UNIVERSITAT DE BARCELONA

La incorporación de prácticas de conservación en cultivos herbáceos extensivos de secano en la región mediterránea: efecto del laboreo mínimo y las enmiendas orgánicas sobre la producción del cultivo, la abundancia de la flora arvense y la calidad del suelo

Integration of conservation agricultural practices in Mediterranean dryland arable fields : effect of reduced tillage and organic amendments on crop production, weed abundance and soil quality

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TESIS DOCTORAL

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Resumen

La intensificación de las actividades agrícolas en los últimos años ha supuesto un uso excesivo de insumos externos y un intenso laboreo del suelo que ha provocado contaminaciones a veces de carácter irreversible, el empobrecimiento y la degradación de los suelos y la pérdida de la biodiversidad de estos sistemas. La tesis doctoral va dirigida al desarrollo de sistemas agrícolas de secano más sostenibles en la región mediterránea con el fin de minimizar los efectos negativos de las prácticas agrícolas como son el laboreo intensivo y los aportes externos de fertilizantes y herbicidas. Los experimentos que forman parte de esta tesis doctoral se han llevado a cabo en *el Parc de l'Espai d'Interès Natural de Gallecs*, un espacio agrario periurbano situado a 15 kilómetros al norte de Barcelona. El capítulo I abarca un experimento de dos años de duración en campos de cereales de invierno con gestión convencional y comprende la introducción de prácticas agrícolas menos intensivas como son el laboreo mínimo y el control mecánico de la flora arvense. Para ello se evaluó el efecto del uso del arado de vertedera comparado con el arado de cincel, y el control mecánico respecto al control químico de la flora arvense sobre el rendimiento del cultivo trigo de invierno, y la abundancia y diversidad de la flora arvense. Los capítulos II, III y IV resumen los resultados de un experimento de cuatro años de duración enfocado al diseño de un sistema de producción de cultivos ecológicos herbáceos extensivos sostenible. En este experimento se estudiaron los efectos del laboreo mínimo, la fertilización orgánica con estiércol y la incorporación de cultivos de cobertura como abono verde en el suelo sobre: a) el rendimiento de los cultivos, la abundancia de la flora arvense, el contenido de carbono orgánico y nitrógeno, la densidad aparente y la biomasa microbiana del suelo (Capítulo II); b) la abundancia y diversidad de lombrices de tierra (Capítulo III); c) y los balances de nitrógeno y los parámetros de la eficiencia del uso del nitrógeno (Capítulo IV), en una rotación de cereales y leguminosas en la región mediterránea. El estudio de las distintas estrategias para disminuir la intensidad de las actividades agrícolas pone de manifiesto que es necesario llevar a cabo experimentos a largo plazo para poder obtener resultados concluyentes sobre cuáles son las mejores prácticas en los cultivos de secano de la región mediterránea. Asimismo, a la hora de aplicar distintas prácticas agrícolas es importante tener en cuenta las condiciones climáticas de la zona, la calidad y fertilidad del suelo y las prácticas de gestión previas. Los suelos de los cultivos de secano de la región mediterránea se caracterizan generalmente por un pobre contenido de nutrientes y una alta tasa de mineralización de la materia orgánica, por ello es imprescindible mantener los niveles de nutrientes en los suelos a través de una adecuada fertilización.

Summary

The agricultural intensification in recent years has led to excessive use of external inputs and intensive soil tillage resulting in water pollution, soils degradation and loss of biodiversity of these systems. The aim of this thesis is the design of more sustainable rainfed agricultural systems in the Mediterranean region in order to minimize the negative effects of agricultural practices such as intensive tillage and chemical inputs (fertilizers and herbicides). The experiments comprising this work have been carried out in the *Parc de l'Espai d'Interès Natural de Gallecs*, a peri-urban agricultural area located 15 kilometers north of Barcelona. Chapter I comprises a short-term experiment of 2 years in winter cereal fields with conventional management and includes the introduction of more sustainable agricultural practices such as reduced tillage and mechanical weed control. In this experiment we evaluated the effect of the use of mouldboard plough vs. chisel plough and the use of mechanical weed control vs. herbicide application on winter wheat crop yields and on weed abundance and diversity. Chapters II, III and IV comprise a medium-term experiment focused on the design of a sustainable organic cereal-legume rotation in Mediterranean dryland arable fields. The effects of reduced tillage, fertilization with farmyard manure and the incorporation of cover corps as green manure was evaluated on a) crop yields, weed abundance and organic carbon content, nitrogen total content and microbial biomass in the soil (Chapter III); b) the abundance and diversity of earthworms (Chapter III); c) and the balance of nitrogen and nitrogen efficiency parameters in the system (Chapter IV). The study of different strategies to reduce the intensity of agricultural practices reflects that it is necessary to perform long-term experiments in order to obtain consistent results on which are the best practices in dryland agrosystems in the Mediterranean region. Furthermore, it is important to be case-specific and take into account the climatic conditions of the area, the quality and fertility of the soil and the previous farming practices. The arable soils of the Mediterranean region are generally characterized by a poor nutrient content and a high rate of mineralization of organic matter, therefore is crucial to maintain soil nutrients levels through a proper fertilization.

