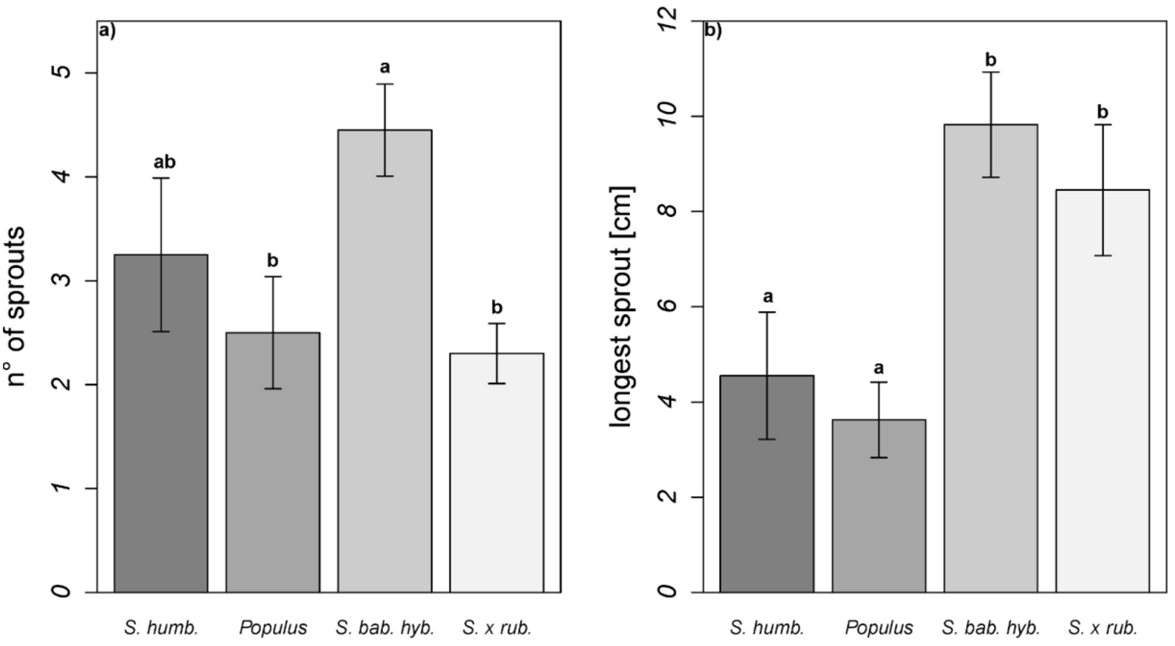

Figure 3 (a,d) Secondary re-sprouting capacities. Secondary re-sprouting capacities measured as a) number of sprouts and b) longest sprout of the four species after 40 days (means±SE). Different letters indicate Tukey's HSD between species (p<0.05). *S. humb* = *Salix humboldtiana*, *Populus* = *Populus* spec., *S. bab*. hyp. = *Salix babylonica* hybrid, *S*. × *rub*. = *Salix* × *rubens*.

Discussion

While all studied taxa were able to reproduce vegetatively, the two invasive *Salix* hybrids clearly out-performed the native *S. humboldtiana* as well as the studied *Populus* species. The questions arise as to how important are the better vegetative reproduction capacities of invasive riparian willows for the ongoing invasion process and whether an out-performance may lead to an out-competing of the native *S. humboldtiana*.

In this context, both regeneration strategies, sexual vs. asexual, have to be taken into consideration. Field observations in the study area revealed that *S. humboldtiana* as well as the *Populus* taxa produce a huge amount of seeds while capsules of the invasive willows contain few or no seeds (personal observation). Simultaneously, vegetative reproduction by broken twigs was observed in all taxa with *S. × rubens* having a great advantage due to the considerable brittleness of the twigs (Beismann et al 2000; Budde et al 2011). These findings suggest profound differences in reproduction strategies among *S. humboldtiana* and *Populus* on the one hand and the invasive willows on the other. Since a considerable number of bare-ground sites suitable for germination are recurrently created by hydrogeomorphologic processes, at first glance, sexual reproduction appears to be a successful strategy to establish self-sustaining *S. humboldtiana* as well as *Populus* stands. However, it is reported that broken twigs of Salicaceae can tolerate broader environmental conditions than their seedlings and usually have higher survival rates. Seedlings are well known to be more susceptible to unfavorable growing conditions such as the mechanical impacts of floods, burial and drought stress as compared to vegetative propagules (Barsoum 2002; Moggridge and Gurnell 2009). Additionally, the establishment of vegetative fragments is not restricted to short time periods such as seedling establishment, which is only possible during the seed-release period. The very short life span of the seeds ranging from several days to few weeks (Karrenberg et al 2002), further restricts successful sexual reproduction. Therefore, in unfavourable years, this process can be a rare event even under natural conditions (Hughes et al 2001) and it can be assumed that under unseasonal flood disturbances, e.g. due to river regulation by dams, the property to establish successfully by vegetative propagation could be a great advantage. Indeed, the intensity of alien willow spreading obviously increased after the onset of river regulation in the feeder rivers in the 70 s of the last century, as reported consistently by residents of the Río Negro region.

Regarding the responses of the four studied taxa in our experiment, due to their vigorous growth, the longer sprouts of the invasive willows may contribute to a more successful establishment in comparison to *S. humboldtiana* and *Populus***.** This property offers the chance of fragment survival even under competition conditions and even under flood events by escaping from shade, being buried and being under water (Radtke et al 2012). With respect to water availability, the invasive willow cuttings performed much better under both drought and sufficient moisture availability, indicating the tolerance to a rather broad range of moisture conditions. In contrast, drought periods probably affect the other two studied taxa to a greater extent. The reason may be that they rely more on sexual reproduction which is per se drought-sensitive. Thus, the poor vegetative reproduction capacity under drought conditions indicates that they cannot compensate for a drought-induced failure in sexual reproduction.

A potential further reason for the invasion success of Eurasian riparian willows at the Río Negro could be that the abundance of *S. humboldtiana* is restricted at this range edge due to unfavourable growing conditions. Its distribution centre is located in the tropical parts of South America, where it forms monospecific stands and flowers the whole year around. Vegetative reproduction occurs in the tropics as well (Liotta 2001; Parolin et al 2002). At the edge of its distribution area a naturally lower abundance and inferior competitive vigour could probably make *S. humboldtiana* more susceptible to competing invasive species in Patagonia than in other regions. However, recent reviews draw the conclusion that the abundance centre hypothesis with decreased abundance and fitness at range edges can not be considered as a general rule (e.g. Sagarin and Gaines 2002; Sexton et al 2009). Further research is necessary, including the tropical centres as well as other range edges of *S. humboldtiana* (e.g. at Mexican rivers as the northern edge) to shed light on this topic.

It needs to be mentioned that extended vegetative reproduction can be accompanied by low clonal diversity or even monoclonality of stands. This harbours a potential disadvantage since it can affect the adaptation potential under changing environmental conditions (Barrett et al 2008). While scenarios of monoclonality have been found at other Patagonian rivers, interestingly, different clones of *S. × rubens* were identified at the Río Negro (Budde et al 2011). Moreover, both sexes as well as viable seeds, although not frequent, were observed for the invasive willow taxa in our study area. Therefore, it cannot be excluded that potential negative impacts of low clonal diversity can be overcome in the future.

Finally, it should be considered that *S. humboldtiana* is known to form hybrids with the studied willow complexes in controlled crosses (Hunziker 1992; Borodowski and Suárez 2004). Since flowering periods are overlapping (personal observation), natural hybridization processes between native and invasive willows are possible which could lead to introgressive gene flow as e.g. Ziegenhagen et al (2008) demonstrated for *Populus nigra* in its European range.

In conclusion, our data gathered through a greenhouse study indicates that the vegetative reproduction capacities could foster the success of invasive willow hybrids along the Río Negro. However, further research is needed to test if the non-native species may outcompete and impact the native under field conditions.

Acknowledgments

We would like to thank Christina Mengel for the molecular taxonomic information on the *S. babylonica* hybrid and *S. × rubens* and the DPA (Departamento Provincial de Aguas Río Negro), who provided data on the water levels. We thank our project partners Leo Gallo, Paula Marcelli and Jorge Bozzi for intensive discussions and support.

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Chapter 3 – Paper 2

Age structure, growth performance and composition of native and invasive Salicaceae in Patagonia

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Plant Ecology (2014) 215, 1047-1056

Abstract

In northern Patagonia, riparian ecosystems are highly modified due to alien plant invasions. The establishment of non-native willows and poplars has unknown effects for *Salix humboldtiana*, the only native floodplain tree species in this region. To clarify establishment processes and to assess whether interspecific competition among the Salicaceae taxa may occur and therefore whether the native species can co-exist in the long term, we analysed age structure and growth performances of the dominant four taxa within mixed adult forest stands along the Río Negro, Argentina.

Low mean ages of < 15 years for all four taxa within 20 stands could be detected and suggest frequent and severe disturbances resulting in the removal of existing vegetation and subsequent creation of sites for establishment. Trees of the same stand showed the same age structure indicating joint establishment events with all taxa involved. A significant better growth performance (basal area, crown diameter) could be proved for the invasive willows. Considering the upper and middle river separately in a total of 88 studied plots, *S. humboldtiana* was more frequent at the middle river stretch while invasive willows showed an opposing pattern suggesting a downstream directed invasion process. The results showed that competition pressure in mature mixed stands could affect *S. humboldtiana.* However, these stands are usually removed by river dynamics creating new sediment bars and islands. These processes enable *S. humboldtiana* seeds to germinate frequently which may compensate for its inferior growth performance and potential impacts by the invasive willows and poplars.

Introduction

Riparian ecosystems are known to be vulnerable to invasion by alien plants mainly due to the hydrological connectivity of river corridors (Ward et al 2002) and to the frequently occurring disturbances (Stohlgren et al 1999; Tabacchi et al 2005; Renöfält et al 2005; Richardson et al 2007). It has been predicted that species invasion along rivers will continue in the future (Tockner and Stanford 2002). Invasive plant species can have severe impacts on river systems. For instance, they can change the composition and structure of vegetation, ecosystem function (Richardson et al 2007) and ecohydrologic processes (Hultine and Bush 2011).

Among riparian invaders, species of Salicaceae introduced for ornamental and functional landscaping play an important role. The Asian weeping willow (*Salix babylonica* L.) and the European Lombardy poplar *(Populus nigr*a L. var. *italic*a) are de facto the most widely planted ornamental trees worldwide (Li 1996). Eurasian willows have naturalised widely, e.g. in Australia (Cremer 2003), New Zealand (Glova and Sagar 1994), South Africa (Henderson 1991) and the USA (Shafroth et al 1994). Species of Salicaceae are known to be invasive alien species around the world. In a global review of invasive trees and shrubs (Rejmánek and Richardson 2013) 14 species of *Sali*x and 5 species of *Populu*s are listed, including *S. alb*a L., *S. babylonic*a L., *S. fragili*s L., *S*. × *ruben*s Schrank, *P. deltoide*s Bartram ex Marsh, *P. nigr*a L. and *P*. × *canadensi*s Moench. In Patagonia, riparian landscapes are dominated by planted and naturalised poplar and willow trees (Naumann 1996). In the past, *Salix humboldtian*a Willd., the only native riparian tree species in the region, was very frequent forming floodplain forests at the river banks of the rivers Negro and Limay (Hauman et al 1947). These gallery forests have vanished and have been displaced by mixed forests (Fig. 1) in which invasive Salicaceae play an important role (Correa 1984; Zalba and Villamil 2002). In the recent two decades especially, floodplain forests structured by invasive willows and poplars have occupied large areas, e.g. in the Río Negro region of Northern Patagonia. They were introduced to Patagonia by European settlers as windbreaks and wood source in the late 19th or early 20th century (Naumann 1996; Peri and Bloomberg 2002). The increase of forests dominated by invasive Salicaceae may have altered the riparian ecosystem of the Río Negro profoundly as it could be shown in other invaded riparian landscapes (e.g. Lester et al 1994; Cremer 2003; Holland-Clift et al 2011). The main question in this context is whether the native *S. humboldtiana* can persist in the future despite the occupation of large areas by these introduced Salicaceae.

Figure 1 Aerial view of a representative section of the upper Río Negro with vegetated islands and sand bars. The Salicaceae forests on the picture are dominated by invasive willows and poplars. In the middle of the picture one of the studied islands is seen with adult stands where all of the four target taxa are present.

In order to answer this question a promising approach is to clarify establishment processes by analysing the age structure of the native and invasive Salicaceae involved in the frequently occurring mixed forests: Is *S. humboldtiana* capable of establishing when invasive forests are already developed or was the native species already present when the invasive ones spread out? Or are the stands even the result of joint establishment events?

Moreover, it is not clear whether invasive Salicaceae have the potential to affect *S. humboldtiana* e.g. through heavy shading and other competition processes. A first investigation revealed a better vegetative reproduction capacity of invasive willow hybrids in comparison to *S. humboldtiana* (Thomas et al 2012). Measurements of growth performance in relation to the age of the trees in mixed forest stands help to assess whether interspecific competition may occur in older life stages in Salicaceae forests and therefore whether the native species can co-exist in the long term.

Therefore, the following questions were addressed in this study:

(1) What is the age structure of adult mixed Salicaceae forests in the active zone of the Río Negro?

(2) Do native and invasive taxa possess different growth performances indicating potential disadvantages for *S. humboldtiana* in the adult life stage?

The Negro River in Patagonia with its dense forests consisting of native and invasive Salicaceae is well suited as a model area for analysing such questions. Moreover, the land-use intensity and invasion history is rather heterogeneous along the course of the river. This aspect is associated with the following question:

(3) Do tree composition and performances of native and invasive Salicaceae change in the course of the river?

Methods

Study area

The research was conducted at the Río Negro in northern Patagonia, Argentina (Fig. 2). The Río Negro drains a catchment area of 65.000 km^2 (Cushing et al 2006) and the valley is surrounded by the dry shrubby Patagonian steppe. The mean annual precipitation is less than 250 mm and the mean annual temperature is 14-20 °C (Gut 2008). The two tributaries, the Río Limay and the Río Neuquén, which rise in the Andean Mountains, join at 225 m a.s.l. to form the Río Negro near the city of Neuquén. From there, it flows approximately 630 km to the Atlantic Ocean crossing the Patagonian high plateau (Cushing et al 2006). The river has a mean annual discharge of 900 m^3 s^{-1} with pronounced water level fluctuations (DPA, Departamento Provincial de Aguas Río Negro). The natural flow regime is governed by snowmelt in the Mountains, but since the onset of dams at the Rivers Limay and Neuquén during the 70s of the last century, flood disturbances are irregular and depend on energy requirements. The Negro River itself is not regulated by dams, but water is withdrawn for intensive agriculture. Human impact along the river is characterized by land use, especially fruticulture and grazing. The river still shows natural dynamics and flows in its natural shape slightly meandering and forming exposed vegetated islands (Fig. 1). The Río Negro is subdivided in the upper (Alto Valle), middle (Valle Medio) and lower stretch (Valle Inferior). The upper river valley is the principle region of Argentina for apple and pear production. Hence the valley is characterised by large fruit plantations, mostly at the northern side of the river, which stretch to the river shores. Plantations are usually enclosed by planted alien poplar and willow rows. Downstream in the middle river valley, large plantations are fewer with farming and cattle breeding being more important. The original vegetation along the river is composed of *S. humboldtiana* as the only native tree species and the natural shrubby steppe vegetation (e.g. *Larrea spp.*, *Prosopis spp.*, *Baccharis spp.*) (Gut 2008).

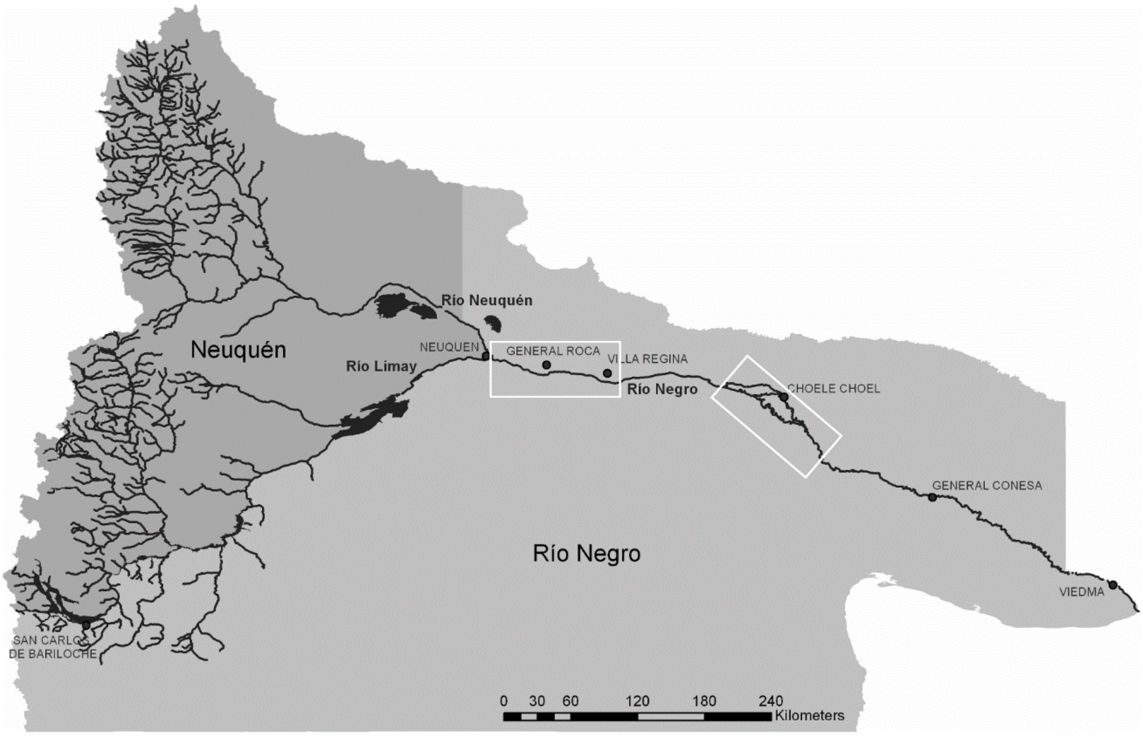

Figure 2 The Río Negro in northern Patagonia, Argentina. All samples were taken in the marked sections in the upper and the middle river stretch.