Introducción general

Introducción general

Los problemas de la intensificación agrícola

Los ecosistemas agrícolas son sistemas antropogénicos debido a que su origen y mantenimiento va asociado a la transformación de la naturaleza para obtener principalmente alimentos por parte del hombre. A mediados del siglo XX tuvo lugar la intensificación agrícola, la llamada Revolución Verde, que ha conllevado el aumento de la producción, pero a su vez ha comportado, en muchos casos, un extraordinario coste ambiental (Foley et al., 2011). La degradación del suelo y los procesos asociados de erosión, la aplicación de un gran número insumos externos (pesticidas y fertilizantes) y el uso intensivo de maquinaria agrícola son algunos ejemplos (Lacasta y Meco, 2006). La intensificación agrícola ha dado lugar a sistemas muy especializados y caracterizados por una escasa complejidad, que constituyen la llamada agricultura convencional (Robinson y Sutherland, 2002; Sans et al., 2013). En el caso de los cultivos herbáceos extensivos, la gestión convencional se basa en el laboreo intensivo, las aportaciones externas de fertilizantes sintéticos y orgánicos y pesticidas, la simplificación de las rotaciones de cultivos y la pérdida de diversidad cultivada a favor de la utilización de pocas variedades híbridas comerciales (Sans et al., 2013). Este tipo de agricultura, aunque ha favorecido el aumento de la producción, ha conllevado importantes problemas desde el punto de vista social, económico y sobre todo ambiental (Lassaletta y Rovira, 2005).

Los cultivos herbáceos extensivos de secano en la región mediterránea

<u>Importancia y limitaciones</u>

La mayor parte de la producción agrícola de la región mediterránea europea se basa en los cultivos de secano. En España los cultivos herbáceos extensivos de secano ocupan el 20,73% de superficie (10.466.100 hectáreas), que representan el 43,12% de la superficie agraria útil y se distribuyen prácticamente por toda la geografía del país (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, 2011). En Catalunya la superficie agraria útil es de 1.167.000 ha (el 36,3% del territorio) y el 27,2% está ocupada por los cultivos herbáceos de secano (317.362 hectáreas) (Figura 1).

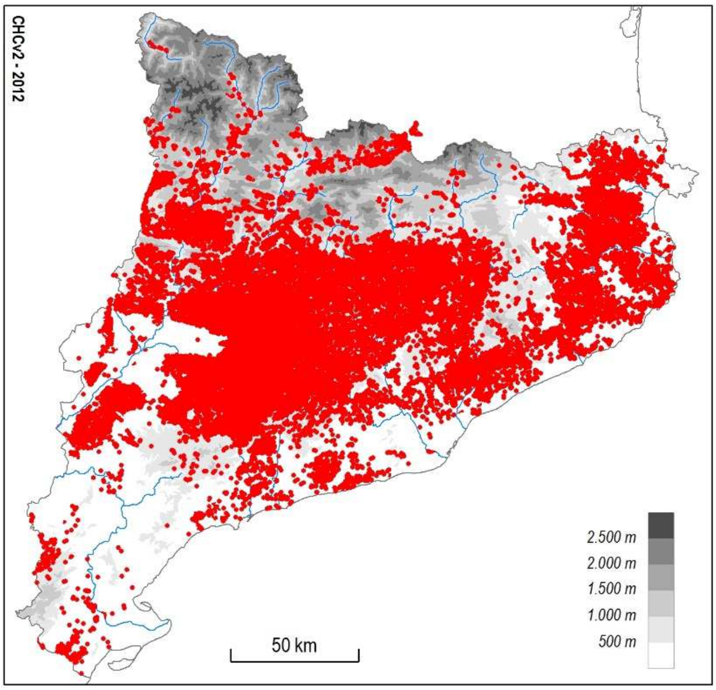

Figura 1. Distribución de los cultivos herbáceos extensivos de secano en Cataluña (en rojo). La gama de colores grises indica la altitud (msnm).

La agricultura de secano se practica según permiten las condiciones naturales de la zona y sin aporte externo de agua (Meco et al., 2011) y por ello las producciones pueden variar interanualmente en relación a las fluctuaciones en las precipitaciones. La climatología de la región mediterránea se caracteriza fundamentalmente por la sequía estival (4-5 meses) que coincide con las mejores condiciones térmicas para el crecimiento de las plantas. En particular, el clima mediterráneo marítimo se caracteriza por inviernos con temperaturas relativamente suaves y veranos secos y calurosos y las precipitaciones se concentran en primavera y otoño (Olarieta et al., 2006). Los cultivos herbáceos mediterráneos de secano se caracterizan por rendimientos potenciales bajos en comparación con las regiones templadas debido al estrés hídrico asociado a las elevadas temperaturas y las escasas precipitaciones de finales primavera y verano (Kassam et al., 2012). La baja precipitación y las variaciones interanuales de la disponibilidad de agua en primavera son factores importantes que afectan a los rendimientos de los cultivos de cereales de las áreas de secano. Según el INE (2007) el rendimiento medio del trigo de invierno y la cebada fueron respectivamente de 3,12 ± 0,181 kg ha^{-1} y 2,82 ± 0,181 kg ha^{-1} durante el período 1997-2003.