Survey method

For all analyses, the most dominant and forest-building taxa were chosen: a) *S. humboldtiana*, b) willows of the *S. alba*-*S. fragilis* complex including the hybrid *S.* × *rubens* *(*hence called *S.* × *rubens*), c) a hybrid of this complex and *S. babylonica* (hence called *S. babylonica* hybrid), as well as d) *Populus* spp. (*P. nigra*, *P. deltoides* and their hybrid *P.* × *canadensis*).

Age structure and Age-DBH-relationship

Wood core samples of trees were taken on a total of 20 islands within the active zone of the Río Negro, Northern Patagonia (Fig. 2). Thereof, 10 islands were located at the upper and 10 at the middle river stretch. Islands were selected since they are the sites with the least human influence across the whole floodplain. All the islands studied were characterized by the occurrence of mixed adult forest stands where all of the four target taxa were present (Fig. 1). In each stand, samples from three individuals per taxon were taken to determine the age of the stands summarizing a total of 60 randomly chosen trees per taxon. Wood cores were taken at breast height (1.3 m) with an increment borer for growth analysis (Suunto, 300 mm). For the exact age determination, a microscope was used for counting the annual rings. Relations between diameter at breast height (DBH) and age from analysed core samples were explored for each taxon by regression analysis with linear and asymptotic models. These models were compared and tested for their suitability using ANOVA. For this purpose, even from the multi-stemmed *S.* × *rubens* only one trunk was measured per individual. Differences in age among the taxa were analysed using one-factorial ANOVA followed by Tukey’s method of honestly significant differences. Additionally, age was related to taxon and river stretch using two-factorial ANOVA followed by Tukey’s method of honestly significant differences.

Growth performance

Measurements of growth performance were carried out in each of fifteen individuals per taxon in five adult mixed forest stands on five islands along the river. Age was determined using wood cores as described above. Tree height, DBH (of all trunks when several existed of one individual), mean crown diameter (average north-south and east-west expansion) and crown base height (the height where the crown of a tree begins) were measured in the field. Basal area of each individual was determined using the DBH and including multiple trunks when present. Differences among the taxa were analysed using ANOVAs followed by Tukey's method of honestly significant differences. Additionally, basal area was related to age and taxon using ANCOVA.

Composition, age and performance along the river

Additionally, 88 randomly distributed plots (10 m^2) were established on 12 islands along the river. To get an idea of the natural age structure, Salicaceae taxa composition, and frequency along the river, these plots were not only located in mature mixed forest stands but reflected a wide range of habitats where minimum one stem > 5 cm DBH occurs. The plots represent different vegetation structures, ranging from almost vegetation-free to dense forests. 54 plots were located at the upper river stretch and 34 plots at the middle stretch (Fig. 2). Within these plots all stems with DBH > 5 cm were measured. In total, 442 trees of *S. humboldtiana*, 430 *Populus* spp., 284 *S. babylonica* hybrids and 177 *S.* × *rubens* were identified. Age was derived from DBH by using the linear model of the regression analyses described above. For all four target taxa, the basal area, i.e. the area covered by stems, within each plot was determined. Furthermore, the following variables were determined for all taxa: mean and maximum age of trees over all individuals, mean number of individuals per plot (only plots counted where the respective taxon was present) and frequency of occurrence in the plots. Age, DBH, basal area and number of individuals were related to taxon and river stretch as explanatory variables using two-factorial ANOVAs followed by Tukey's method of honestly significant differences. All analyses were made with the software package R (Version 2.12.2, R Development Core Team 2011). Constancy of variance and normality of errors were checked using diagnostic plots (Crawley 2007).

Results

Age structure and Age-DBH-relationship

Age-DBH-relationship is best explained by linear models. Regression analyses revealed significant linear relationships of age and DBH for each taxon (Tab. 1). Two factorial ANOVA results with taxon and river stretch as explanatory variables and age as response variable only revealed significant differences between the taxa, but not between the upper and middle river stretch. Furthermore, no interactions between the explanatory variables could be detected. Thus, only the results of the one-factorial ANOVA with age and taxon as variables were considered ($F_{3,235} = 5.304$, $p = 0.0014$). For all four taxa, the studied individuals on the 20 densely-forested islands exhibited a mean age of < 15 years. *S. humboldtiana* (12.8 ± 3.5 years, means ± SE), *Populus* spp. (13.1 ± 2.4 years) and *S.* × *rubens* (13.7 ± 3 years) did not differ significantly in age. The *S. babylonica* hybrid showed a slightly higher mean age (14.8 ± 2.9 years), significantly different from *S. humboldtiana* and *Populus* spp. The oldest *S. humboldtiana* was 29 years old, *Populus* spp. 19 years old while the oldest *S. babylonica* hybrid and *S.* × *rubens* were 23 years old.

Table 1 Regression analysis of DBH and age for each taxon (n= 60 individuals per taxon). Levels of significance (p) are denoted with *p<0.05, **p<0.01 and ***p<0.001.

species	**Parameter**				
	a	**b**	**d.f.**	**F-statistic**	**p-value**
Salix humboldtiana	0.692	1.121	1,58	89.9	2.148e-13***
Populus spp.	-0.002	1.72	1,58	26.84	3.121e-06***
Salix babylonica hybrid	-6.934	2.698	1,58	72.12	9.205e-12***
Salix × *rubens*	-5.711	1.679	1,58	43.98	1.135e-08***

Growth performance

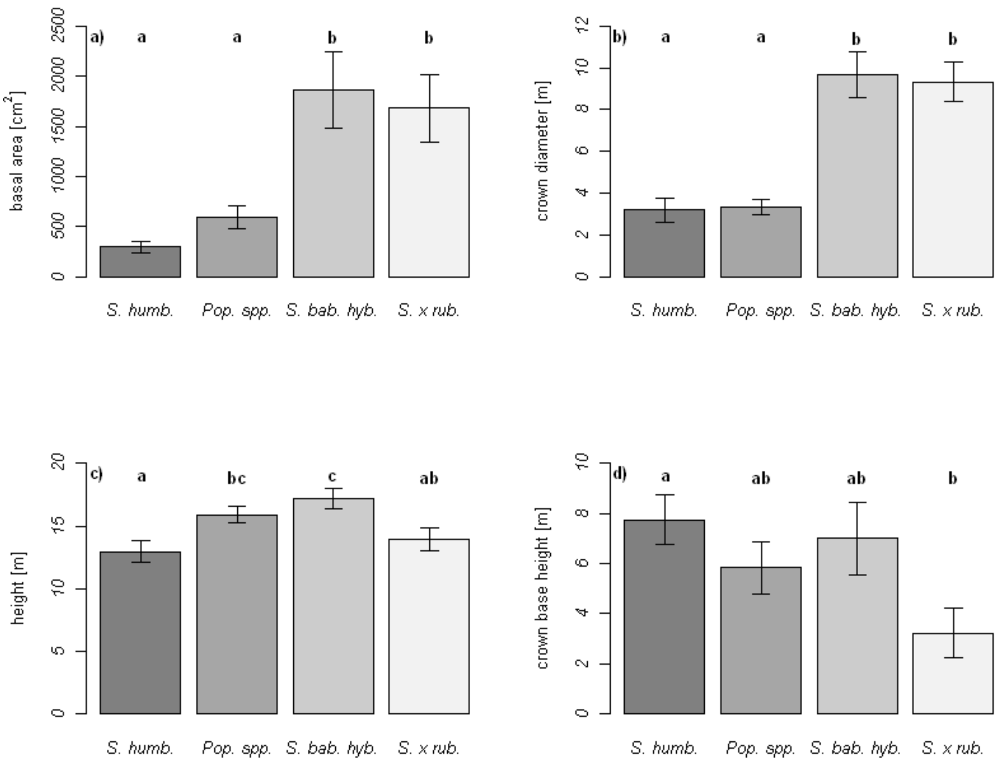

Figure 3 a-d Growth performance of different taxa in mixed forest stands (means ± SE). Different letters indicate significant differences (p<0.05) among taxa according to Tukey's method of honestly significant differences.

Regarding the individuals used for growth performance measurements, the age did not differ significantly between taxa ($F_{3,56}$= 0.600, p= 0.6185) with means of 14.4 - 15.9 years. However, great differences could be observed concerning basal area ($F_{3,56}$= 4.461, p= 3.979e-05) and crown diameter ($F_{3,56}$= 22.055, p= 1.478e-09) (Fig. 3 a,b). The *S. babylonica* hybrid achieved the highest means concerning basal area and crown diameter followed by *S. x rubens,* which could reach high basal areas due to multiple stems. Their performances differed significantly from *Populus* spp. and *S. humboldtiana*. Likewise, for tree height ($F_{3,56}$= 5.937, p= 0.00137) and crown base height ($F_{3,56}$= 3.289, p= 0.02714), significant differences could be observed (Fig. 3 c,d). *S. humboldtiana* reached a significantly lower tree height than *Populus* spp. and the *S. babylonica* hybrid, which was not different from *S.* × *rubens*. However, *S.* ×

rubens had a significantly lower crown base height than *S. humboldtiana.* ANCOVA results of basal area in relation to taxon and age revealed significant differences between both explanatory factors, and significant interactions between them (Tab. 2). Considering the increase of basal area along the age gradient, *S. humboldtiana* and *Populus* spp. performed significantly worse in comparison to the two invasive willow hybrids (Fig. 4).

Table 2 ANCOVA results of basal area in relation to taxon and age. Levels of significance (p) are denoted with *p<0.05, **p<0.01 and ***p<0.001.

	d.f.	**SS***	**MS****	**F-statistic**	**p-value**
Taxon	3	27287202	9095734	12.1555	3.842e-06***
Age	1	7548012	7548012	10.0871	0.002511**
Taxon:Age	3	7739224	2579741	3.4476	0.023103*
Residuals	52	38910653	748282		

* SS = Sum of squares

** MS = Mean sum of squares

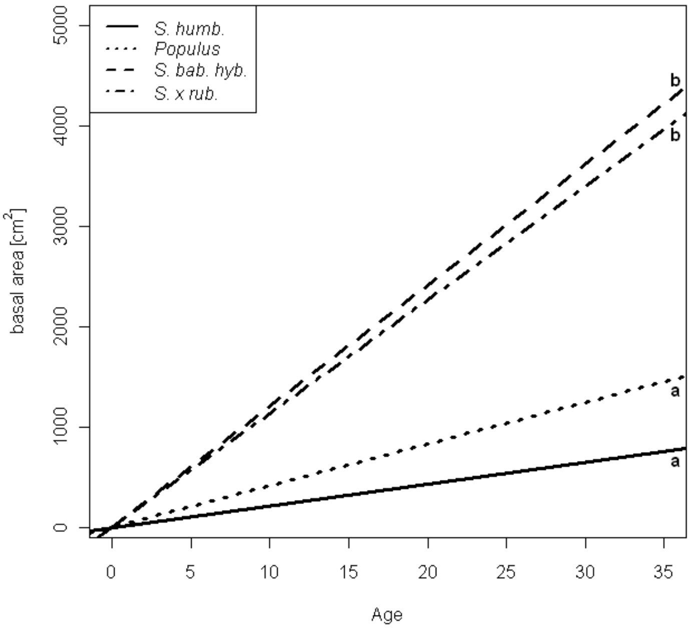

Figure 4 Basal area related to age of different taxa in mixed forest stands explained by linear function. Different letters indicate significant differences (p<0.05) among taxa according to Tukey's method of honestly significant differences.

Composition, age and performance along the river

ANOVA results revealed significant differences for the response variables age, DBH and basal area among taxa in the randomly distributed plots along the river (Tab. 3, Tab. 4).

Table 3 ANOVA results of age, DBH, basal area and number of individuals in relation to taxon and river stretch along the Río Negro. Age and DBH refer to all measured individuals, basal area and number are means measured per plots. Levels of significance (p) are denoted with *p<0.05, **p<0.01 and ***p<0.001.

		d.f.	**SS***	**MS****	**F-statistic**	**p-value**
Age	Taxon	3	147.4	49.12	3.0388	0.028135*
	River stretch	1	1286.8	1286.75	79.5985	< 2.2e-16***
	Taxon:River stretch	3	220.8	73.61	4.5535	0.003511**
	Residuals	1325	21419.4	16.17		
DBH	Taxon	3	7497	2498.9	49.8854	< 2.2e-16***
	River stretch	1	3500	3500.5	69.8806	< 2.2e-16***
	Taxon:River stretch	3	991	330.4	6.5965	0.0002005***
	Residuals	1325	66373	50.1		
Basal area	Taxon	3	32610169	10870056	3.2064	0.02479*
	River stretch	1	8913779	8913779	2.6293	0.10690
	Taxon:River stretch	3	1658992	552997	0.1631	0.92105
	Residuals	158	535639020	3390120		
Number	Taxon	3	550.8	183.599	2.5706	0.05621
	River stretch	1	16.1	16.052	0.2247	0.63611
	Taxon:River stretch	3	299.9	99.966	1.3996	0.24499
	Residuals	158	11284.9	71.423		

* SS = Sum of squares

** MS = Mean sum of squares

Low mean ages could be observed on the 88 plots for all taxa (Tab. 4). The river stretch influences the age of the taxa significantly (Tab. 3). For all taxa the mean age was higher in the middle river stretch with significant results for *S. humboldtiana* and *Populus* spp. (after post hoc comparisons using the Tukey HSD test). Concerning the DBH of all individuals, significant differences between the taxa and river stretch could be proved (Tab. 3). *S. humboldtiana* and *S. × rubens* reached significantly lower diameters than the *S. babylonica* hybrid and *Populus* spp. The mean DBH of the *S. babylonica* hybrid and *Populus* spp. were significantly higher at the middle river stretch.

Regarding mean basal area per plot, where the respective taxon was present, only *Populus* spp. and *S.* × *rubens* showed significant differences, with *Populus* reaching higher mean values. Although all taxa reached higher mean basal areas in the middle than in the upper valley, the results were not significant. The mean number per plot did not differ significantly between the taxa and between in the upper and middle river stretch. In contrast, differences could be observed in terms of frequency. *S. humboldtiana* was much more frequent in the middle than in the upper stretch. Also *Populus* spp. was slightly more frequent at the middle valley while the *S. babylonica* hybrid and *S.* × *rubens* showed converse results (Tab. 4).

Table 4 Age, DBH, basal area, number and frequency of the four target taxa on 88 plots along the Río Negro (54 plots in the upper valley, 34 in the middle valley). Mean age, maximum age and DBH refer to all measured individuals, basal area and number of individuals are means measured per plot.

	taxon	mean age ± SE of individuals	max age of individuals	mean DBH [cm] ± SE of individual	mean basal area [cm²] per plot	mean number of individuals per plot	Frequency in plots [%]
Upper Valley	*Salix humboldtiana*	7.4 ±2.7	18.6	8.97 ±3.00	639.03	9.11	35
	Populus spp.	7.3 ±4.5	34.4	12.55 ±7.76	1553.99	9.09	60
	Salix babylonica hybrid	8.0 ±3.2	17.5	14.56 ±8.68	1552.59	6.89	67
	Salix × rubens	9.0 ±2.1	16.2	9.56 ±3.53	558.55	6.85	37
Middle Valley	*Salix humboldtiana*	8.9 ±4.3	39.5	10.68 ±4.85	1383.62	12.95	65
	Populus spp.	10.7 ±6.1	50.2	18.45 ±7.96	2134.22	6.00	65
	Salix babylonica hybrid	9.8 ±4.2	20.2	19.48 ±11.39	1787.42	4.63	24
	Salix × rubens	10.3 ±3.1	16.5	11.74 ±5.33	742.96	5.71	21

Discussion

Trees of the same stand showed the same age structure indicating one single or very few establishment events with native and invasive Salicaceae involved. Small variations in age could be explained by different reproduction strategies with the invasive willows depending more on vegetative propagation (Budde et al 2011; Thomas et al 2012) which occurs throughout the year. In contrast, sexual reproduction is restricted due to a short period of seed release and seed viability. The detected low age of adult mixed stands suggests frequent and severe disturbances within the active zone of the Río Negro. Major floods may allow for the establishment of stands by removing existing vegetation or by creating new islands when sediments are deposited (Bendix and Hupp 2000). Indeed, all states of island development could easily be detected in current aerial views of the Rio Negro with small shallow islands with no or almost no vegetation establishment to large islands of higher elevations covered by dense Salicaceae forests (Fig. 1). Also land loss by erosion leading to the disappearance of adult forests can be observed on islands. The low mean age of the floodplain forest stands coincides with the results of e.g. Karrenberg et al (2003) who found a mean age of <10 years of willow stands within the active zone of the near-natural Tagliamento in Italy.

S. humboldtiana is known to be the first colonizing pioneer tree of newly formed sediment banks along river margins. In the Paraná delta, Argentina, for example, *S. humboldtiana* forests generally represent the first successional stage and are replaced by *Tessaria integrifolia* (South American alder) stands after less than 20 years (Casco et al 2010). Likewise, S. *humboldtiana* forms monospecific stands in the Amazonian floodplain followed by *Cecropia latiloba* after 10-20 years (Parolin et al 2002). In Patagonia, in contrast, there is no successive tree species that displaces *S. humboldtiana* while invasive Salicaceae co-occur as first colonizers. Despite the absence of successional followers it seems that this particular Salicaceae composition is not forming older stands due to strong natural disturbance events. Beyond the active floodplain zone, in the absence of disturbances by floods, solitary trees of all the four studied taxa that grow on pastures can reach higher ages (> 40 years according to ring core analyses, data not shown).

Changes in light quality due to heavy shading by invasive species turned out to be an important factor in the suppression of native vegetation (Reinhart et al 2006). In the study, both the introduced willow hybrids form crowns about three times larger than *S. humboldtiana.* Additionally, the native willow has sparser crowns due to the slim, gracile leaves in contrast to the invasive hybrids and their broader leaves. This may have a severe effect not only on *S. humboldtiana* which is highly intolerant to shade (Parolin et al 2002), but also on native shrubs and herbaceous vegetation. It is known that the biomass productivity can increase in ecosystems due to the invasion by highly productive species (Vilà et al 2011). Although no data for biomass production is available it is obvious that the superior performance of the invasive willow trees and the increase of Salicaceae forests, dominated by invasive taxa, result in higher biomass production. For example, the *S. babylonica* hybrid achieved a basal area that was six times higher than that of *S. humboldtiana*.