El importante papel que tiene el clima conlleva que el efecto de los aportes externos de fertilizantes tenga a veces, poca incidencia sobre el rendimiento (Meco et al., 1998). La mayor parte de los suelos agrícolas de la región mediterránea tienen un contenido bajo de materia orgánica y una pobre estructura de agregados, en consecuencia el empleo de prácticas agrícolas muy intensivas empeoran la situación (Kassam et al., 2012). Además, las proyecciones científicas sobre las consecuencias del cambio climático en las áreas mediterráneas señalan la disminución de las precipitaciones en las próximas décadas (Meco et al., 2011). Por esta razón, la gestión de esta parte del territorio tiene una destacada importancia ambiental tanto desde el punto de vista de la producción como de la conservación de los recursos naturales. Durante los últimos años, los bajos precios del grano del cereal, los cambios en las políticas ambientales y los programas gubernamentales han incentivado que los productores opten por sistemas de producción alternativos en los que la calidad del suelo y la protección del medio ambiente adquieren mayor importancia (Campiglia et al., 2015).

Problemas en la gestión

La excesiva intensificación de las actividades agrícolas ha supuesto, en muchos casos, el empobrecimiento de los suelos y la aceleración de procesos irreversibles de erosión. Los suelos agrícolas han sufrido una pérdida importante en el contenido de materia orgánica ya que actualmente, la mayoría no son manejados de manera sostenible (Fließbach et al., 2007). Tradicionalmente, los agricultores han realizado un laboreo intensivo del suelo, a menudo llamado laboreo convencional, que consiste habitualmente en la inversión de las capas de suelo mediante el uso del arado de vertedera (Holland, 2004; Plaza et al., 2011). El laboreo convencional modifica la estructura del suelo y en consecuencia favorece la degradación de la materia orgánica dando lugar a la compactación y eventualmente a la pérdida de suelo, así como la consiguiente pérdida de su biodiversidad y muchas de las funciones ecosistémicas mediadas por el suelo que proporcionan, regulan y protegen los servicios ambientales (Holland, 2004; Montgomery, 2007).

Asimismo, el efecto negativo se ha acentuado en los últimos cincuenta años con el uso de métodos químicos para la protección de los cultivos que ha originado contaminaciones, a menudo de carácter irreversible. La intensa aplicación de fertilizantes y pesticidas ha incrementado la lixiviación de estas sustancias que afectan a las aguas subterráneas y superficiales, contaminando ríos y acuíferos, afectando los hábitats acuáticos y la salud humana (Tilman et al., 2002). Del mismo modo, la aplicación de fertilizantes y otras acciones antrópicas han alterado las condiciones básicas del ciclo natural del nitrógeno contribuyendo a la contaminación por nitratos de los ecosistemas terrestres y acuáticos con graves riesgos para la salud humana (Fernández-Pascual, 2002). Además, el excesivo uso de herbicidas para el control de la flora arvense ha dado lugar al desarrollo de resistencias de algunas especies, a la pérdida de la biodiversidad de estos sistemas, y en consecuencia a la eliminación o modificación de estos hábitats (Heap, 2014). Asimismo, la concentración espacial de explotaciones ganaderas desvinculadas de la producción agrícola ha creado problemas de contaminación de los acuíferos, de erosión y de producción de residuos (Sans et al., 2013).

El cambio de gestión de los sistemas agrícolas que se ha producido durante los últimos decenios ha motivado que la agricultura y ganadería tradicional haya sido desplazada por un tipo de producción muy intensivo basado exclusivamente en criterios económicos con graves consecuencias medioambientales. Los paisajes rurales mediterráneos, muy diversificados, de un extraordinario valor cultural y ambiental resultado de la ancestral gestión armónica con el entorno se han convertido, en buena parte del territorio, en paisajes monótonos y banales como consecuencia de la excesiva roturación y el uso intensivo. Por este motivo, el papel de la agricultura - alrededor del 50% de la superficie de la Unión Europea es de uso agrícola - en la conservación del paisaje y el entorno rural es un hecho cada vez más necesario como reflejan diversas iniciativas comunitarias directamente relacionadas con la agricultura y el paisaje (Sans et al., 2013). En este contexto, es importante el desarrollo de prácticas que permitan aumentar y regenerar la diversidad, reforzar el equilibrio ecológico y mejorar la sostenibilidad de los agrosistemas desde el punto de vista agronómico y medioambiental.

Importancia de la conservación del suelo en la región mediterránea

El suelo es un recurso natural no renovable y es esencial para el desarrollo de los ecosistemas terrestres, ya sean naturales o cultivados. La calidad del suelo es muy importante en los sistemas agrícolas debido a que la producción depende, en buena medida, del estado de conservación del suelo (Meco et al., 1998, 2011). La conservación del suelo es uno de los temas principales que deben abordarse actualmente en los sistemas agrícolas. Aproximadamente el 20% de los suelos europeos están sujetos a la degradación; estudios recientes estiman que alrededor de 130 millones de hectáreas de suelo están afectadas por erosión en la Unión Europea (European Environment Agency, 2012). En los sistemas agrícolas mediterráneos, el contenido de materia orgánica de los suelos es especialmente pobre y las prácticas agrícolas intensivas de los últimos años han provocado la pérdida de materia orgánica y la degradación de estos suelos (Kassam et al., 2012; Romanyà y Rovira, 2011). Además, los sistemas herbáceos extensivos de secano gestionados mediante prácticas muy intensivas están sometidos a graves riesgos de desertificación a causa de las elevadas tasas de erosión y la disminución del contenido de materia orgánica del suelo. Por ello la prevención de la degradación de los suelos requiere cambios en la gestión encaminados a conservar la calidad y fertilidad en estos sistemas (Holland, 2004; Kassam et al., 2012).

Estrategias para mejorar la gestión de los cultivos herbáceos extensivos de secano

La tesis doctoral se enmarca en la necesidad de desarrollar sistemas agrícolas de secano más sostenibles en la región mediterránea con el fin de minimizar los efectos negativos de las prácticas agrícolas como son el laboreo intensivo y los aportes externos de fertilizantes y herbicidas. En este contexto, este trabajo pretende por un lado implementar prácticas encaminadas a disminuir la intensidad de las prácticas agrícolas en fincas cerealistas convencionales y, por el otro, diseñar sistemas herbáceos extensivos ecológicos sostenibles mediante la incorporación del laboreo mínimo y los cultivos de cobertura que se incorporan al suelo como abono verde antes del cultivo principal.

Disminución de la intensidad de las prácticas agrícolas

En los últimos años han surgido alternativas a la agricultura convencional destinadas a preservar el suelo y el medio ambiente, una de ellas es la agricultura de conservación, desarrollada por la FAO (Food and Agriculture Organization of the United Nations, 2015). La agricultura de conservación se caracteriza por tres principios básicos: la mínima perturbación del suelo, la cobertura permanente del suelo y la diversificación y rotación de los cultivos. Estas prácticas no solamente van encaminadas a reducir la degradación de los suelos, sino que también pretenden contribuir a la producción agrícola sostenible, especialmente en las zonas donde los suelos son frágiles y de baja calidad (Hobbs et al., 2008). Las prácticas de la agricultura de conservación pueden mejorar el desarrollo de los sistemas agrícolas, así como los aspectos socio-económicos y ambientales. Por lo tanto, pueden ser una alternativa a la agricultura convencional, principalmente en las áreas dominadas por cultivos herbáceos extensivos con clima mediterráneo donde las condiciones climáticas ya son, por sí mismas, una importante limitación para la producción (Meco et al., 1998).

1. Reducción de la intensidad de laboreo

El laboreo del suelo es importante en los sistemas agrícolas para la incorporación de los residuos orgánicos al suelo que facilite la mineralización y la liberación de nutrientes para el cultivo, y para el control de las poblaciones de especies arvenses (Bàrberi, 2002; Peigné et al., 2007). Sin embargo, el laboreo excesivamente intenso que invierte las capas del suelo acarrea cambios en la estructura y acelera la degradación de la materia orgánica. Para revertir esta situación, se han propuesto prácticas de manejo del suelo encaminadas a minimizar la intensidad de la perturbación asociada al laboreo (Post et al., 2001). Los regímenes de manejo de los suelos basados en el laboreo de conservación (no laboreo y laboreo mínimo) sin inversión del perfil del suelo son adecuados para conservar la fertilidad del suelo y prevenir la erosión (Berner et al., 2008; Gadermaier et al., 2011). Sin embargo, el laboreo de conservación puede ser un factor limitante en regiones con períodos de sequía y suelos pobres en materia orgánica como en la región mediterránea (Dorado y López-Fando, 2006). La estructura frágil del suelo y el predominio de las arcillas puede conllevar la compactación del suelo y afectar la disponibilidad de agua, la dinámica de los nutrientes, el desarrollo de las raíces y en consecuencia limitar el crecimiento de los cultivos (Dorado y López-Fando, 2006; Légère et al., 2008). Asimismo, el laboreo mínimo sin inversión de las capas del suelo, puede afectar la distribución y viabilidad de las semillas de la flora arvense en el perfil del suelo y, en consecuencia, aumentar la emergencia y supervivencia de las plántulas, la producción de semillas y la supervivencia de las estructuras vegetativas de especies perennes (Bullied et al., 2003; Gruber y Claupein, 2009). Sin embargo, la combinación del laboreo mínimo con prácticas adecuadas para el control de la flora arvense en post-emergencia, puede permitir diseñar sistemas agrícolas eficientes.

2. Prácticas menos agresivas para el control de la flora arvense

Uno de los principales motivos de preocupación de los agricultores al implementar el laboreo de conservación es el incremento de la abundancia de la flora arvense y su potencial competencia con el cultivo y la necesidad de realizar un manejo adecuado de la flora arvense en post-emergencia en estos sistemas (Armengot et al., 2012; Plaza et al., 2011). El control químico ha sido eficaz para reducir las pérdidas de rendimiento y minimizar las infestaciones de flora arvense. Sin embargo, la aplicación de herbicidas ha provocado la aparición de resistencias y ha conllevado negativos efectos secundarios sobre el medio ambiente (Boutin et al., 2002). La intensa aplicación de herbicidas se ha manifestado por la disminución de la abundancia y la diversidad de la flora arvense y ha modificado extraordinariamente la composición de las comunidades arvenses (Sans et al., 2013). La flora arvense es un componente importante de la diversidad vegetal en los agroecosistemas y desempeña un importante papel en el apoyo de la diversidad biológica en todos los niveles tróficos superiores y en la provisión de servicios ecosistémicos como la fertilidad del suelo, la polinización y el control biológico (Marshall et al., 2003; Meco et al., 1998). El importante papel ecológico de la flora arvense requiere el desarrollo de alternativas al uso de herbicidas que permitan mantener las poblaciones de las especies arvenses por debajo de los umbrales de competencia y que hagan compatible la producción con la conservación de la biodiversidad (Armengot et al., 2012). En este sentido, la grada de púas flexibles es uno de los métodos de control mecánico más utilizados en cultivos herbáceos extensivos ecológicos. Este sistema consiste en un apero ligero que se utiliza para eliminar la flora arvense mediante la perturbación mínima de la superficie del suelo, de manera que desentierra las plántulas y éstas se secan al quedar sobre la superficie del suelo (Pardo et al., 2008).