Thicker and older trees could be observed for all taxa in the plots at the middle river stretch. This fact may be explained by lower and less frequent disturbances by flood events due to increased meandering and branching of the river. *S. humboldtiana* exhibited a higher abundance and frequency in the middle valley whereas it was vice versa in the case of the invasive willow hybrids. This could reflect invasion history suggesting an early invasion in the upper river stretch since agriculture, i.e. fruit plantations, is more intensive in the upper valley with, consequently, more Salicaceae planted next to the river shores. A later downstream colonization due to the spread of downstream floating twigs can be assumed.

High floods that remove vegetation create new space for establishment of alien species (Richardson et al 2007) but in our case also for indigenous *S. humboldtiana* seedlings. *S. humboldtiana* as well as the *Populus* taxa which produce a large amount of viable seeds rely more on sexual reproduction than the invasive willows. The latter generate no or only few seeds in capsules and only few male individuals of the *S. alba-fragilis*-comlex and *S. babylonica* hybrids could be observed along the Río Negro (personal observation). The spread of vegetative dispersal units is supposed to play a key role in the rapid distribution of invasive willows (Shafroth et al 1994; Budde et al 2011; Thomas et al 2012). While sexual reproduction requires bare-ground sites and sufficient humidity without intensive inundations for establishment, vegetative recruits are less dependent on environmental conditions and have higher survival rates (Moggridge and Gurnell 2009).

However, strong disturbances with the creation of new sediment bars and islands enable *S. humboldtiana* seeds to germinate frequently. Stands of older life stages, where competition due to the great growth performance of the invasive willow hybrids would probably affect *S. humboldtiana*, are removed by river dynamics. These considerations let us assume that, despite its inferior growth performance and potential impacts by the invasive Salicaceae, *S. humboldtiana* will not become an endangered species at the Río Negro in the nearby future if water levels and flow conditions allow for natural disturbance processes and therefore seedling establishment. However, these natural and necessary processes are susceptible to river regulations preventing the creation of bare-ground habitats and the removal of mature vegetation (Catford et al 2011).

For a long-term prediction of co-existence other properties have to be taken into account such as the question as to what extent *S. humboldtiana* is threatened via hybridization processes with invasive willows. Willows are known to frequently hybridize (e.g. Argus 1974; Thiebault 1998; Adair et al 2006). The flowering-periods of the different willow taxa along the Río Negro overlap (in September and October, pers. observation). Hybridizations between *S. humboldtiana* and *S. babylonica* as well as willows of the *S. alba-fragilis*-complex are possible (Hunziker 1992; Borodowski and Suárez 2004). Thus natural hybridizations could be possible in the study area with the consequence that the willow hybrids could expand their range by introgression. Moreover, new species such as e.g. *S. matsudana* could be observed at the margins of the River Negro and could affect the floodplain system in the future.

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Chapter 4 – Paper 3

River dynamics and invasion: Distribution patterns of native and invasive woody vegetation at the Río Negro, Argentina

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Manuscript

Abstract

Salicaceae are typical invaders of riparian ecosystems throughout the world and they have the potential to change much of the invaded habitats. Along Patagonian streams, riparian softwood forests composed of non-native Salicaceae are increasing in abundance, area and tree species diversity. Especially in the last decades, dense floodplain forests dominated by invasive willows and poplars and additionally Russian olive and tamarisk spread almost explosively e.g. along the Río Negro in northern Patagonia.

This study focuses on the identification of ecological niches and niche overlaps of the native and invasive woody species in order to assess the impact on the native *Salix humboldtiana* Willd. Data on species (presence/ absence) and explanatory variables were gathered in the field using a grid-based, stratified-randomized sampling design. Different environmental variables were then related to species occurrence in different life stages (adult, juvenile, seedling) using habitat distribution models.

In the final models, flood duration, the amount of gravel and the location (upper or middle river valley) were included to describe the probability of occurrence of native and invasive taxa. For all life stages, a strong niche overlap could be observed for *S. humboldtiana* and the invasive taxa with no remaining exclusive habitats indicating a potential threat to the native willow. The study contributes to a better understanding of *Salix* invasion and its consequences for riparian ecosystems.

Introduction

Willows and poplars of riparian softwood forests are typical pioneer species and adapted to the high dynamics of rivers. Frequency of inundation as well as sedimentation and erosion processes are the main factors that determine species distribution in riparian habitats (Osterkamp and Hupp 2010). Major floods change the morphology of riparian landforms and consequently have impacts on riparian plant communities (Bendix and Hupp 2000; Asaeda et al 2011). These dynamic processes, particularly the natural disturbances and the hydrological connectivity (Ward et al 2002), are considered as the main reasons for the high invasibility of rivers.

In Patagonia, alien plant invasions have led to significant changes in riparian ecosystems. Historically, the occurrence of Eurasian willows and poplars in Argentina followed importation patterns of European settlers. Concurrent to expanding settlements, the occurrence of non-native species increased along the rivers. Since the late 19th century Salicaceae were planted to protect river banks, as windbreaks and wood source (Naumann 1996; Peri and Bloomberg 2002) and some clones are extensively cultivated in Argentina (Borodowski and Suárez 2004; Garau et al 2008). However, only in recent decades, the natural spread of alien willows and poplars has dramatically increased so that today invasive Salicaceae can be observed along many rivers in Patagonia (Budde et al 2011). Invasive willows are able to change much of the natural environment of river systems as it could be observed e.g. in Australia where non-native willows are now declared as “Weeds of National Significance” (Cremer 2003; Adair et al 2006). For example, displacement of native vegetation (Cremer 2003), differences in litter quality and changed decomposition rates (Serra et al 2013), alteration to river profiles (Adair et al 2006) including higher development and growth of sand bars and islands (Moggridge and Gurnell 2009), can be the consequences for native ecosystems. Furthermore, *Salix* invasions can have negative effects on the fauna such as aquatic invertebrates (Lester et al 1994) and riparian birds (Holland-Clift et al 2011).

It can be assumed that in former times the only native tree species, *Salix humboldtiana* Willd., as well as different shrub species (e.g. *Larrea spp.*, *Baccharis spp.*; Gut 2008) have structured riparian softwood forests in the otherwise mostly treeless steppes in northern Patagonia. Nowadays, *S. humboldtiana* is one of several willow and poplar species forming river accompanying woodlands. Beside Salicaceae species *Tamarix* as well as *Elaeagnus* are prominent invaders along the Río Negro, where this study was conducted.

A characteristic of *Salix* species is the ability to regenerate either sexually or asexually under a range of environmental conditions (Karrenberg et al 2002). The reproduction via vegetative fragments is considered a key factor in non-native plants becoming invasive (Kolar and Lodge 2001). Indeed, Budde et al (2011) revealed the great importance of vegetative reproduction for the spread of a single willow clone along Patagonian rivers. Beyond that, Lamarque et al (2011) showed that, once established, growth rate of trees explained best their invasive success. Given that invasive willow taxa show better vegetative reproduction and resprouting capacities and a better growth performance of the adult life stages (Thomas et al 2012; Thomas and Leyer 2014), negative impacts on *S. humboldtiana* can be expected. Hence, it is important to gain information on the factors determining the occurrence of native and invasive species and species-habitat relations. Different niches for the different species would imply a spatial coexistence of these species while a strong niche overlap with the occupation of the same habitat would point to a threat for the native willow due to competitive advantages of the invasive species. Therefore, the study focuses on the identification of ecological niches of the native and invasive woody species occurring in the active zone of the Río Negro. Habitat distribution models were developed in order to relate environmental variables to the probability of occurrence of native and invasive taxa. We included different life stages to account for possibly varying competition processes between young and old individuals and different habitat requirements.

In this study, we (1) show how environmental factors relate to the occurrence of native and invasive woody species, (2) analyse if native and invasive species exhibit strong ecological niche overlaps taking different life stages into account, and (3) evaluate if there are indications that *S. humboldtiana* and the riparian ecosystem could be negatively affected by the ongoing invasion process along the Río Negro.

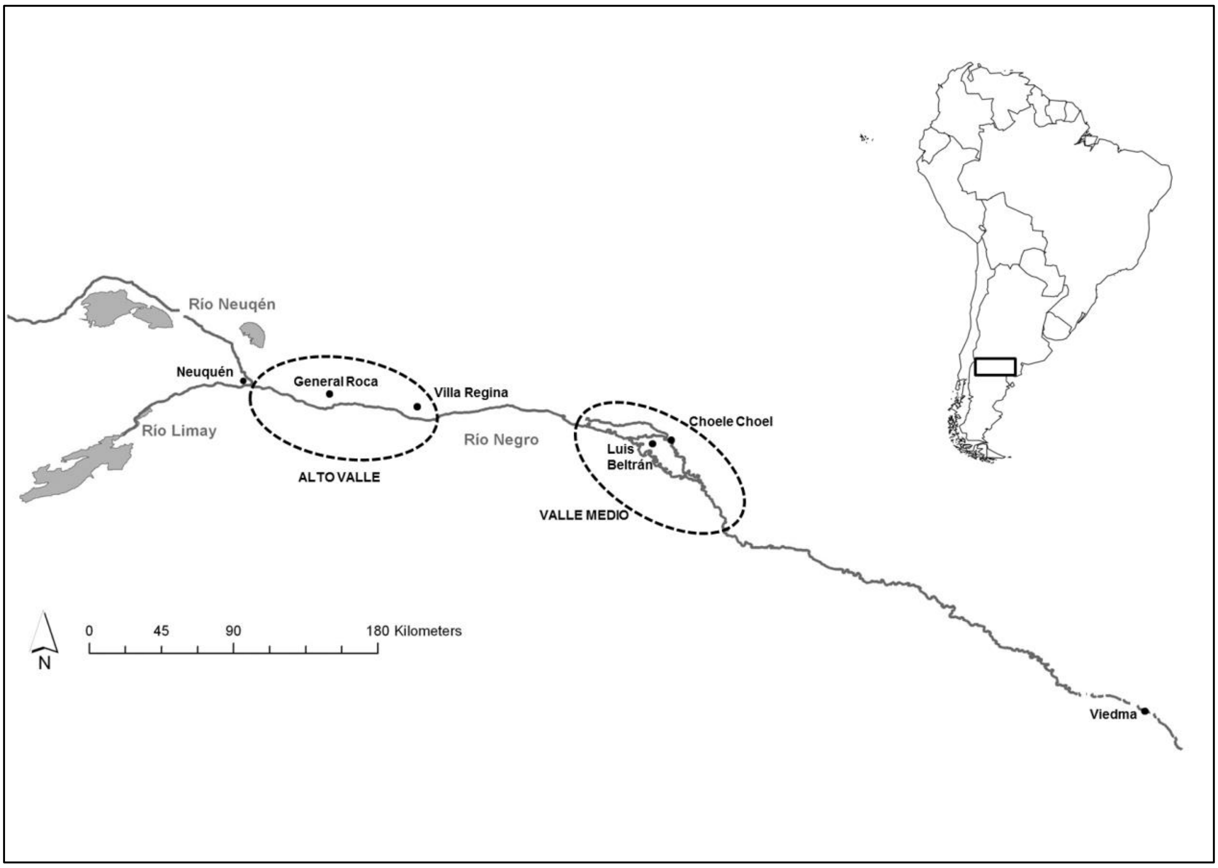

Figure 1 Map of the study area along the Río Negro in northern Patagonia, Argentina. All samples were taken in the marked sections in the upper and the middle river stretch.

Methods

Study area

The Río Negro, where this study was conducted, is located in northern Patagonia in Argentina and is surrounded by dry steppe (Fig. 1). The river is approximately 630 km long, originates near the city of Neuquén as a confluence of the rivers Limay and Neuquén and flows southeast to the Atlantic Ocean. It has a mean annual discharge of 900 $m^3 s^{-1}$ near the city of Neuquén with pronounced water level fluctuations up to 2.52 m at the water gauge near Mainqué in the upper valley in the years 2006-2010 (DPA, Departamento Provincial de Aguas Río Negro). The Negro River itself is not regulated by dams but water is withdrawn for intensive agriculture and the flow regime depends on regulation by dams of the two tributaries. Despite bank stabilisation by rip-raps to protect farmland it still exhibits natural morphodynamics, flowing in large meanders and forming depositional gravel bars. The study area comprised two sections of the Río Negro located in the upper (Alto Valle) and middle river valley (Valle Medio). Compared to the upper valley, in the middle valley the river is more branching and meandering and thus flood disturbances are lower. Furthermore, land use changes in the course of the river. While large fruit plantations characterize the upper valley, smaller fields and livestock breeding are more important in the middle river valley.

Study species

This study includes the most dominant and forest building woody species that occur along the Río Negro*,* comprising *S. humboldtiana,* two invasive *Salix* hybrids and *Populus* taxa, different *Tamarix* species, and *Elaeagnus angustifolia* Willd. *S. humboldtiana* is the only native willow in Argentina as well as the only native tree species along the Río Negro. It occurs from Mexico to South America but has become relictual in some places due to competition with invasive species (Zalba and Villamil 2002). Its southernmost occurrence is located at the river Chubut in the Santa Cruz province, about 500 km south of the study area. In warmer regions of South America, it is known to be the first colonizer of new habitats before other species take over (Parolin et al 2002; Casco et al 2010). *Salix* × *rubens* Schrank is the hybrid of *Salix alba* L. and *Salix fragilis* L. which belong to the most frequent hybridizing *Salix* species in Central Europe (Kehl et al 2008). In northern Patagonia, the distribution of willows belonging to the *S. alba*-*S. fragilis* complex has increased substantially along river margins. The reasons for their successful invasion are most likely the highly effective asexual reproduction (Budde et al 2011) with easily breaking twigs acting as hydrochorous propagules which fall into the water and can be carried some distance downstream colonising new habitats (Mosner et al 2012).

The second dominant invasive *Salix* taxon along the Río Negro is a hybrid of the *S. alba*-*S. fragilis* complex and S*alix babylonica* L. (hence called *S. babylonica* hybrid). *S. babylonica* is native in East Asia but it was introduced to many countries as an ornamental tree (Li 1996) and was brought to Argentina around 1850 (Borodowski and Suárez 2004). Different hybrids of *S. babylonica* have been cultivated in the Paraná Delta since 1953 including a hybrid of *S. babylonica* and *S. alba* (Borodowski and Suárez 2004). Moreover, several non-native *Populus* taxa occur in Patagonia (Ares et al 2002; Diaz et al 2003). Along the Río Negro the taxa that naturalised widely and where included in this study are *P. nigra* L., *P. deltoides* Bartram ex Marsh and their hybrid *P. × canadensis* Moench.

Besides invasive Salicaceae taxa, other alien woody species, tamarisk (*Tamarix* spec.) and Russian olive (*Elaeagnus angustifolia* Willd.*)*, are spreading strongly in the Río Negro valley. These species too can alter riparian ecosystems profoundly. Riparian landscapes in western USA have been changed greatly due to tamarisk invasion. As a consequence, restoration programs have been developed that remove this alien plant (Stromberg et al 2009). Three *Tamarix* species, native to Eurasia and Africa, are reported to occur along the Río Negro: *T. gallica* L., *T. ramosissima* Ledeb. and *T. chinensis* Lour. and probably their hybrids (Natale et al 2008; Natale et al 2010). In this study they are only listed as *Tamarix* without further differentiation. *Elaeagnus angustifolia*, native to southern Europe and central and eastern Asia, is invading and altering semi-arid riparian ecosystems in the USA (Katz and Shafroth 2003; DeCant 2008; Mineau et al 2011). According to Klich (2000) *E. angustifolia* notably expanded in the middle Río Negro valley during the last 20 years. Russian olive most frequently occurs on former pastures, forming dense stands aside the active zone of the river. In adult Salicaceae forests *Tamarix* and *Elaeagnus* both usually occur as understory vegetation.

Vegetation sampling

Field data were gathered during two periods in spring/summer of 2009/2010 and 2010/2011. As it was the ambition to examine species distribution under natural conditions with as little human influence as possible, all plots were set up on islands in the active zone of the river (Fig. 2). By doing so, plantings could be excluded which are frequent next to the riverbanks to protect e.g. fruit plantations. Another important factor that influences natural development along the river are local management strategies by farmers comprising logging, burning and the use of pesticides to remove mainly *Elaeagnus* and *Tamarix*. However, with the focus on islands, we were able to consider the natural colonisation processes.

Figure 2 Aerial view of a representative section of the upper Río Negro with vegetated islands and sand bars. The southern shore of this section is characterized by the uprising high plateau and grassland, the northern shore by fruit plantations with enclosing planted willow and/or poplar rows.

To cover the range of different life stages and environmental gradients, a stratified-randomized sampling design was employed (Hirzel and Guisan 2002). For plot selection, Google Earth pictures were imported to ArcGIS 9.3 (ESRI), and with Hawth's Analysis Tools (Beyer 2004) regular grids were used to randomly select single plots with a more or less flat surface. Each plot had an area of 10m x 10m. Per island, a minimum of 4 and a maximum of 10 plots was randomly selected (depending on island sizes), comprising a total of 167 plots on 23 islands. Thereof, 116 plots on 13 islands were located at the upper river valley, 51 plots on 10 islands at the middle valley. In each plot, the presence/absence of the target taxa, *S. humboldtiana, S.* × *rubens,* the *S. babylonica* hybrid, *Populus spp., Tamarix* spp. and *Elaeagnus angustifolia* was recorded. Individuals of each taxa were divided into three age classes: 'seedling', 'juvenile', and 'adult'. The age class 'seedling' comprised plants that germinated in the year of data collection and could be clearly identified as generative dispersal units by digging up the saplings. Trees were classified as juvenile when their diameter at breast height was smaller than 10 cm and the tree height smaller than 3 m. Since differentiation between *S.* × *rubens and* the *S. babylonica* hybrid in juvenile life stages was not assured by means of morphological characteristics, they were defined as 'invasive *Salix*'. In contrast, adult *S. babylonica* hybrids can be easily distinguished from *S.* × *rubens* by the weeping habitus of the trees. Combined with the earlier development of leaves of the *S. babylonica* hybrid during the vegetation period, these two features permit a differentiation between these two invasive willow hybrids in the adult life stage.

Environmental variables

Multiple abiotic factors were related to the occurrence patterns of the species in order to assess competition potential and to understand processes structuring riparian woody vegetation. Hydrological conditions are known to have a strong influence on distribution patterns of woody floodplain species (Bendix and Hupp 2000; Francis and Gurnell 2006). The average water level and the average flood duration as hydrological variables influencing floodplain vegetation have long been recognised (Van Splunder et al 1995; Karrenberg et al 2002; Leyer 2005). Mosner et al (2011) showed that the distribution patterns of floodplain willows can be explained by hydrological variables. Therefore, we calculated the mean flood duration and the average water level to describe the hydrological conditions on the studied plots along the Río Negro. To calculate mean flood duration, firstly, the water level in relation to the elevation of each plot was measured using a theodolite. The average flood duration as days per year was then calculated from daily water level measurements of three water gauges along the river near Paso Cordoba (near General Roca), Mainqué (near Villa Regina) and Beltrán (near Choele Choel) (DPA, Departamento Provincial de Aguas Río Negro) and the difference in elevation of a plot and the water surface of the river. Additionally, the average water level (AWL), i.e. depth to the water table (0 = level of top ground surface, negative values = below surface, positive values = above surface) was calculated. The average flood duration and AWL were calculated for all study plots over five years (2006-2010) and refer to the nearest water gauge. Since elevations of the plots lying permanently under water could not be measured and hence AWL could not be calculated, fixed values of 1 and 1.5 were assigned to extent the gradient of the variable, since plots that were not inundated only a few days reached AWL-values close to 1. Values of 1.5 were given for plots lying in deep water in the middle of the stream, whereas 1 was assigned when the plots were not so deep and located close to the riverbank.

Another important factor influencing species occurrence and composition along rivers is the flow velocity (Naiman and Décamps 1997; Steiger et al 2005). For example, mechanical impacts through floods can prevent a successful establishment (Beismann et al 2000; Moggridge and Gurnell 2009) and the adaptation to disturbance intensities can differ between the species. We defined flow strength as an ordinal variable representing shear stress in times of flooding ranging from 1 (low flow-strength) to 4 (very high). Plots in the category 1 are well protected from the current and lie in dense forests. Category 2 plots are protected from the stream current and are located behind protecting vegetation or at the downstream end of the islands. Plots of category 3 lie in the current (when flooded) but are located within the islands, whereas plots in the category 4 lie near the upper end of the islands directly in the stream current. The categories were assigned during field survey.

Soil samples were taken in each plot (except when under water). Soil probes comprising a volume of 500 ml from the top 20 cm of the soil were sampled at three locations within a plot (south-western corner, centre, north-eastern corner). The samples of each plot were mixed and stored in plastic bags until analyses. The samples were air-dried and the amount of clay/silt, sand and gravel was determined. Therefore, the samples were sieved with 0.063 and 2 mm mesh sizes and weighted.

Because even on islands human influence cannot be excluded land use was included as a further predictor variable (present or absent) although only slight grazing occurred on the studied plots.

The predictor variable location (upper and the middle river stretch) was included since river dynamics, i.e. stronger meandering in the middle river stretch, and landuse vary along the river. The upper river stretch of this study represents the first 100 km, the middle river stretch the section between 200 and 300 km of the river (Fig. 1).

Data analyses

A DCA (Detrended Correspondence Analysis, CANOCO 4.5; ter Braak and Smilauer 2002) was performed to determine the relationship of species occurrence and environmental variables and to assess the position of different life stages along the environmental gradients. For this, species presence/absence data on 167 plots were analysed and the environmental variables (average water level, flood duration, flow strength, amount of gravel, amount of sand, and amount of clay/silt) were treated as supplementary variables in the same analysis. The location upper river valley (Alto Valle) and middle river valley (Valle Medio) and land-use were treated as nominal supplementary variables. All supplementary variables were added post-hoc to the ordination by projection.

Generalized linear models (GLM) are a frequently used method for species distribution modelling of presence/absence data (Guisan and Zimmermann 2000; Austin 2007) to predict the probability of species occurrence in relation to predictor variables (Guisan and Edwards 2002). Hence, we chose GLMs for habitat distribution modelling to describe species-habitat-relations. All three life stage classes were analysed for all taxa using logistic regressions (GLM with logit link and binomial error structure; Crawley 2007). Firstly, all predictor variables were tested for multicollinearity using two-sided Spearman rank-correlation test. There was a strong positive correlation between flood duration and AWL (r_s=0.98, p-value<0.001). Furthermore, flow strength was highly correlated with gravel (r_s=0.75, p-value<0.001) and gravel was negatively correlated with the amount of silt/clay (r_s=-0.84, p-value<0.001). AWL had less explanatory power in the single models of the adult and seedling life stage, but a slightly better model fit in the juvenile life stage. However, to make the models of the different life stages comparable, AWL was excluded from further analyses and flood duration was used as variable in the final models. Due to the ordinal nature of the variable 'flow strength' and its high correlation to the actually measured variable 'amount of gravel', the latter was used for the models although a better model fit was achieved by flow strength. Consequently, 'amount of silt/clay' was excluded, too, due to its less explanatory power in comparison to gravel. A stepwise backward selection was applied for model simplification using ‘step’ and AIC (Akaike’s Information Criterion) as a measure for the fit of a model (Crawley 2007) where non-significant variables were excluded from the models. For the explanatory variable ‘flood duration' additionally the quadratic term was tested since unimodal responses of the species were expected. The explanatory power of the models was evaluated calculating Nagelkerke's R^2_N (Nagelkerke 1991). For model discrimination we used the AUC-value (area under curve) of the receiver operating characteristic (ROC) (Hanley and McNeil 1982; Fielding and Bell 1997; Elith et al 2006). The AUC ranges from 0 to 1, with 0.5 = random models without any power of prediction, 0.7-0.8 = acceptable models, 0.8-0.9 = excellent models, and >0.9 = outstanding models (Hosmer and Lemeshow 2000). For model validation we performed a bootstrapping procedure with 1000 iterations to correct for potential overoptimistic performance criteria due to the evaluation of models based on the training data set (Guisan and Zimmermann 2000). In a last step, we applied hierarchical partitioning (Chevan and Sutherland 1991) to separate the explanatory effects of each predictor variable into independent effects on the probability of occurrence of the species.

Additionally, differences in the occurrence of *S. humboldtiana* and the invasive taxa were tested applying One-way ANOVA for all life stages (adult, juvenile and seedling) with the flood duration of the presence data as response variable followed by Tukey's method of honestly significant differences. Constancy of variance and normality of errors were checked using diagnostic plots (Crawley 2007).

All analyses concerning the habitat distribution models, model discrimination and model validation were made with R (R Development Core Team, Vienna, AT). The HMISC and the rms packages were used for model estimation and validation (Harrell 2001). Hierarchical partitioning was conducted using the hier.part package (Mac Nally and Walsh 2004).

Results

Across all life stages similar frequencies for native and invasive Salicaceae could be detected (Tab. 1). *Populus* spp. was a bit more frequent in the adult life stage and invasive willows were more frequent in the juvenile life stage than all other taxa. For both, *S. humboldtiana* and *Populus* spp., more seedlings could be found than for the invasive willows. *Tamarix* achieved lower percentages in occurrence than the Salicaceae in the adult and juvenile life stages, but a higher frequency of seedlings. No *Elaeagnus* seedlings could be found at all. In addition, 6.6% of the plots were permanently flooded and another 6.6% were without any woody vegetation (bare ground).

Table 1 Percentage occurrence of the different taxa distinguished for the three life stages for a total of 167 plots along the Río Negro.

	S. humboldtiana	invasive *Salix*		*Populus* spp.	*E. angustifolia*	*Tamarix* spp.
		S. × rubens	*S. babylonica* hybrid			
adult	27%	26%	28%	35%	13%	25%
juvenile	30%	38%		34%	8%	22%
seedling	8%	5%		7%	-	14%

Community patterns

The DCA depicted an eigenvalue for the first axis of 0.679, and 0.263 for the second axis, the lengths of gradient were 6.15 and 4.24 SD for the first two axes. The environmental variables flow strength, flood duration, AWL and the amount of gravel showed the highest correlation with the first DCA axis, while upper/middle valley (AV, VM) correlated best with the second axis (Fig. 3). However, the location of the plots in the upper or middle river valley could only explain trends in species composition.

All species showed a clear separation of the different life-stages (seedling, juvenile, adult) along the first axis (Fig. 3). Seedlings occurred on low elevated sites with high flood durations/average water levels, high amounts of gravel and high disturbance rates reflected by high levels of flow strength. In contrast, adult life stages were found on higher elevated sites with high amounts of clay and silt. The juvenile stages were located in between.

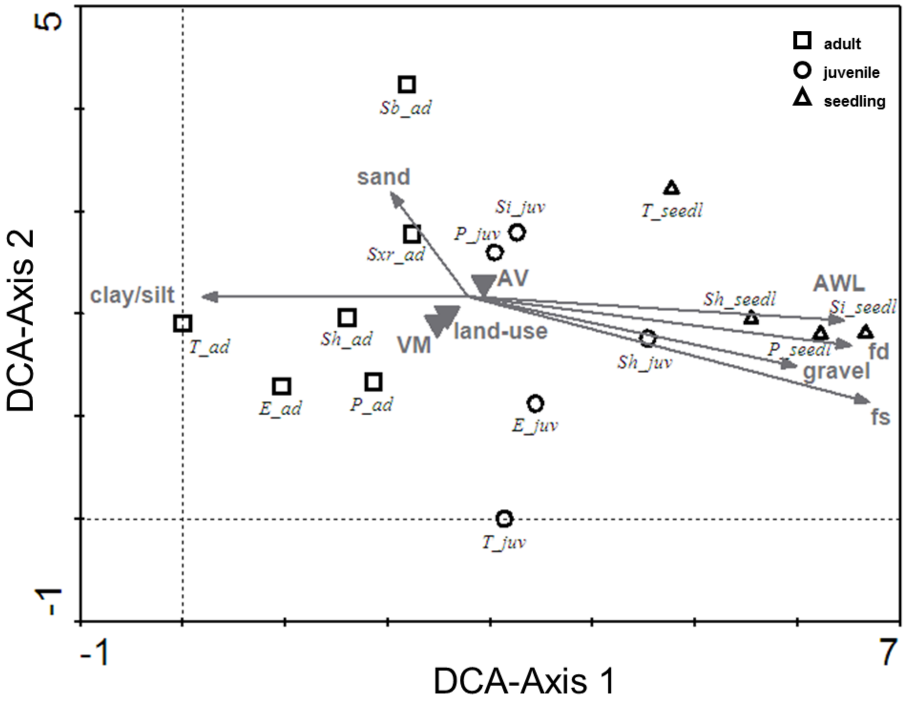

Figure 3 Detrended Correspondence Analysis (DCA) of species and supplementary environmental variables. First and second axis eigenvalues: 0.679/ 0.263; first and second length of gradient: 6.145/ 4.240. AV (Alto Valle = upper river valley), VM (Valle Medio = middle river valley) and land-use are nominal environmental variables. Used abbreviations: fd = flood duration, AWL = average water level, fs = flow strength, Sh = S. humboldtiana, Sb = S. babylonica hybrid, Sxr = S. × rubens, Si = invasive Salix (including S. × rubens and the S. babylonica hybrid), P = Populus, E = Elaeagnus, T = Tamarix; small types stand for life stages: ad = adult, juv = juvenile, seedl = seedling.

Habitat models

In general, flood duration, the amount of gravel and the location (upper or middle river valley) were included as final predictor variables in the models (Tab. 2). For the adult life stages of all taxa AUC-values of the final models ranged between 0.71 and 0.89. Regarding the juvenile life stages, only models of *S. humboldtiana* and the jointly considered invasive *Salix* exceeded the critical AUC-values. This was also the case for models of the seedling stages where additionally the *Populus* model generated an excellent AUC.

All final models comprised flood duration as predictor variable either in a unimodal (adult *S. babylonica* hybrids, juvenile willows, seedlings) or linear way (all others). Flood duration proved to be the only significant predictor variable for Salicaceae seedlings. Figure 4 demonstrates the strong niche overlap of invasive Salicaceae with the native willow in the adult, juvenile, as well as in the seedling life stage along the flood duration gradient. The strongly overlapping curves indicate that *S. humboldtiana* exhibits the same optimum as the other invasive taxa. Moreover, the occurrence of adult trees on low inundated sites in contrast to seedlings occurring on frequently flooded sites represent the different elevations on which the different life stages occur. *Elaeagnus* and *Tamarix* only achieved adequate models in the adult life stage.

Additionally, ANOVA-results revealed no significant differences in the occurrence along the flood duration gradient of *S. humboldtiana* and invasive taxa concerning different life stages except for seedlings (one-way ANOVA: $F_{3,53}$=6.781, p<0.001) with a significant observed difference in the occurrence of *S. humboldtiana* and *Tamarix* spp.

The predictor variable ‘location’ only had significant influence on the occurrence of *E. angustifolia*, *Tamarix* spp., *Populus* spp., and the *S. babylonica* hybrid in the adult life stage, as well as of juvenile invasive *Salix* (Tab. 2). While the *S. babylonica* hybrid occurred more often in the upper river valley than in the middle valley, the other three taxa were more frequent in the middle river valley. The variables ‘land use’ and ‘amount of sand’ had no significant effects and were thus not included in the models.

The hierarchical partitioning analysis showed that almost for all final models which included different explanatory variables, flood duration had the highest independent effect on the probability of occurrence of the different taxa (Tab. 3).

Table 2 Regression coefficients, standard errors (SE) and p-values as well as performance criteria, R^2_N and AUC, of the final binomial GLMs for the different species and life stages. The category 'invasive Salix' within the juvenile and seedling life stages comprise *S.* × *rubens* and the *S. babylonica* hybrid without differentiation. fd = flood duration; R^2_N = Nagelkerke's R^2; AUC = Area Under Curve.

life stage: adult

	S. humboldtiana			*Salix* × *rubens*			*S. babylonica* hybr.			*Populus* spp.			*E. angustifolia*			*Tamarix* spp.		
	Coeff.	SE	P	Coeff.	SE	P	Coeff.	SE	P	Coeff.	SE	P	Coeff.	SE	P	Coeff.	SE	P
Intercept	0.439	0.337	ns	0.147	0.133	ns	-0.590	0.522	ns	0.470	0.305	ns	-1.713	0.519	***	0.564	0.421	ns
fd	-0.010	0.004	**	-0.009	0.003	**	0.033	0.014	*	-0.017	0.004	***	-0.028	0.008	***	-0.023	0,006	***
fd+fd^2							-2,03E-01	8,22E-02	*									
gravel	-0.026	0.008	**	-0.016	0.007	*	-0.027	0.008	**							-0.035	0.016	**
location							-1.471	0.492	**	1.633	0.445	***	3.584	0.729	***	1.766	0.531	***
	Original	Validated		Original	Validated		Original	Validated		Original	Validated		Original	Validated		Original	Validated	
Performance criteria																		
R^2_N	0.270	0.253		0.195	0.178		0.333	0.291		0.367	0.352		0.465	0.446		0.474	0.444	
AUC	0.781	0.775		0.716	0.707		0.787	0.766		0.804	0.798		0.893	0.890		0.877	0.869	

life stage: juvenile

	S. humboldtiana			invasive *Salix*		
	Coeff.	SE	P	Coeff.	SE	P
Intercept	-3.470	0.588	***	-1.579	0.398	***
fd	0.040	0.007	***	0.027	0.006	***
fd+fd^2	-9,73E-02	2,01E-02	***	-7,87E-02	1,87E-02	***
location				-0.991	0.396	*
	Original	Validated		Original	Validated	
Performance criteria						
R^2_N	0.286	0.272		0.196	0.168	
AUC	0.790	0.787		0.722	0.709	

life stage: seedling

	S. humboldtiana			invasive *Salix*			*Populus* spp.		
	Coeff.	SE	P	Coeff.	SE	P	Coeff.	SE	P
Intercept	-9.552	2.729	***	-15.860	5.321	**	-19.290	5.817	***
fd	0.069	0.024	**	0.116	0.043	**	0.146	0.047	**
fd+fd2	-1,21E-01	4,93E-02	*	-2,15E-01	8,36E-02	*	-2,68E-01	8,77E-02	**
	Original	Validated		Original	Validated		Original	Validated	
Performance criteria									
R^2_N	0.375	0.323		0.249	0.239		0.312	0.307	
AUC	0.906	0.902		0.876	0.875		0.894	0.894	

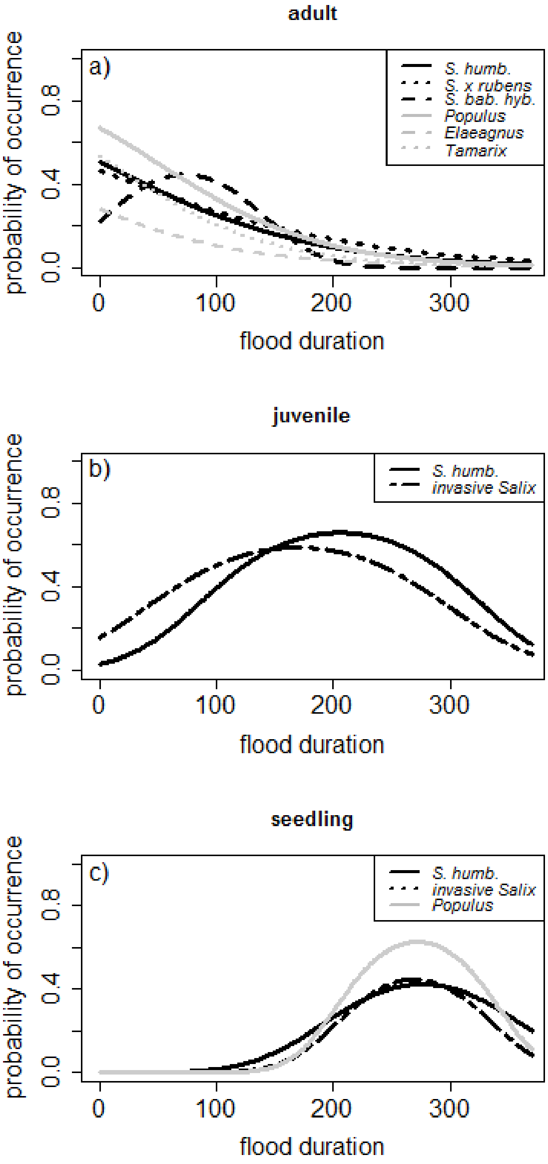

Figure 4 Probability of occurrence of the studied taxa in different life stages: a) adults, b) juveniles, and c) seedlings in relation to flood duration.