Diseño de sistemas herbáceos extensivos ecológicos sostenibles

La agricultura de conservación y la agricultura ecológica son dos estrategias complementarias destinadas a mejorar la calidad del suelo y la fertilidad de los sistemas herbáceos. El diseño de sistemas herbáceos extensivos ecológicos pretende equilibrar las demandas de la producción de alimentos con la sostenibilidad ambiental (Peigné et al., 2015). La agricultura ecológica emplea un conjunto de prácticas con el fin de minimizar los efectos negativos sobre el medio ambiente, preservar la fertilidad del suelo, incrementar el uso de recursos internos del sistema y conservar la biodiversidad. Los sistemas ecológicos tienen por objetivo ser ambientalmente adecuados, altamente productivos y económicamente viables. La gestión ecológica se caracteriza por prohibir el uso de productos de síntesis química y basar la fertilización en la aplicación de materia orgánica y la incorporación de leguminosas y abonos verdes en las rotaciones de cultivo (Sans et al., 2013). Estos sistemas se enfocan en mantener la productividad de los cultivos y a la vez preservar la máxima biodiversidad cultivada y espontánea posible. Por ello, las actuaciones se enfocan a favorecer los procesos naturales e interacciones biológicas que optimizan sinergias de modo tal que la agrobiodiversidad sea capaz de subsidiar por si misma procesos claves tales como la acumulación de materia orgánica, los mecanismos de regulación biótica de plagas y mantener o mejorar la fertilidad del suelo (Altieri, 1999; Gliessman, 2000).

1. Mantener la fertilidad del suelo

Los principales factores limitantes para el desarrollo de los cultivos herbáceos extensivos en las áreas con clima mediterráneo son la disponibilidad hídrica y el contenido de materia orgánica del suelo. Por ello la mejora de la funcionalidad de estos sistemas se debe basar en la conservación de la fertilidad del suelo. Según Meco et al. (1998, 2011) la fertilidad se define como "*el potencial de un suelo de cultivo, para mantener de manera perdurable un nivel de producción optimo y de calidad, conservando un estado de alta estabilidad frente a procesos que implicarían su* *degradación o pérdida, y dentro de unos límites determinados por un manejo específico y por un contexto ambiental y socioeconómico concreto*".

La evaluación de la sostenibilidad en estos sistemas requiere estudiar el rendimiento de los cultivos a lo largo de la rotación en relación con la fertilidad del suelo. Algunos indicadores clave para la evaluación de la fertilidad y calidad del suelo son el contenido de carbono orgánico del suelo (SOC), el contenido de nitrógeno total (N) y la densidad aparente del suelo (Berner et al., 2008; Peigné et al., 2007). Además, las prácticas agrícolas pueden causar cambios en el contenido de carbono del suelo, por ello, la reserva de carbono es un indicador del equilibrio entre las entradas y salidas de carbono en el suelo y dependiendo de los cambios en las reservas de carbono, el suelo puede actuar como una fuente de carbono o como un sumidero de carbono (Guo y Gifford, 2002).

Por otro lado, los organismos edáficos son actores fundamentales para garantizar las propiedades físicas y químicas necesarias para la fertilidad del suelo (Lavelle, 1996; Mäder y Berner, 2011). En la fauna del suelo pueden reconocerse diversos grupos funcionales, dependiendo de su actividad, su modo de alimentación y sus efectos sobre el suelo: los remineralizadores, los ingenieros del suelo y los transformadores de hojarasca (Decaëns et al., 2006). Las lombrices de tierra participan de manera muy activa en la transformación de las materias vegetales en humus, necesario para aglomerar las partículas de arcilla, limo y arena del suelo y formar una estructura que permita la circulación del agua y del aire (Crittenden et al., 2014; Ernst y Emmerling, 2009; Metzke et al., 2007). Asimismo, la actividad microbiana del suelo es primordial en la agricultura ecológica, ya que la liberación de nutrientes para el cultivo depende principalmente de la degradación de la materia orgánica por los microorganismos del suelo (Vian et al., 2009). La biomasa microbiana del suelo puede ser un mejor indicador de los cambios en el manejo del suelo a corto plazo, ya que los cambios en la biomasa microbiana puede ser mayores y más rápidos comparados con el SOC y el N (Fließbach et al., 2007). Estos indicadores dependerán de las aportaciones de materias orgánicas en estos sistemas, y en función de ello se podrá mantener y mejorar la vida y en consecuencia la fertilidad del suelo (Decaëns et al., 2006; Freckman, 1994).

2. Cobertura permanente del suelo

Las prácticas de la agricultura de conservación tienen como objetivo mantener una cubierta vegetal durante todo el año, y una opción puede ser la siembra de cultivos de cobertura durante todo el año o durante los periodos entre los cultivos anuales de la rotación. Los cultivos de cobertura se pueden dejar en la superficie del suelo para que se incorporen al suelo de manera progresiva o se pueden gestionar como abonos verdes. Los abonos verdes son cultivos de cobertura que no se cosechan si no que se incorporan en el suelo y se siembran con la finalidad de proteger al suelo contra la erosión, mejorar su estructura y captar más agua, pero también tienen otros beneficios ambientales importantes como el aumento de la biodiversidad, la creación de fuentes alternativas de polen para las abejas o de hábitat para la nidificación de aves, además de ser hospedantes alternos para determinadas plagas (Meco et al., 1998). Los abonos verdes pueden reducir la flora arvense mediante la competencia por la luz, el agua, el espacio y los nutrientes. Además, la liberación de sustancias alelopáticas y la descomposición de los residuos de los abonos verdes pueden influir en la germinación y el crecimiento de las especies arvenses (Hobbs et al., 2008; Masilionyte et al., 2017).