Table 3 Relative importance of explanatory variables in relation to the amount of explained deviance of the final models by means of hierarchical partitioning. Percentage indicates the relative independent effect of each predictor variable fd = flood duration, g = gravel, loc = location (upper or middle river valley).

	final model	explained deviance	hierarchical partitioning
life stage: adult			
S. humboldtiana	fd+g	18%	47%+53%
S. × *rubens*	fd+g	12%	60%+40%
S. babylonica hybrid	fd+g+loc	22%	50%+35%+15%
Populus spp.	fd+loc	24%	80%+20%
E. angustifolia	fd+loc	38%	40%+60%
Tamarix spp.	fd+g+loc	34%	43%+38%+19%
life stage: juvenile			
invasive *Salix*	fd+loc	12%	83%+17%

Discussion

The identification of ecological niches and occurring niche overlaps are required information to understand invasion processes and to assess the consequences for native species. Along the River Negro invasive woody trees, particularly Salicaceae, have the same optima as *S. humboldtiana* regarding flood duration, water level, flow strength, and soil texture gradients. Thereof, flood duration, the amount of gravel and the location proved to be the best explanatory variables to describe the occurrence of the different life stages and where thus included in the final models.

Distribution patterns of woody vegetation

Riparian woody vegetation interacts with environmental factors structuring the riparian zone. Hydrological disturbances and plant succession lead to a fluctuating habitat mosaic over time (Whited et al 2007). The different successional stages create their own hydrogeomorphic conditions by changing their environment. In this context, the required conditions for a successful establishment of riparian softwood species (e.g., Barsoum 2002; Karrenberg et al 2002; Francis and Gurnell 2006) and the importance of hydrological conditions with frequent and severe disturbances have been identified by several authors (e.g., Karrenberg et al 2003; Vreugdenhil et al 2006; Stromberg et al 2007). The combined effect of vegetation and sedimentation succession leads to a vertical aggradation, reduces inundation duration and changes soil texture from the seedling to the adult life stage. These characteristic processes of riparian ecosystems with natural disturbances could also be observed along the river Negro: Low lying bare ground sites with a high amount of gravel arising after long time of inundation exhibit ideal moist sites for seedlings to establish. Further in the process, tree growth decreases flow velocity due to an increase in surface roughness leading to intensified sediment deposition and thus to the aggradation of the floodplain (Steiger et al 2005; Gurnell and Petts 2006; Gurnell 2014). Thus, with increasing age, trees are found on higher elevated sites with reduced flood durations. Consequently, proceeding spread of invasive species might accelerate the vertical and lateral extent of islands and lead to a higher total area of Salicaceae forest both along the river shores and on islands and might change the processes in the riparian ecosystem of the Río Negro.

River margins in the Patagonian steppe are apparently susceptible to invasion by alien tree species. This may be explained by the fact that naturally only one native riparian softwood species, *S. humboldtiana*, occurs without any other competitor or successive follower. Along other rivers in the otherwise treeless Patagonian steppes where *S. humboldtiana* is not present or of low abundance, invasive *S.* × *rubens* apparently successfully occupied empty niches via vegetative reproduction due to the absence or low abundance of native riparian woody vegetation (Budde et al 2011). The alien taxa have the same habitat optima like *S. humboldtiana* across all life stages. Due to their high competitiveness because of a better growth performance invasive Salicaceae have the potential to push back the native species and thus imply a potential threat to *S. humboldtiana.*

The frequency of *Elaeagnus* with a higher probability of occurrence in the middle valley corresponds to the invasion history and its first introduction along the Río Negro in the middle valley (Klich 2000). In contrast, the *S. babylonica* hybrid is more frequent in the upper river valley. It can be assumed that planting of Salicaceae is more common in this agriculturally strongly used area. A downstream directed invasion is likely as it was supposed by Budde et al (2011) for *S.* × *rubens.* A further hint on an advanced invasion in the upper valley is the significantly higher occurrence of juvenile invasive *Salix* compared to the middle valley.

River regulations and possible consequences

River regulations by dams in the rivers Limay and Neuquén cause changes for the Río Negro in river flow, flood dynamics and water levels. It was reported by local people (oral communication), that the number of *S. humboldtiana* individuals has decreased drastically since the onset of the dams during the 70s of the last century. Simultaneously, the distribution area of invasive species has increased significantly in the last decades. Indeed, the distribution of invasive species and the increase of floodplain forests could be a result of these modified flow regimes that can create the conditions for a successful establishment (Mortenson and Weisberg 2010; Catford et al 2011).

Although high floods that remove vegetation are considered to be the main influencing factor for plant invasion along river systems (Richardson et al 2007), they may also provide conditions for the preservation of native species such as *S. humboldtiana.* In contrast to the invasive willows, which are mostly females, both, male and female individuals of *S. humboldtiana* occur frequently along the Río Negro. In conjunction to the observation, that *S. humboldtiana* exhibits distinct higher seed numbers than the invasive willow hybrids it can be concluded, that the native species relies more on sexual reproduction than the invasive willows. Yet, also invasive willow seedlings occurred in the plots, even though to a lesser extent. It seems possible, that sexual reproduction of invasive willows will increase during the ongoing invasion process when mating partners either from their own taxa or from interbreeding congeners become available. The required destructive floods that remove existing vegetation and create open sites for seedling establishment are still available at the Río Negro suggesting further successful establishment of *S. humboldtiana* seedlings. However, the enormous increase of floodplain forests in the last decades indicates that the spread of invasive tree species is a fast proceeding process that cannot be inhibited by the existing flood disturbances.

Age structure analyses of mixed adult forest stands revealed low mean ages of < 15 years of all studied Salicaceae on islands in the active zone of the river (Thomas and Leyer

2014). Trees of the same stands had the same age indicating joint establishment events of the species. These results are in line with other studies, e.g. Karrenberg et al (2003) detected low mean ages of < 10 years within the active zone of the Tagliamento river which are frequently disturbed by flood events. *S. humboldtiana* stands in other regions, too, are known to reach only low mean ages < 20 years before they are replaced by other species (Liotta 2001; Parolin et al 2002; Casco et al 2010). On the one hand, the low mean ages along the Río Negro could be explained by frequently occurring destructive floods that periodically erase established woody vegetation. On the other hand, it is possible, that prior to the establishment of the existing forests no dense forest stands appeared despite scattered *S. humboldtiana* occurrences. This assumption would lead to the conclusion that dense Salicaceae forests only occurred in the recent past representing the first invasion wave. However, on higher elevated sites beyond the river stream, of all Salicaceae (*S. humboldtiana*, *S.* × *rubens,* the *S. babylonica* hybrid and *Populus* spp.) solitary trees > 40 years could be found (Thomas and Leyer 2014).

River regulations without destructive floods can push the establishment of floodplain forests at the cost of pioneer vegetation (Wenger et al 1990). A possible scenario for the Río Negro could be the establishment of other non-native hardwood species. While *S. humboldtiana*, as typical pioneer tree, is restricted to the active channel of the river with frequently occurring floods, farther from the active channel or on higher situated places plant communities may be composed by older vegetation and other species (Gregory et al 1991; Hupp and Osterkamp 1996). Non-native *Acer negundo* L., *Fraxinus pennsylvanica* Marshall and *Eucalyptus spp.* already occur along the river, and thus a further shift in plant communities composition and an expansion of the area covered by floodplain forests is possible. Future studies will be necessary to monitor other non-native plants species and their effects for the ecosystem.

Conclusion

The transformation of the riparian ecosystem of the Negro river probably will continue. The upper river valley is a principal region for fruit cultivations and its increase is most likely. Thus, it can be assumed, that riparian land will be converted to agricultural land or urban use. Apart from being threatened by human activities, the vegetation along the Río Negro might be further subject to changes in consequence of alien invasive species and shifts in plant community composition. In our study, only the dominant species were considered, but other alien woody floodplain species could be observed along the river (e.g. *Salix viminalis* L., *Salix matsudana* Koidz., *Populus alba* L., *Eucalyptus* spp., *Acer negundo* L., *Fraxinus pennsylvanica* Marshall) which also have the potential to become frequent in the floodplain forests. Other non-native willows of both sexes and consequently new natural hybridizations can provoke a further invasion step with an increase of sexual reproduction. Several genotypes could be found so far only along the Río Negro, in contrast to other Patagonian streams, where almost exclusively one female of *S.* × *rubens* forms monoclonal stands (Budde et al 2011). With the appearance of male individuals and other species or hybrids comparable evolutionary processes to those at the Río Negro with sexual regeneration can be expected along other rivers. Particularly for the *S. babylonica* hybrid, whose spread is probably a development of the recent years, a further spread along other rivers is likely.

Finally, the knowledge, that the native willow exhibits a strong niche overlap with invasive woody species in all life stages (seedling, juvenile and adult) with no remaining exclusive habitats can aid to assess the future impact on the native species. Based on the assumptions, that the invasion is still ongoing and that the highly competitive alien Salicaceae will probably continue to expand their range, it can be expected that these processes indicate a serious threat to the native species.

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Chapter 5 – Paper 4

Detection of land cover and vegetation change by invasive woody species spread at the Río Negro, Argentina

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Manuscript

Abstract

Context: Riparian ecosystems are considered to be especially vulnerable to plant invasions and among riparian invaders, Salicaceae species play an important role. It can be expected that invasive willows and poplars change much of natural riparian environments although large gaps in knowledge exist.

Objectives: This study aimed at detecting land-cover changes in the riparian ecosystem of the Rio Negro, Patagonia, due to woody species spread. The following questions were addressed: 1) How has land-cover in the upper Rio Negro valley changed since 1986? And 2) Have forest areas, dominated by invasive species, increased in size at the loss of other land-cover types?

Methods: Multispectral satellite images from 1986 and 2003 were employed to classify images of the upper Río Negro valley using classification tree analysis and to detect land-cover changes between them via post classification comparison.

Results: Major land-cover transformations were detected in the study area. Forests, dominated by invasive species, increased by almost 150 % in 17 years and currently cover about 20 % of the riparian ecosystem. Major gains were also detected for areas in agricultural use, whereas land-cover types dominated by native species decreased by more than 30 %.

Conclusions: The results confirmed observations made by local people that willows and poplars started to spread intensively two decades ago. The alarming increase of forests dominated by invasive species will probably have significant consequences for native species and ecosystem properties. A coordinated and comprehensive management is needed in order to limit ecological and potential economic impacts of invasion processes.

Introduction

Biological invasions are among the most important drivers of biodiversity loss worldwide (Mack et al 2000, Vilà et al 2011), and can have ecological, evolutionary and economic consequences (Primack et al 2010). South America is strongly affected by biological invasions. According to Speziale et al (2012) at least 41 out of the 100 world's worst invasive species (Lowe et al 2000) have been established on the continent. Nevertheless, these processes as well as their impacts have been poorly studied and governments in the affected countries have not given much attention to the problem (Pauchard et al 2004; Speziale et al 2010).

Riparian ecosystems are considered to be especially vulnerable to plant invasions because of the frequently occurring disturbance events (Brown and Peet 2003; Stohlgren et al 1999; Ward et al 2002) and the hydrological connectivity of river corridors, which do not only cause high species diversity but also facilitate the dispersal of invasive plants and other invasive species connected to rivers (Renöfält et al 2005; Ward et al 2002).

Among riparian invaders, Salicaceae species introduced for ornamental and functional landscaping play an important role (Thomas and Leyer 2014). Eurasian willow and poplar species have naturalised widely, e.g. in Australia (Cremer 2003), New Zealand (Glova and Sagar 1994), South Africa (Henderson 1991) and the USA (Shafroth et al 1994). In riparian ecosystems of Patagonia, they have probably been introduced by European settlers in the late 19th or early 20th century as windbreakers and source of fuel wood or timber (Peri and Bloomberg 2002). Nowadays, they form large gallery forests e.g. in the floodplains of the Ríos Limay, Neuquén and Negro (Budde et al 2011). These forests can also comprise the only native riparian tree species in the region, *Salix humboldtiana* (Thomas et al 2012, Thomas and Leyer 2014), which is distributed throughout the warm regions of South America (Liotta 2001), but has become relictual in some places due to competition with invasive species (Zalba and Villamil 2002). Beside species of the Salicaceae, silver berry (*Elaeagnus angustifolia*), native to southern Europe and central and eastern Asia, as well as tamarisk species (*Tamarix* spp.), native to Eurasia and Africa, are prominent invaders of Patagonian riparian ecosystems.

The distribution area of all these invasive woody species has apparently increased in the last few decades along Patagonian river systems as numerous farmers and land owners of the region reported (oral communication). However, little information on the qualitative and quantitative changes of river accompanying vegetation is available. Datri and Maddio (2008) and Datri et al (2010) have investigated land-cover composition and ecosystem changes in a small stretch of the Lower Río Neuquén and the area where Rio Neuquen and Rio Limay converge, but land-cover change and invasive woody species' spread in the area have not been detected on a large spatial scale yet.

Remote Sensing has the potential to be an important tool in assessing the extent of plant invasions and their impact on landscapes (Civille et al 2005; Richardson and Rejmanek 2011). Indeed, invasive species have been mapped with Remote Sensing in a variety of studies in both terrestrial (e.g. Bradley and Mustard 2006; Huang and Asner 2009; Mosher et al 2009) and aquatic (e.g. Albright and Ode 2011) ecosystems and it has even been used to inventory riparian areas (e.g. Akasheh et al 2008).

Consequently, this study aimed at detecting land-cover changes in the riparian ecosystem of the largest Northern Patagonian river system by making use of remotely sensed Landsat TM/ETM+ satellite image data of the years 1986 and 2003. The study had a special focus on vegetation types structured by invasive Salicaceae species as well as by silver berry and Tamarisk species.

Specifically, the following questions were addressed:

(1) How has land-cover in the upper Río Negro valley changed since 1986?
(2) Have forest and woodland areas, dominated by invasive woody species, increased in size at the loss of other land-cover types?

The study wants to contribute to a better understanding of invasion dynamics in Patagonian riparian landscapes and to assist in assessing the impact of woody species invasion on the riparian ecosystem with its native vegetation.

Methods

Study Area

The Río Negro in Patagonia, Argentina, is formed by the confluence of the Río Limay and the Río Neuquén and discharges into the Atlantic Ocean. It is about 600 km long and has a mean annual discharge of about 900 m^3/s with pronounced water level fluctuations (DPA, Departamento Provincial de Aguas Río Negro). We investigated a 100 km stretch of the upper Río Negro, from its origin at the confluence of the Ríos Limay and Neuquén at 38°59’35’’S, 68°00’07’W, near the city of Neuquén, to the village of Valle Azul near the town of Chichinales at 39°09’14’’S, 66°47’13’’W (Fig. 1). The Upper Río Negro forms a valley in the Patagonian tableland with a width up to 10 km and a depth around 100 to 250 m. It is framed by the uprising high plateaus, called *bardas* or *mesetas*. The area is situated in a transition zone between the subtropical and the temperate zones on the South American continent. It has a semi-arid to arid climate with an annual mean precipitation of less than 250 mm and an annual mean temperature of roughly 15 °C (Ayesa et al 2002; Gut 2008).

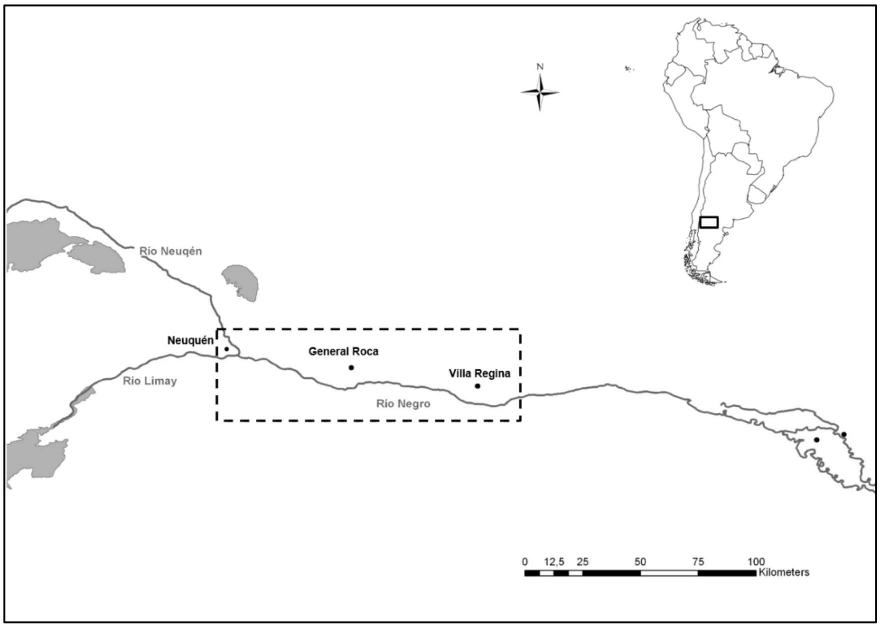

Figure 1 Río Negro river system with its feeder rivers Rio Limay and Rio Neuquén. Dashed rectangle: study area.

The former natural vegetation of the valley was a mixture of the typical vegetation of the surrounding dry shrub steppe with grasses and shrubs adapted to semi-arid or arid conditions and, in the more moist areas close to the river braids, gallery forests of the native *Salix humboldtiana*, interspersed with shrub species from the surrounding steppe and pioneer species like the shrub *Baccharis salicifolia* (Gut 2008). However, nowadays the upper river valley is the principle region of Argentina for apple and pear production. Hence it is characterised by large fruit plantations, mostly at the northern side of the river, which stretch to the river shores (Thomas and Leyer 2014). Plantations are usually enclosed by planted alien poplar and willow rows. The study area itself includes the river braids and their shores and is limited by the *bardas* or the beginning of the intensely used agricultural area within the river valley.

Input data and software

This change detection study aimed to cover a possibly long period of time while at the same time availability of freely accessible scenes was a limiting factor. A Landsat TM image from 23/01/1986 and a Landsat ETM+ image from 14/01/2003 were chosen for the study. Both pictures have a cloud cover of less than 10 % and were obtained from the United States Geological Survey (USGS).

All processing of Landsat image data, including pre-processing, classification and change detection was done with the digital image processing software IDRISI^{TM} Taiga.

Pre-processing

No distortions or pixel shifts were found in the scenes, thus co-registration of both images was not necessary. Thermal infrared bands' were resampled to 30m spatial resolution.

Correction of atmospheric attenuation was performed by using the COST Model approach of Chavez (1996). This model combines a dark object subtraction and a procedure to minimize the effects of absorption and Rayleigh scattering in the atmosphere to calculate absolute reflectance (Goettlicher et al 2009). The model was implemented by using the module ATMOSC in IDRISI^{TM} Taiga. For dark object subtraction, a water reservoir in the investigation area which contained the lowest spectral reflectance values of all water bodies in the scene was chosen.

A topographic correction was not necessary since the investigation area has a more or less flat terrain with elevation differences of a few metres only.

Since a considerable part of the Landsat images cover steppe vegetation on top of the *bardas* and include the rivers Limay and Neuquén, which are not in the focus of this study, these parts were masked out, so that only the actual investigation area, i.e. the riparian ecosystem with the braided river and its connected floodplains was analysed.

Land-cover classification

Training data collection for land-cover classification of the 2003 Landsat image

Training data was collected in the field from September 2010-January 2011, thereby choosing the same season (summer) as that of scene capture. In advance, the land-cover classes of interest were identified with the help of expert knowledge and field observations and by examining 2003-2010 Google Earth^{TM} images, which were available for the complete study area. Eight land-cover classes were selected to be identified in the classification (Table 1).

180 sites were recorded, ranging between 13 and 35 sites per class. The position of training sites was captured with a Mobile Mapper^{TM} CX GPS in autonomous GPS mode (deviation approx. 2-5 m) with the help of the software ArcPad. One or few reference points within each training site were captured by averaging over 50 measurements. In each training site, species composition as well as amount and composition of understory vegetation and ground vegetation layer was recorded. Other observations that could diminish a site's suitability as training data, like flooding, estimated age of vegetation, or soil texture, were also recorded.

After capturing, position data was imported into GIS software ArcMap and checked for position errors and size. Suitable tracks were then imported into IDRISI^{TM} Taiga as vector files. These were compared to Landsat true and false colour composites, the *Normalized Difference Vegetation Index* (*NDVI*) (Rouse et al 1973), and cluster maps from an unsupervised classification, before training sites were digitised within the boundaries of the tracks. Additional training sites were selected on-screen for the Fruticulture land-cover class because field data on those was sparse. However, they were reasonably well identifiable by comparison with the colour composites. Training sites for the land-cover class Water were completely elected on-screen via comparison with *NDVI*, false colour composite of Landsat bands 1,2,4, and the cluster map. Altogether, 147 training sites were digitised (Table 1).

Table 1 Land-cover classes for supervised classification as well as number of training sites and training site pixels per class in the 2003 supervised classification.

Category	**Class**	**Description**	**No of Training Sites**	**No of Pixels**
Vegetation	Fruit Plantation	Standard fruit trees, mainly apple, pear, peach and cherry	17	287
	Salicaceae Forest	Adult stands of mainly invasive *Salix* (including native *S. humboldtiana*) and *Populus* taxa	21	309
	Elaeagnus angustifolia Woodland	Woodlands dominated by *E. angustifolia*	10	244
	Dry Shrub Vegetation	Dry shrubs of the natural *monte* vegetation, occasionally including invasive *Tamarix spec*. individuals	21	376
	Green Herbaceous Vegetation	Pastures and other gramineous or herbaceous vegetation, comparatively green and healthy	14	145
	Dry Herbaceous Vegetation	Pastures and other gramineous or herbaceous vegetation, dry, often over-grazed	8	80
Non-Vegetation	Bare Ground	Mostly mixtures of sand and gravel with no or very sparse vegetation	14	106
	Water	River braids and backwaters of the Río Negro	30	917

Training data collection for the land-cover classification of the 1986 Landsat image

Hardly any information such as maps or aerial photography of the upper Río Negro valley was available for collecting training data for classification of the 1986 image and because of the large time difference, only very few training sites from the 2003 classification could be adapted. Therefore, training data was mainly elected on the basis of the cluster map from unsupervised classification, which could be compared with a 1985 infrared aerial photograph of the area around the city of Neuquén (property of the land-registry office of the province of Neuquén), in order to identify the land cover classes of interest. Colour composites and *NDVI* maps were used to support decisions on training data. Thus, between 7 and 33 training sites per year and class were chosen (Table 2). However, it was not possible to discriminate certainly between the land-cover classes Dry Shrub Vegetation, Dry Herbaceous Vegetation, and Green Herbaceous Vegetation which is why only one class "Shrub and Herbaceous Vegetation" was created. Also, no cluster could be identified as *E. angustifolia* Woodland, thus, all clusters that could be associated with forest and woodland were put into one class with that same name. Fruticulture was not represented by any cluster in the unsupervised classification, probably due to its inhomogeneous texture. Consequently, training sites were chosen on the basis of their geometrical structure in the colour composites.

Table 2 Number of training sites and training site pixels per class in the 1986 supervised classification.

Land-cover Class	**No of Training Sites**	**No of Pixels**
Fruticulture	36	411
Forest and Woodland	28	552
Shrub and Herbaceous Vegetation	33	572
Bare Ground	14	146
Water	30	944

Classification tree analysis

We used a supervised hard non-parametric classification approach to classify both images, namely a classification tree analysis (CTA), sometimes also referred to as decision tree analysis.

The IDRISI^{TM} Taiga CTA classifier used in this study is a univariate tree classifier which works by taking known training sites for each class to construct a decision tree based on the measured attributes (i.e. spectral radiance). The entropy splitting algorithm was chosen for our CTAs.

In order to counteract overfitting of data, a common problem in CTAs (Friedl and Brodley 1997), we did an auto-pruning of 1% in order to eliminate meaningless leaves. Landsat bands 1-7 were used to classify both images. After classification, a 3x3 mode filter was applied to the classification results in order to flatten them and the previously constructed mask was applied to the respective classification maps.

Accuracy assessment

We assessed classification accuracy by calculating *Overall*, *User's*, and *Producer's Accuracy* from an error matrix, as well as the Kappa Index of Agreement (KIA) (Cohen 1960). Since independent ground truth information was not available for this study, we used the ground data collected in 2010/2011 that served as training data for the classification of the 2003 image for accuracy assessment, assuming it to be the most reliable information available. For the classification of the 1986 image, sample sites for accuracy assessment were obtained the same way as were the training sites, i.e. via comparison with the respective cluster maps and colour composites. An average of 13 sample sites and 244 pixels per class and year could thus be collected. Based on suggestions of Justice and Townshend (1981) and Mc Coy (2005) sample sites had a minimum size of 60x60 m² and were as equally distributed throughout the investigation area as possible.

Change detection

Land-cover change in the investigation area was detected in a per-pixel post-classification comparison (PPC) using the IDRISI^{TM} Taiga Land Change Modeler Tool. In a PCC, categorical changes of land-cover in time are detected by comparing independently produced classification maps (Rogan and Chen 2004). This comparison results in a complete matrix of changes that enables the analyst to observe any subset of changes of interest (Singh 1989) as well as the nature of the change (Rogan et al. 2003). PCC has proven to deliver good results in a variety of change detection studies (Abd El-Kawy et al 2011; Chen and Wang 2010; Jensen et al. 1995; Kesgin and Nurlu 2009; Munyati 20000; Peterson et al 2004) and it was suggested by Lu and Weng (2007) especially for "detailed `from-to´ detection". Since this study specifically aimed at detecting the change in woody species distribution and the contribution of other land-cover classes to this change, PCC thus promised to deliver the best results. Quantitative as well as spatial change was detected between the years 1986 and 2003. Since different numbers of land-cover classes were mapped in each of both year's classifications, with the 2003 classification being more detailed than the 1986 classification, they had to be adjusted to one another in order to make change detection possible. Thus, the shrub and herbaceous categories in the 2003 classification map were reclassified into one class "Shrub and Herbaceous Vegetation". The same way, Salicaceae Forest and *E. angustifolia* Woodland were reclassified into "Forest and Woodland" in order to be able to detect changes between the 2003 and the 1986 classification.

Results

Land-cover classification of the 2003 Landsat image

Very good classification results could be obtained with the non-parametric CTA, with an *Overall Accuracy* of 0.99 and KIA of 0.98. *User's* and *Producer's Accuracy* for the individual land-cover classes were very high, too, ranging between 0.91 and 1.00 for the different land-cover classes (Table 3). Furthermore, visual comparison of the classification map with an aerial photograph of the confluence region near Neuquén (property of the land-registry office of Neuquén) also indicated towards a high accuracy of the classification, although Fruticulture seemed very slightly overestimated (Fig. 2).

Water covered the largest area in the studied part of the Río Negro valley (Table 4). Among the land classes, Fruticulture, Dry Shrub Vegetation and Salicaceae Forest showed the highest coverage, while Bare Ground was the smallest land-cover class. Thus, the classes dominated by invasive woody species, namely Salicaceae Forest and *E. angustifolia* Woodland, covered 25 % of land surface (excluding Water), whereas 22 % were covered by Dry Shrub Vegetation.

Table 3 Error matrix for classification tree analysis with 8 land-cover classes in 2003. Columns: training data (truth). Rows: mapped.

Class	F	SF	BG	EA	DS	GH	DH	W	Total	Acc_U
F	**287**	9	0	0	0	3	0	0	299	0.96
SF	0	**295**	0	1	0	0	0	1	297	0.99
BG	0	0	**103**	0	0	0	0	0	103	1.00
EA	0	0	0	**243**	0	0	0	0	243	1.00
DS	0	0	1	0	**370**	0	0	0	371	1.00
GH	0	0	0	0	0	**141**	3	0	144	0.98
DH	0	1	0	0	6	1	**77**	0	85	0.91
W	0	4	2	0	0	0	0	**916**	922	0.99
Total	287	309	106	244	376	145	80	917	2464	
Acc_P	1.00	0.95	0.97	1.00	0.98	0.97	0.96	1.00		

Acc_U: *User's Accuracy*
Acc_P: *Producer's Accuracy*
F: Fruticulture; SF: Salicaceae Forest; BG: Bare Ground; EA: *E. angustifolia* Woodland; DS: Dry Shrub; GHV: Green Herbaceous Vegetation; DHV: Dry Herbaceous Vegetation; W: Water

Table 4 Area coverage of land-cover classes 2003.

Land-cover class	**Area (ha)**	**Area (%)**	**Area (% of land surface*)**
Fruticulture	4697	19.82	25.30
Salicaceae Forest	3518	14.85	18.95
E. angustifolia Woodland	1213	5.12	6.53
Dry Shrub Vegetation	4125	17.41	22.22
Green Herbaceous Vegetation	2709	11.43	14.60
Dry Herbaceous Vegetation	1712	7.22	9.22
Bare Ground	587	2.48	3.16
Water	5137	21.68	
Total	23697		

*excluding Water

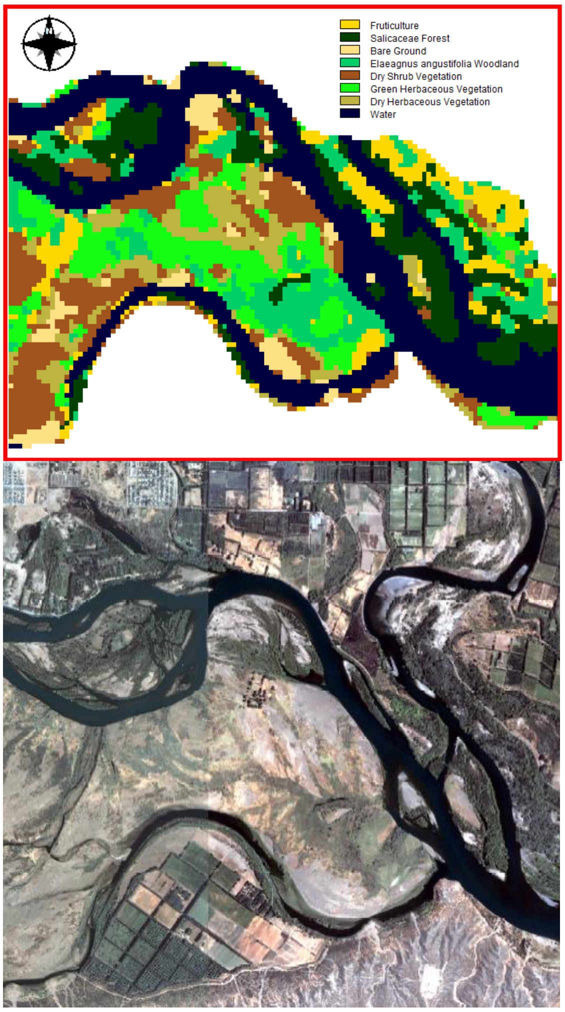

Figure 2 Comparison of cta classification map (above) with aerial photograph of the confluence region near Neuquén, 2001 (property of the land-registry office of the province of Neuquén).

Land-cover classification of the 1986 Landsat image

Classification results were also very good for the 1986 Landsat image, with very high *User's* and *Producer's Accuracy* for the individual land-cover classes (Table 5), and a high *Overall Accuracy*, which was but slightly lower than that of the 2003 classification (Table 6). High within-class variability remained for the class Fruticulture, expressed by the numbers of leaves generated in the CTA (data not shown).

Table 5 Error matrix for classification tree analysis of the 1986 Landsat image. Columns: truth. Rows: mapped.

Class	F	FW	BG	SH	W	Total	AccU
F	**123**	11	1	6	0	141	0.87
FW	3	**262**	0	0	2	267	0.98
BG	0	0	**280**	0	0	280	1.00
SH	1	0	30	**276**	0	307	0.90
W	0	0	3	0	**318**	321	0.99
Total	127	273	314	282	320	1316	
AccP	0.97	0.96	0.89	0.98	0.99		

Acc_U: *User's Accuracy*
Acc_P: *Producer's Accuracy*
F: Fruticulture; FW: Forest and Woodland; BG: Bare Ground; SH: Shrub and Herbaceous Vegetation; W: Water

Table 6 Comparison of accuracy statistics for classification tree analyses of the 1986 and 2003 images.

	1986	**2003**
Overall Acc	0.96	0.99
Acc_U	0.95	0.98
Acc_P	0.96	0.98
KIA	0.95	0.98

Land-cover change between 1986 and 2003

Singh (1989) stated that the accuracy of land-cover change in a PCC would be completely dependent on the classification accuracies and is likely to be close to the product of these, which leads to an accuracy estimate of 0.95 for the overall change in this change detection study.

Land-cover changes between 1986 and 2003 were vast, especially in the classes Shrub and Herbaceous Vegetation, Forest and Woodland, and Fruticulture (Fig. 3, Appendix 2).

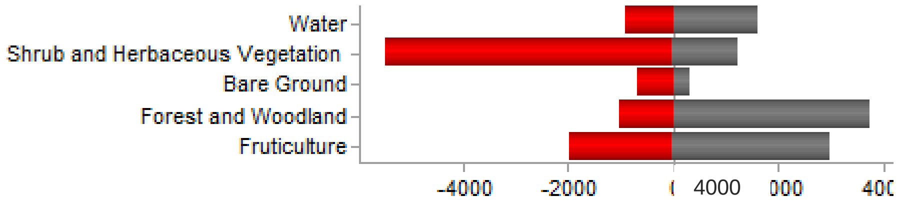

Figure 3 Gains and losses of land-cover classes between 1986 and 2003 in hectares.

Figure 4 Area exchanges between Forest and Woodland and Water in a section of the upper Río Negro valley between 1986 and 2003.

Forest and Woodland cover more than doubled its size, with a gain of over 2700 ha (Table 7). This class increased all over the investigation area, with no obvious pattern apparent, and as good as no persistence (Appendix 3). Fruticulture had the second largest increase with 1085 ha. Although Forest and Woodland cover, like Fruticulture, mostly replaced former Shrub and Herbaceous areas, Water also contributed to the change, even though area covered by Water was generally higher in 2003 (Table 8). A look on the exchange map of Water and Forest and Woodland shows that the areas transformed into Forest and Woodland almost exclusively appear at the river braids' shores and on islands (Fig. 4). The greatest reduction was suffered by Shrub and Herbaceous Vegetation, which lost a third of its former area, mainly due to the increase in both Forest and Woodland, and Fruticulture (Tables 7+ 8, Appendix 4).

Table 7 Net area changes of land-cover classes between 1986 and 2003.

Land-cover Class	**change (ha)**	**change (%*)**
Fruticulture	+1085	+ 33.5
Forest and Woodland	+2744	+149.5
Shrub and Herbaceous Vegetation	- 4194	- 34.0
Bare Ground	- 343	- 37.1
Water	+ 709	+ 16.5

*in % of former area covered by the same class

Table 8 Cross-tabular of changes (ha) between land-cover classes 1986-2003. Numbers in grey show persistence.

Year		**2003**				
	Class	F	FW	SH	BG	W
1986	F	1285.83	853.02	890.91	17.91	191.16
	FW	758.07	835.47	96.93	1.35	143.82
	SH	2206.8	2077.74	6879.51	190.8	995.94
	BG	40.59	114.03	248.04	249.48	272.34
	W	32.76	698.94	41.04	121.86	3408.48

F: Fruticulture; FW: Forest and Woodland; EA: *E. angustifolia* Woodland; SH: Shrub and Herbaceous Vegetation; BG: Bare Ground; W: Water

Discussion and Conclusions

The results of this study provide the first extensive description of land-cover and land-cover changes in the upper Río Negro riparian ecosystem, and an assessment of the spread of the invasive woody species in this area. Interpretation of the detected change patterns will help to comprehend their impact on native biota and ecosystem properties.

Error sources and potential improvement of methods

In this study, exceptionally high classification accuracies were achieved by implementing CTA, especially for the classification of the 2003 Landsat image. However, accuracy for this classification was calculated with the same ground truth data that had already been used for training and which was thus not independent from the classification. Use of independent ground truth data might have lowered classification accuracy to some extent. Additionally, CTAs are known to be sensitive to overfitting of data, which is corrected by employing pruning measures. Pal and Mather (2003) stated that “the choice of an appropriate pruning method has a positive effect in improving classification accuracy”. The pruning mechanism used in this study was a very simple approach and might not have been sufficient to completely correct for overfitting of data. Approaches employed by other scientists include cross-validation (Friedl and Brodley 1997; Rogan et al 2003) and manual pruning (Hansen et al 2000). Future employments of CTA for mapping land-cover in the investigation area should therefore evaluate methods of pruning in order to get the best possible results.

CTA is also considered to be sensitive to the number of training samples and to large discrepancies in these numbers among individual classes (Rogan et al 2003). Pal and Mather (2003) found that univariate trees worked best with a number of at least 300 pixels per class. Training data collection in this study was difficult because of limited accessibility to sites, varying occurrence of land-cover types and, most of all, because of the great time difference between image acquisition and field data collection. This was especially problematic because changes of land-cover are inherently fast in a riparian ecosystem with natural dynamics, and because all of the studied woody species are relatively fast-growing. However, all this was kept in mind during data collection and even though there are some rather large discrepancies in the number of training pixels among the individual classes, the finally used composition of training data proved to be the most efficient when mapping land-cover in the investigation area.