Los cultivos de cereales de secano de las áreas mediterráneas generalmente se siembran en otoño y se cosechan entre finales de primavera y principios del verano. Tras la siega los rastrojos se incorporan al suelo o se mantienen hasta el otoño. Las escasas precipitaciones y las elevadas temperaturas no permiten el establecimiento de cultivos de cobertura que maximicen la protección del suelo de las temperaturas extremas y minimicen la erosión. Sin embargo, los cultivos de cobertura se pueden establecer en otoño e incorporase al suelo como abonos verdes antes de los cultivos de leguminosas anuales de primavera-verano.

3. Rotación de cultivos

La rotación de cultivos diversificada y con especies con características complementrarias es esencial para mejorar la fertilidad del suelo. Además, para garantizar la producción a corto plazo y la sostenibilidad a largo plazo es fundamental mantener los niveles de nutrientes en el suelo. Una rotación adecuada de cultivos que incluya leguminosas puede generar excedentes en los balances de nitrógeno (N) y de esta manera contribuir a la sostenibilidad de estos sistemas (Gadermaier et al., 2011). Meco et al. (1998) sostienen que la rotación de cultivos es el eje en el cual se sustenta la eficiencia productiva y ambiental de los sistemas cerealistas extensivos de secano en el mediterráneo, por su influencia sobre la dinámica de nutrientes, su eficiencia en la conservación del agua, la protección del suelo y sobre el control de la flora arvense y de posibles plagas. Además, las rotaciones de cultivo en el secano permiten gestionar los recursos hídricos necesarios para mantener el proceso de mineralización en verano y asegurar la liberación de nutrientes (Meco et al., 1998). Por lo tanto, la rotación de cultivos junto con el uso de abonos verdes puede reducir las pérdidas de rendimiento en los sistemas de cultivos ecológicos de secano (Masilionyte et al., 2017).

Objetivos

Objetivos

Objetivos generales

Actualmente, el desarrollo de modelos más sostenibles de agricultura que armonicen la producción con la conservación de los recursos naturales es una necesidad urgente. Esta tesis doctoral pretende aportar información que permita el desarrollo de una agricultura más sostenible a pequeña escala en el *Parc de l'Espai d'Interès Natural de Gallecs*. El establecimiento de un modelo de gestión agroecológico que potencie los valores económicos, ecológicos y sociales de este *Espai d'Interès Natural* constituye un proyecto pionero en Cataluña que tiene un extraordinario valor para la población rural de Gallecs, la comarca del Vallès Oriental y para la sociedad catalana en general. En el año 2005 se inició el proyecto de reconversión a la gestión ecológica bajo la dirección del Consorcio del *Parc de l'Espai d'Interès Natural de Gallecs* y el apoyo científico y técnico del Grupo de Investigación Consolidado "Ecología de los Sistemas Agrícolas" de la Universidad de Barcelona. Este proyecto responde a la necesidad de desarrollar modelos alternativos de gestión de los espacios agrícolas y a la generación de los conocimientos para mejorar el manejo de las áreas agrícolas de Cataluña. En el marco de este proyecto se estableció un experimento a medio plazo encaminado al desarrollo de un sistema de producción ecológica de los cultivos herbáceos extensivos que integre las técnicas de la agricultura de conservación con el objetivo de aumentar la productividad y el uso eficiente de los recursos, gestionar de manera adecuada las poblaciones de la flora arvense y aumentar la biodiversidad y disminuir la huella ecológica del carbono. Asimismo, se han llevado a cabo diversos experimentos en campos convencionales que pretenden implementar mejoras en la gestión agrícola y favorecer la transición a la agricultura ecológica en las fincas que todavía mantienen como modelo la gestión convencional.

En este contexto, el objetivo general de la tesis doctoral es estudiar la incorporación de prácticas más sostenibles en cultivos herbáceos extensivos de secano de la región mediterránea para mejorar la gestión de estos sistemas mediante dos estrategias:

Objetivos

1. La disminución de la intensidad de laboreo del suelo y el uso de métodos mecánicos de control de la flora arvenses en campos de cereales convencionales de secano.

2. La integración de prácticas agrícolas de conservación como son el laboreo mínimo, la aportación de abonos orgánicos y la introducción de los cultivos de cobertura durante el otoño y el invierno (que se incorporarán al suelo como abono verde antes del establecimiento del cultivo de primavera) a lo largo de una rotación de cuatro años de cereales y leguminosas ecológicas para el consumo humano.

Objetivos específicos

A continuación, se detallan los objetivos específicos de cada capítulo de la tesis:

Capítulo I:

1. Analizar el efecto del tipo de laboreo del suelo (vertedera -con inversión de las capas del suelo- vs. cincel -sin inversión de las capas del suelo-) sobre el desarrollo y el rendimiento del cultivo de cereal y sobre la abundancia y la riqueza específica de flora arvense durante dos años consecutivos en cultivos convencionales de trigo.