Classification accuracy estimates for the classifications of the 1986 Landsat image was also very high and indicates towards high map accuracy, even though discrepancies in numbers of training data pixels and pruning procedures must be considered again. A specific problem in the classification of the older image was the lack of historic reference information which resulted in drawing both training and truth data from unsupervised classification results. Obviously, this method cannot be as reliable as the collection of data in the field or drawn, e.g., from aerial photographs, and it did not allow for a classification as detailed as that of the 2003 image. This was especially problematic when electing training sites for Fruticulture since this class was not represented by any one cluster in the unsupervised classification, probably due to its inhomogeneous texture. Consequently, training sites were chosen on the basis of their geometrical structure in the colour composites, but this approach is prone to errors. As a result, Fruticulture showed rather high within-class variability in the CTA.

Land-cover composition

Mapping from the 2003 Landsat image revealed that forests and woodlands cover almost 25 % of the terrestrial part of the riparian ecosystem. They are mainly composed of two invasive willow hybrids as well as poplar hybrids from Eurasian origin although *S. humboldtiana* occurred as well. Thomas and Leyer (2014) found that 173 of 849 counted adult Salicaceae tree individuals in 54 plots were *S. humboldtiana*, and these had a smaller average basal area than the other ones. Therefore, invasive willow and poplar taxa dominate the forests and woodlands that covered almost a quarter of the land surface in the riparian ecosystem in 2003. Similar to these results, Datri and Maddio (2008) also found nearly 20 % of a 14 km stretch of the Río Limay near Neuquén to be covered by Salicaceae woods and forests.

Among the remaining vegetation classes, Dry Shrub Vegetation is assumed to represent native vegetation type and species better than any other class because of the comparatively small number of non-native species that were found in the training sites for this class (data not shown). However, Dry Shrub Vegetation only covered 22 % of land surface, less than both Forest and Woodland and Fruticulture. Despite the rather natural species composition in this class, it also comprised the invasive shrub *Tamarix* spp. This species could not be mapped independently because it typically occurred in small groupings and stripes in the investigation area that seldom reached widths more than 20 m and could thus not be captured by Landsat pixel resolution of 30 m. *Tamarix* spp. was also frequent in the understory of *Salicaceae* woods and forests and it occasionally grew in co-dominant stands with *E. angustifolia.* This implies that the species is among the important woody invaders in the Río Negro valley or might at least have the potential to become so.

Land-cover changes

Between 1986 and 2003, the land-cover class Forest and Woodland not only increased by almost 150 %, but there was also as good as no persistence, which implies that almost all of the woody vegetation in 2003 has developed after 1986. However, it has to be considered that in the 2003 classification map the Forest and Woodland class represented the two original classes Salicaceae Forest and *E. angustifolia* Woodland, which were summed up after classification to enable comparison with Forest and Woodland in the 1986 classification. This could have led to a slight overestimation of the detected increase if some of the woody vegetation in 1986, where land-cover classification was not as exact as in 2003, was misclassified as Shrub and Herbaceous Vegetation or Fruticulture.

Age structure analysis of the forests composed of willows and poplars on islands along the upper and middle Rio Negro revealed low mean ages of < 15 years for all taxa involved (Thomas and Leyer 2014). This could suggest frequent and severe disturbances resulting in the removal of existing vegetation and subsequent creation of new sites for establishment as it is observed in near natural dynamic river systems (Karrenberg 2002). In this study, this is reflected by the change from Forest and Woodlands to Water. However, change detection also showed that the main process was vice versa: Woody vegetation has developed from Water after 1986 (fig. 4) despite an overall gain of Water between 1986 and 2003. It is thus probable, that the main part of the recent adult stands of Salicaceae forest on the islands are the representatives of a first invasion wave. This would confirm statements of local farmers and land-owners that invasive species distribution has increased notably only in the last one or two decades (oral communications).

Remaining questions are, which factors might have caused the enormous increase of forests and woodlands, and why there were no persistent woods. Among the important factors may be changes in river flow, flood dynamics and water levels, caused by regulation of dam reservoirs in the Río Limay and Río Neuquén and irrigation water diversions. Even more important may be land-use and land-use changes in the region. These include the plantings of invasive species for protection against wind and water erosion or for ornamental purposes, logging and clearing, and grazing (Vila and Ibanez 2011). Life traits of the invasive species will also be important, especially reproduction. For instance, vegetative reproduction has been found to be an important factor in the spread of invasive willows in North Patagonia (Budde et al 2011) and in the outperforming of *S. humboldtiana* (Thomas et al 2012).

Next to Forest and Woodland, Fruticulture had the greatest gains over the study period. When interpreting the changes in Fruticulture in the river valley, it has to be minded that most of this land-use type was excluded from the study area. Thus, this study cannot claim to detect the dynamics of it. However, an increase and intensification of agricultural use has been observed in other studies, too, both in Argentina (Baldi and Paruelo 2008), and in riparian landscapes in other parts of the world, like, e.g., the Nile delta (Abd El-Kawy et al 2011). The magnitude of the increase of Fruticulture detected in this study suggests that agricultural use has also intensified in the Alto Valle region and that the riparian ecosystem has been affected by this as well.

Land coverage of Bare Ground was also subject to considerable changes between the studied years. These changes are inherent because bare ground areas are rapidly colonized by vegetation and almost all of them are close to the river and thus subject to changing water levels. For instance, in January 2003 (time of scene capture) water levels were high in comparison to other years (DPA, Departamento Provincial de Aguas Río Negro) which will explain the great losses of Bare Ground in this year.

Impact on the riparian ecosystem

Invasive species can have the ability to change the habitat in which they invade (Civille et al 2005) and to cause the impoverishment of native biota (Baldi and Paruelo 2008). In this context, it is essential to notify that all major invasive species in the study area have traits that may alter the ecosystem profoundly. Salicaceae are known to alter fluvial dynamics and to facilitate the development and growth of gravel bars and islands (Moggridge and Gurnell 2009). *E. angustifolia* has been found to alter nitrogen content and dynamics in riparian ecosystems (Decant 2008; Mineau et al 2011; Shah 2010). While *Tamarix* spp.'s tentative ability to accumulate salt content in soils remains a subject of discussion among scientists (Glenn and Nagler 2005; Sher et al 2002), it does have been found to lower water tables (Lindgren et al 2010). Furthermore, the introduced Salicaceae species and *E. angustifolia* form extremely dense canopy covers in comparison to both native *S. humboldtiana* and native shrubs. This may have a severe effect on native herbaceous and understory vegetation. Indeed, many non-native species were found in plots of Salicaceae Forest but further studies are necessary to evaluate the influence of the invasive trees and shrubs on the ground vegetation layer. Altogether, the dramatic increase in the forests and woodlands, dominated by invasive species, may have altered the riparian ecosystem of the Río Negro profoundly and may continue to do so since the invasion process is probably still ongoing. Although results from this study as well as field observations suggest that Salicaceae species currently occupy most of the habitat that is suitable for them, they will probably continue to colonize newly formed islands and may even establish in yet unfavourable habitats if ecosystem conditions change for the better. *E. angustifolia*, on the other hand, may continue its spread further, since it is able to tolerate a broad range of physical conditions (Katz and Shafroth 2003) and can thus invade habitats that are unfavourable for Salicaceae species. It is also able to tolerate shade and can establish underneath the canopy of other riparian tree species (Katz and Shafroth 2003), which was also observed in the study area and may lead to the species taking over sites that are yet dominated by Salicaceae species. The same might be true for *Tamarix* spp. This species, too, profits from a number of adaptations against drought, salinity, fire and, at a young age, even flooding (Glenn and Nagler 2005; Lindgren et al 2010; Sher et al 2002) and may thus spread in areas that are too dry or otherwise unsuitable for Salicaceae trees. It has further to be considered that non-native hardwood species like *Fraxinus pennsylvanica* and *Acer negundo* were frequently observed in the understory of Salicaceae Forest. Thus, it is probable that invasion processes will continue by the establishment of non-native hardwood forests. A coordinated and comprehensive management together with a monitoring of vegetation and environmental change is urgently needed in order to limit both ecological as well as potential economic consequences of invasion processes in Patagonian river landscapes.

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Supplemental material

Appendix A

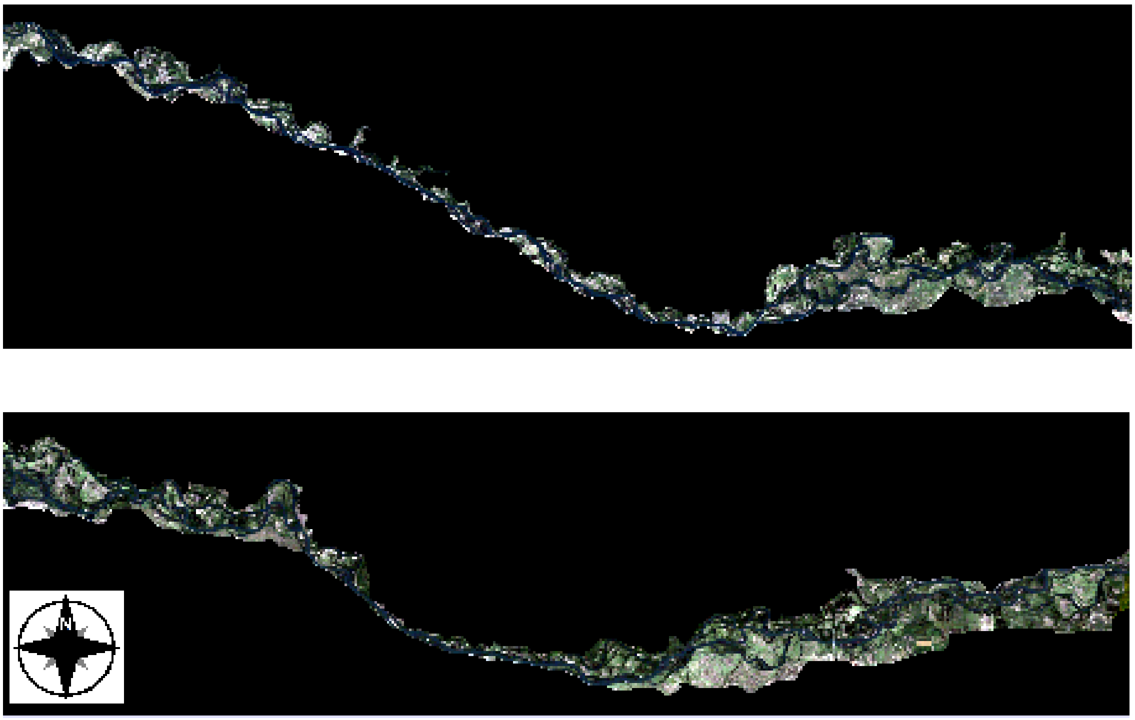

Figure A1 The Upper Río Negro floodplain 2003. Colour composite of the Landsat ETM+ channels 1,2,3. Upper image: Western half of the investigation area. Lower image: Eastern part of the investigation area.

Appendix B

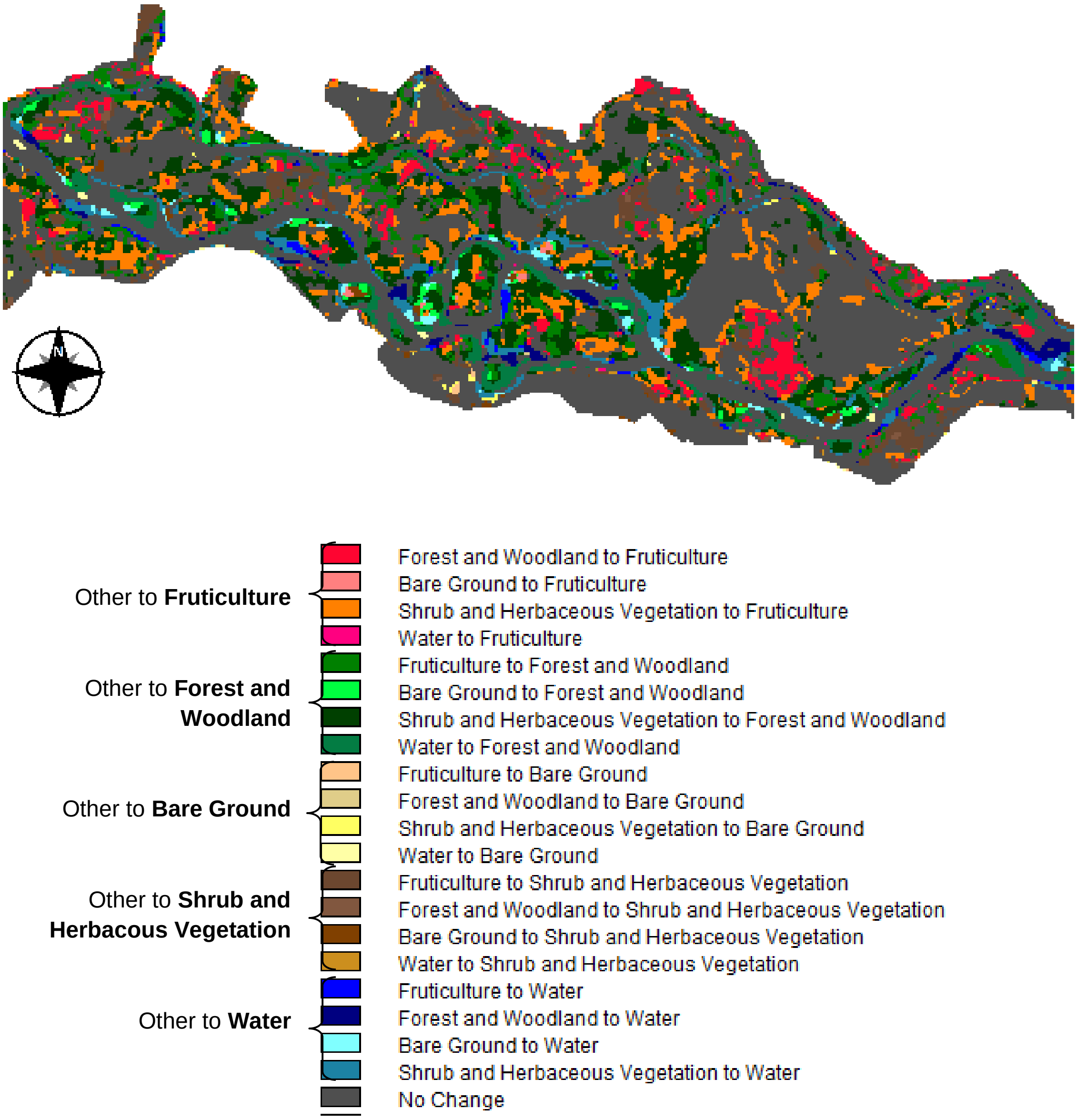

Figure B1 Mapped changes of land-cover classes in a section of the upper Río Negro valley between 1986 and 2003.

Appendix C

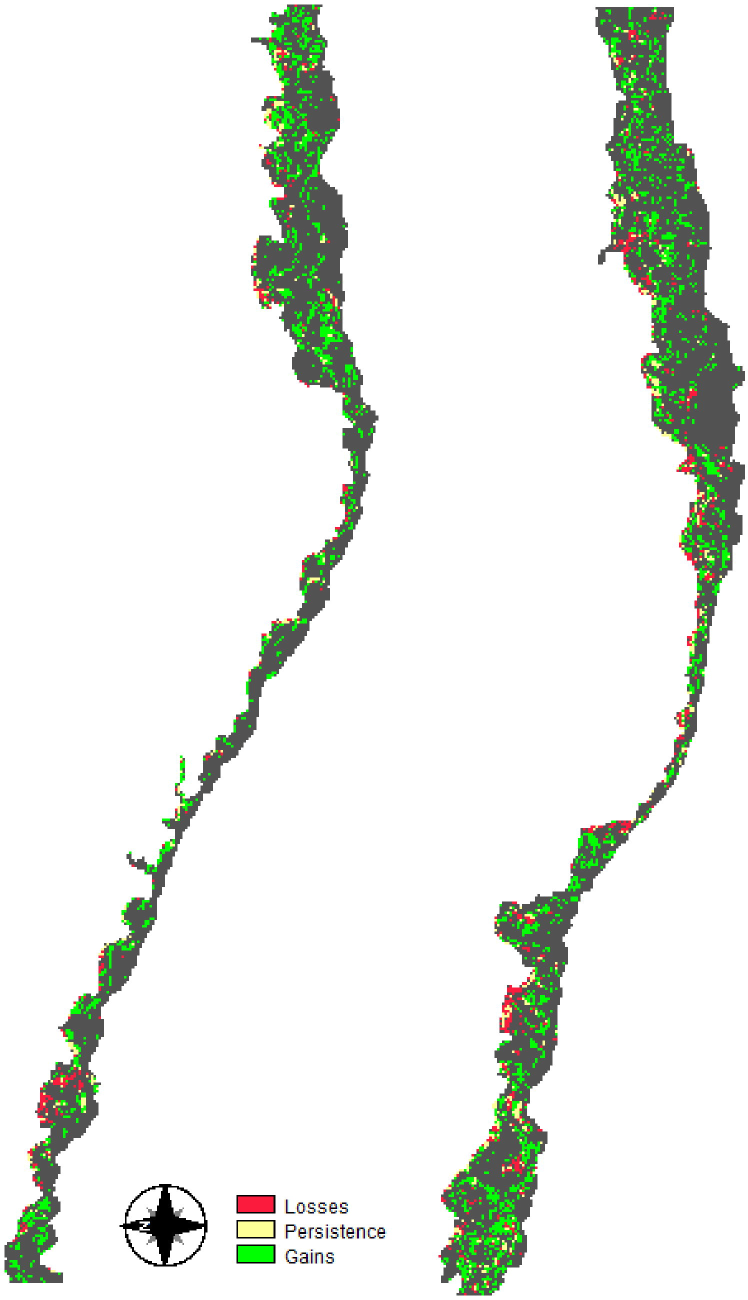

Figure C1 Losses, gains, and persistence in Forest and Woodland between 1986 and 2003. Please note that in order to improve illustration, the investigation area was divided into two halves and each half is oriented to east. Left side: Western part of the investigation area, right side: Eastern part of the investigation area.

Appendix D

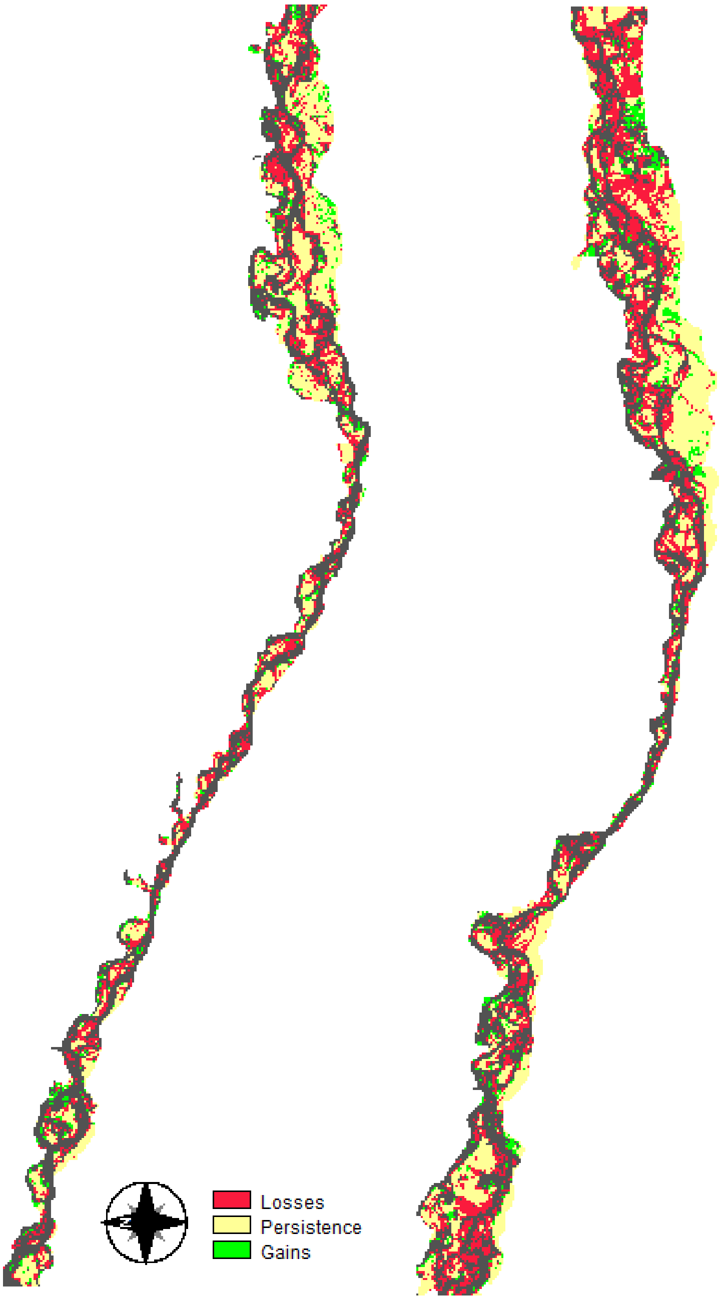

Figure D1 Losses, persistence and gains of Shrub and Herbaceous Vegetation between 1986 and 2003. Please note that in order to improve illustration, the investigation area was divided into two halves and each half is oriented to east. Left side: Western part of the investigation area, right side: Eastern part of the investigation area.

Chapter 6 – Synthesis

The results of my thesis, i.e. the ecology of both invasive and native species in a community context along the Río Negro, assist in understanding the invasion developments of the recent decades. They provide a sound basis for the conservation of *S. humboldtiana*, the only native willow, and the river ecosystems as well as for the management of invasive Salicaceae in general. Not only *Salix humboldtiana* needs a conservation strategy, but a general management strategy is needed how to deal with explosively spreading invasive willows and poplars.

6.1 Establishment of native and invasive Salicaceae

In natural river systems seed release is timed with the flood regime so that optimal nutrient and soil moisture conditions exist for germination and rapid growth (Van Splunder et al. 1995; Karrenberg and Suter 2003). Although until now, vegetative reproduction clearly is a main strategy of invasive willows along the Río Negro that also explain their successful rapid invasion, evolutionary processes could already be observed with invasive willows (Budde et al. 2011). Viable seeds were found in some catkins and the habitat models I developed identified the same niches for non-native willow seedlings as for the *S. humboldtianas*' (**Chapter 4**), even though seedlings from non-natives occurred to a lesser extent than from *S. humboldtiana* and *Populus*. It seems possible, that sexual reproduction of invasive willows will increase during the ongoing invasion process when mating partners either from their own or other taxa become available. However, the success of generative regeneration is not guaranteed since it depends on suitable regeneration sites. Drought as well as inundation may critically affect the survival of Salicaceae seedlings (Selequist et al. 1993; Karrenberg et al. 2002; Braatne et al. 2007) and, thus, sexual establishment can be a rare event when optimal conditions are lacking (Hughes et al. 2001). In contrast, vegetative fragments cope with broader environmental conditions (Moggridge and Gurnell 2009). Vegetative reproduction units not only have a higher survival rate than seedlings, but they also exhibit higher growth rates (Moggridge and Gurnell 2009; Asaeda et al. 2011). Hence it is not surprising, that reproduction via vegetative fragments plays a key role in the invasion success of willows (Shafroth et al. 1994; Cremer 2003; Budde et al. 2011), since broken twigs or branches enable Salicaceae a long-distance dispersal by water. Using habitat models I could show, that, concordant with other studies (e.g. Barsoum 2002; Karrenberg et al 2002; Francis and Gurnell 2006), seedlings only can establish on low elevation sites where inundation events are very frequent (**Chapter 4**). However, mortality of juvenile plants can be higher at lower elevations with longer flood durations (Stokes 2008). Hence, asexual reproduction that may occur more frequently on higher situated sites can be a distinct advantage (Stokes 2008).

In this context, it is important to know the vegetative reproduction potential of *S. humboldtiana* at its distribution edge compared to invasive willows and poplars since vegetative regeneration is not equally well successful for all species (e.g. because of different degrees of brittleness of twigs and success of vegetative regeneration itself). According to Liotta (2001) *S. humboldtiana* shows both a high generative and vegetative reproduction capacity. This is confirmed by its male clone *S. humboldtiana* 'Pyramidalis' which is invading Australian waterways only by vegetative reproduction (Cremer 2003). I could show in an experimental approach that invasive willow taxa have better vegetative reproduction and resprouting capacities and clearly out-performed the native *S. humboldtiana* and non-native *Populus* under all tested soil moisture and soil composition gradients (**Chapter 2**). In contrast to the invasive willow hybrids *S. humboldtiana* and *Populus* both produce a distinct higher seed number than the willow hybrids and, thus, have higher generative reproduction capacities when suitable establishment sites exist. Due to river dynamics and hydro-geomorphic processes bare-ground sites suitable for seedling establishment occur frequently so far and, thus, allow a successful sexual reproduction strategy. Consequently, the superior vegetative reproduction success of the invasive willows does not necessarily cause a better establishment. Nevertheless, the results of **Chapter 5** indicate that forest establishment is a more rigorously advancing process than their occasional removal by destructive floods.

As shown by other studies (Van Splunder et al. 1995; Kollmann et al. 1999; Karrenberg et al. 2003b) regeneration sites can be composed of a mixture of seedlings and resprouts which underlines the importance and use of the combination of both strategies for successful regeneration. For a further remaining of the young plants the ability for an effective root anchorage may be crucial to persist during disturbances by floods (Karrenberg et al. 2003a). As shown in **Chapter 2**, *S.* × *rubens* and the *S. babylonica* hybrid not only produced a higher shoot biomass, but also a significant higher root biomass than *S. humboldtiana* and *Populus* which may be an indication for a better uprooting resistence via anchorage during high flood events after establishment. Likewise, a rapid development of a high root biomass is essential to keep up with a falling water table and protects the plant from drought mortality (Karrenberg et al. 2002).

6.2 Interspecific competition processes

Other studies identified growth rate as the most important determinant of tree invasiveness (see for a review Lamarque et al 2011) and changes in light quality and quantity are known to negatively affect native vegetation (Reinhart et al. 2006). Consequently, in order to assess the impact of invasive non-native woody species, it is a promising approach to focus on competitive interactions between alien and native riparian plant species when they occupy the same ecological niches and, thus, niche overlap occurs. To find out whether and when interspecific competition occurs, the question arose, whether species composition of mixed forests is the results of joint establishment processes or whether *S. humboldtiana* established before or after the invasive species (**Chapter 3**). It could be proved that joint establishment events occur but invasive willows exhibit a significant better growth. With increasing age, the invasive willow hybrids develop dense canopies with significant larger and denser crowns than *S. humboldtiana*. Besides, they reach in short times much bigger diameters and de facto more area is covered by a single tree. The profound changes in light quality due to havy shading will not only have severe effects for the shade-intolerant *S. humboldtiana* (Parolin et al. 2002) but also for native shrubs and herbaceous vegetation. Consequently, *S. humboldtiana* could be negatively affected by competition pressure in mature mixed stands.

It can be concluded, that there is a significant competition potential by invasive Salicaceae which could affect the native species. Under natural conditions without invasive competitors, *S. humboldtiana* would not be disturbed by competition processes, or, as it has been shown for Amazonian rivers (Parolin et al. 2002) and in the Paraná delta (Casco et al.

2010), represents the first successional woody stage that will later be replaced by other species.

6.3 Distribution patterns and invasion development

All taxa that were included in this study are known to modify their environment by driving hydro-geomorphological processes, e.g. interactions with water and sediment flow during floods and accumulation of sediments that leads to an aggradation of fluvial islands (reviewed by Gurnell 2014). Accordingly, this modifications may lead to habitat construction with suitable new sites for seedling establishment (Corenblit et al. 2014). Strong disturbances due to erosion and deposition (Hupp and Osterkamp 1996; Bendix and Hupp 2000; Karrenberg et al. 2002; Osterkamp and Hupp 2010) account for low ages < 10 years in woody patches within the active zone of rivers (Karrenberg et al. 2003b), and *S. humboldtiana* stands in other regions, too, are known to reach only low mean ages < 20 years before they are replaced by other species (Liotta 2001; Parolin et al. 2002; Casco et al. 2010). Indications of invasion history, development and existing dynamic processes that frequently remove adult vegetation can be obtained by using age structure analyses. Within mixed adult forest stands on islands low mean ages of < 15 years could be detected (**Chapter 3**) and, thus, the findings from other studies were confirmed along the Río Negro. On the one hand, the low mean ages along the Río Negro could be explained by frequently occurring destructive floods that periodically erase established woody vegetation. On the other hand, it is possible, that prior to the establishment of the existing forests no dense forest stands appeared despite scattered *S. humboldtiana* occurrences. This assumption could lead to the conclusion that dense Salicaceae forests occurred only in the recent past representing the first invasion step. However, in the absence of disturbances by floods, beyond the active zone of rivers e.g. on pastures and higher situated places, of all studied Salicaceae taxa including *S. humboldtiana*, the *S. babylonica* hybrid, *S.* × *rubens* as well as *Populus* spp. solitary trees with ages > 40 years were found (**Chapter 3**).

The establishment of Salicaceae stands dominated by invasive species, in addition with *Tamarix* and *Elaeagnus*, could explain the enormous increase of floodplain forests along the Río Negro, with invasive species establishing along the rivershores and islands. Corresponding to observations by local farmers and land owners (pers. communication) it could be proved that floodplain forests expanded their distribution area considerably only in the recent decades by studying land cover and vegetation change in the upper Río Negro valley (**Chapter 5**). Older individuals of all Salicaceae were found, which indicates that they had been introduced earlier. An exception however is the *S. babylonica* hybrid which probably is the latest introduced invasive willow showing the best growth performance with high basal areas and large dense crowns (**Chapter 3**). The *S. babylonica* hybrid was found to be significantly more frequent in the upper river valley than in the middle river valley (**Chapter 4**). It seems plausible, that this finding is based on a downstream directed invasion due to floating twigs as it was suggested by Budde et al (2011) for *S.* × *rubens* along other Patagonian streams. In addition, *S. humboldtiana* has a higher abundance and frequency in the middle than in the upper valley (**Chapter 3**), which could be another indication of an advanced invasion in the upper river stretch and a possible suppression of the native willow. It is possible that the *S. babylonica* hybrid was firstly planted in the highly agriculturally used upper Río Negro valley from were it started its range expansion. Furthermore, the new appearance of the *S. babylonica* hybrid could explain the strong increase of invasive spread and underlines that this is an ongoing process with a probable expansion in the future. With increasing sexual regeneration and hybridization with other *Salix* species and hybrids, a possible scenario is also an upstream directed invasion through wind dispersal and, consequently, a further spreading along streams that are so far only invaded by *S.* × *rubens*. In general, multiple factors cause the high dynamic and succession of softwood forests concerning age, area and species composition over time and long-term changes in these pioneer forests are common in riparian ecosystems (Friedman and Lee 2002; Stromberg et al. 2010; Lamarque et al. 2011; Reynolds et al. 2014). However, it is unknown, which processes or events triggered the alien species invasion as well as the native *S. humboldtiana* establishment and, thus, are responsible for the enormous forest increase.

6.4 Perspectives and applications

Various methods have been developed in order to understand invasion processes and to predict the distribution of invasive species (Gallien et al. 2012). The number of ecological niche models of invasive species in different spatial scales is increasing (Barbosa et al. 2012). Regression models such as GLM (generalized linear model) are simple to implement and thus, as species distribution models (Guisan and Zimmermann 2000) that relate species probability of occurrence to environmental variables, are a valuable method to understand e.g. potential distributions of invasive species. In the context of invasion ecology, however, it is not only important to identify potential habitats, but to assess the effects on native species using several methods, as it was done in this thesis using different approaches (**Chapters 2-5**). It could be shown that the developed habitat models (**Chapter 4**) that revealed the same habitat optima for native and invasive species are, in combination with a higher competition potential of invasive willows, able to identify the potential threat to the native *S. humboldtiana*.

However, the studies were only conducted along the Río Negro and, thus, represent insights into invasion processes on a rather local scale. Along streams in the otherwise treeless Patagonian steppe, a similar increase of softwood galery forest structured by a variety of *Salix* species and hybrids is possible. Other non-native willows of both sexes and, consequently, new natural hybridizations can provoke a further invasion step with an increase of sexual reproduction. Several genotypes could be found so far only along the Río Negro. In contrast a single female *S.* × *rubens* almost exclusively forms monoclonal stands at other Patagonian streams (Budde et al. 2011). With the appearance of male individuals and other species or hybrids comparable evolutionary processes to those observed along the Río Negro with sexual regeneration can be expected along other rivers, too. Particularly for the *S. babylonica* hybrid, whose explosive invasion is a development of the recent years, a further spread along other rivers is likely. It is highly recommendable to document exactly these processes and the invasion status of Eurasian floodplain willows and the degree of naturalization and possible sites for a further invasion should also be identified. However, an application of the developed models might probably be limited in several ways. Firstly, *S. humboldtiana* is more frequent along the Río Negro than along other Patagonian streams, where it occurs only occasionally or is not to be found. Secondly, the environmental conditions such as climate and disturbance differ along other river systems. The Río Negro is the largest Patagonian river and different discharge and flooding regimens can be expected along other rivers. Moreover, human impact, such as planting, is extremely high in the Río Negro valley and thus the invasion process is probably faster than in other regions which are less populated by humans. Additionally, exotic species are known to occur more frequently within areas that are disturbed by humans (Mack et al. 2000; Speziale and Ezcurra 2011). Thirdly, other native riparian tree species boarder lakes and rivers which are located closer to the Andes, e.g. *Nothofagus* spp., *Eurcryphia cordifolia* Cav., *Luma apiculata* (DC.) Burret and *Myrceugenia exsucca* (DC.) O. Berg (Gut 2008). Thus, other interspecific competition processes might occur, that make these habitats perhaps not similarly prone to plant invasion. Indeed, habitats shadowed by *Nothofagus* dominated forests, seem to be not so susceptible to plant invasions as it is the case for rivers whithin the treeless Patagonian steppes (Budde et al. 2011).

In general, further research in a variety of spacial and temporal scales is necessary before the dynamics of Salicaceae invasion along Patagonian river systems and their impacts on native species and ecosystems can be properly understood.

Shafroth et al (2008) pointed out, that restoration projects for invaded riparian ecosystems need a careful planning with site-specific methods, whether for controlling, removal or re-vegetation practices. For an effective management, clear goal-definition as well as monitoring and maintenance are needed, and a careful review of literature is essential (Shafroth et al. 2008; Stromberg et al. 2009). These recommendations may partly also apply to other woody invasions and cost-intensive projects should be planned carefully. Giljohann et al (2011) developed a method that links species occurrence with a decision model to optimize willow management by identifying priority locations for control.

In Australia, e.g., invasions of several willow species are controlled at heavy costs in coordinated efforts. A combination of chemical and mechanical control is used and even biological control is considered there since willows are able to resprout after being cut down (Cremer 2003; Adair et al. 2006). Moreover, restrictions in selling and planting of willows have been established (Cremer 2003). *Tamarix* and *Elaeagnus*, too, belong to the most dominant invaders of riparian habitats (Reynolds et al. 2014). Restoration programs and the control or removal with mechanical, chemical or biological means lead to high costs (Shafroth et al. 2008).

So far, there are no co-ordinated or large-scale strategies for management or control of invasive woody species along the Río Negro or other Patagonian rivers. Existing management efforts by local farmers or land-owners are done on a small-scale with the aim to create or maintain agricultural land and include mostly manual and herbicidal clearing or burning. General recommendations for nature conservation authorities for adequate management strategies are difficult or even impossible to give. At the Río Negro, on the one hand, native and invasive Salicaceae including their hybrids have similar properties and the same habitat optima. On the other hand, *Elaeagnus* and *Tamarix* have different characteristics and adaptation systems and thus need a separate management approach. As a consequence, it is comprehensible that for each riparian ecosystem and even for each species special management strategies are required. Nevertheless, it has to be mentioned that invasive woody species are already widespread and dominant along the Río Negro, and hence, their invasion is certainly an irreversible situation. So, strategies have to be developed in order to keep up the status quo and to avoid a further spreading of alien tree species and a simultaneous suppression of the native *S. humboldtiana*. A first step could be that further planting of non-native willows, especially male individuals, should be avoided along Patagonian watercourses since this could provoke a drastical increase of sexual reproduction.

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Erklärung

Hiermit versichere ich, dass ich meine Dissertation

„Ecological effects of willow and poplar invasions
at the Río Negro in northern Patagonia, Argentina“

selbstständig und ohne fremde Hilfe angefertigt habe und mich keiner als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe. Alle vollständig oder sinngemäß übernommenen Zitate habe ich als solche gekennzeichnet. Diese Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

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