2. Evaluar el efecto del tipo de control la flora arvense (mecánico –grada de púas flexibles- vs. químico –herbicidas-) sobre el desarrollo y el rendimiento del cultivo y sobre la abundancia y diversidad de la flora arvense durante dos años consecutivos en cultivos convencionales de trigo.

Capítulo II:

1. Analizar el efecto del laboreo del suelo (vertedera -con inversión de las capas del suelo- vs. cincel -sin inversión de las capas del suelo) sobre el desarrollo y el rendimiento de los cultivos y sobre la abundancia de la flora arvense en una rotación de cuatro años de cereales y leguminosas ecológicos para el consumo humano.

2. Evaluar el efecto de la fertilización orgánica sobre el desarrollo y el rendimiento de los cultivos y sobre la abundancia de la flora arvense en una rotación de cuatro años de cereales y leguminosas ecológicos para el consumo humano.

3. Analizar el efecto de la incorporación al suelo de los cultivos de cobertura, establecidos durante el otoño y el invierno, como abono verde sobre la abundancia de la flora arvense y el desarrollo y el rendimiento del cultivo de leguminosas posterior.

4. Evaluar la influencia de la fertilización, el sistema de laboreo y los abonos verdes sobre la calidad y fertilidad del suelo, estimada a partir del contenido de carbono orgánico total (SOC) y el nitrógeno total (N) del suelo, la densidad aparente del suelo y las reservas de carbono.

5. Evaluar la influencia de la fertilización, el sistema de laboreo y los abonos verdes sobre la abundancia de la biomasa microbiana del suelo (C_{mic} y N_{mic}).

Capitulo III

1. Evaluar el efecto de la fertilización, el sistema de laboreo y los abonos verdes sobre la abundancia de las lombrices de tierra.

2. Estudiar la diversidad de lombrices de tierra y cómo afecta la fertilización, el sistema de laboreo y los abonos verdes a los distintos ecotipos de lombrices.

Capítulo IV

1. Evaluar el efecto de la fertilización, el sistema de laboreo y los abonos verdes sobre el uso y la disponibilidad del N del suelo y de los cultivos a lo largo de una rotación de cuatro años de cereales y leguminosas ecológicos para el consumo humano.

2. Identificar la mejor combinación de prácticas agrícolas en términos de balance de N y de la eficiencia del uso del N a lo largo de una rotación de cuatro años de cereales y leguminosas ecológicos para el consumo humano.

Área de estudio y diseño experimental

Área de estudio y diseño experimental

<u>Área de estudio</u>

Los experimentos que forman parte de esta tesis doctoral se han llevado a cabo en el *Parc de l’Espai d’Interès Natural de Gallecs*. Gallecs es un espacio agrario periurbano de 753 hectáreas, situado en la comarca del Vallés Oriental, a 15 kilómetros al norte de Barcelona (41°33'31.9"N 2°11'59.5"E) (Figura 2).

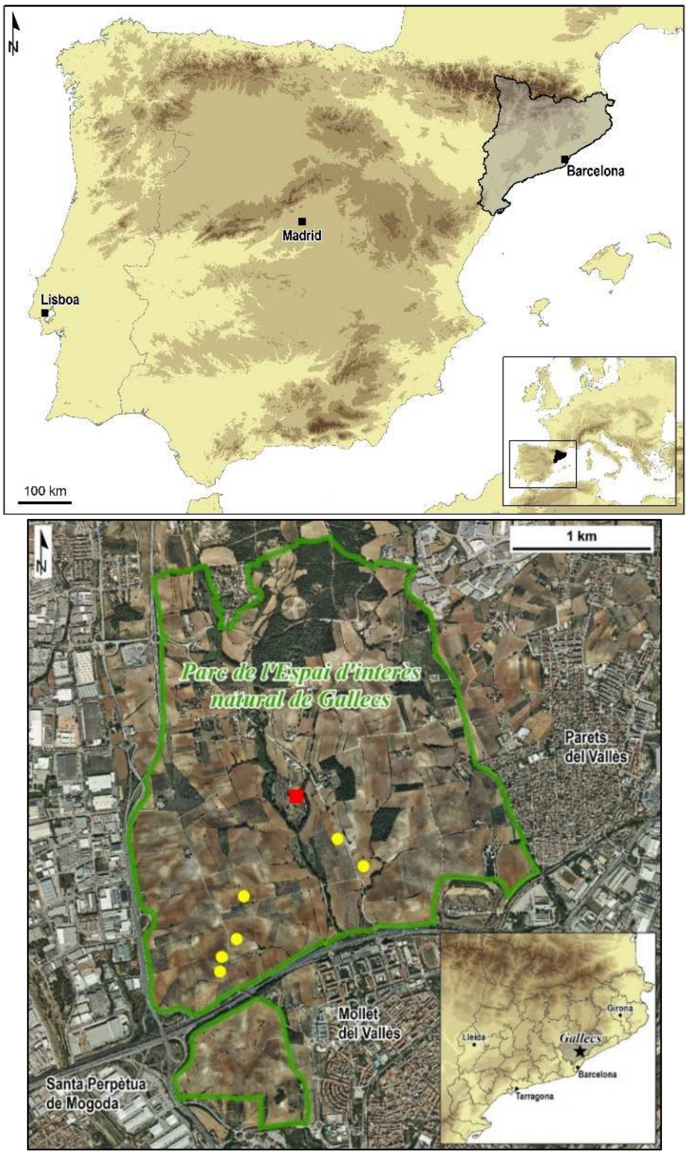

Figura 2. Área de estudio. Figura superior: mapa de localización de Cataluña en España. Figura inferior: mapa del *Parc de l’Espai d’Interés Natural de Gallec*s, los puntos amarillos indican la ubicación de las parcelas experimentales del estudio que recoge el capítulo I y el cuadrado rojo representa la ubicación de la parcela experimental de los estudios que recogen los capítulos II, III y IV.

Área de estudio y diseño experimental

El clima del área de estudio es de tipo mediterráneo, con veranos secos y calurosos y las precipitaciones concentradas en otoño-invierno; la precipitación y la temperatura media anual de la zona es de 647 mm y 14.9 °C respectivamente (Figura 3).

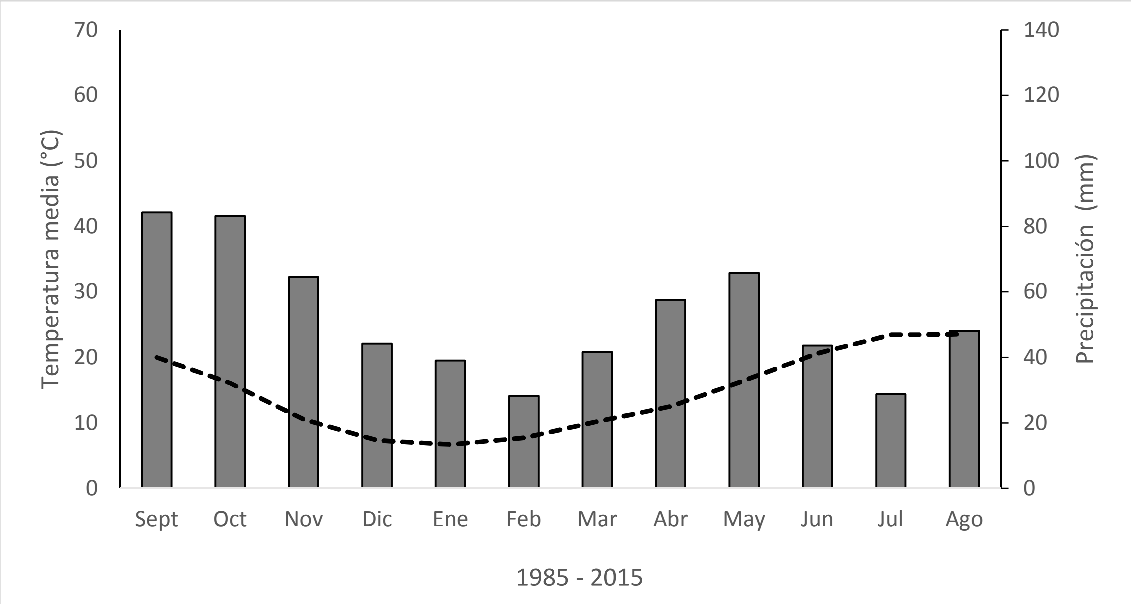

Figura 3. Temperatura media mensual (°C) y precipitación total mensual (mm) de un promedio de 30 años (1985-2015) medidos en la estación experimental Caldes de Montbui (Vallés Oriental, Cataluña), cercana al área de estudio (20 km). Fuente: Ruralcat: *La comunitat virtual agroalimentaria i del món rural* (https://www.ruralcat.net/web/guest/agrometeo, acceso el 10 de noviembre 2016).

El paisaje de Gallecs se caracteriza por el predominio de cultivos herbáceos extensivos de secano (70,82%), mientras que la vegetación natural y semi-natural ocupa el 16,23% del territorio, el resto del área está ocupada por cultivos arbóreos y arbustivos, huertos y yermos (4,17%) y habitáculos e infraestructuras viarias (8,79%). El área está rodeada de importantes extensiones de áreas industriales y residenciales, y de vías de comunicación, iniciadas en los años ochenta y consolidadas en los últimos veinte años. Actualmente, constituye una de las escasas reservas de espacio rural en el área metropolitana de Barcelona y posee un papel fundamental en la ordenación del territorio y la conservación del paisaje, y actúa como pulmón verde en el contexto de un territorio muy urbanizado. En 2005 Gallecs fue clasificado como suelo no urbanizable, calificado como sistema de espacios públicos y fue protegido y nombrado *Parc de l'Espai d'Interés Natural de Gallecs*. En 2009 Gallecs se incorpora a la lista de espacios protegidos del Pla d'Espais Naturals Protegids (PEIN). En noviembre de 2005 once agricultores junto con el Consorcio del *Parc de l'Espai d'Interés Natural de Gallecs*, iniciaron la reconversión a la agricultura ecológica de los cultivos extensivos de secano. A principios de 2005, periodo anterior a la reconversión, el monocultivo de cereal ocupaba el 90,03% de la superficie. La actual diversificación de los cultivos, que se manifiesta por una importante reducción del cultivo de cereales (48,15%), se ha basado en la progresiva incorporación de diversas leguminosas con el objetivo de favorecer la diversidad cultivada en el espacio (policultivos) y en el tiempo (rotaciones), así como la fertilidad del suelo (Safont et al., 2007). Desde 2005 el equipo de investigación "Ecología de los Sistemas Agrícolas" de la Universidad de Barcelona realiza el seguimiento y la evaluación del proceso de transición desde una perspectiva agronómica, ambiental y económica mediante un convenio de colaboración que mantiene con el Consorcio del *Parc de l'Espai d'Interés Natural de Gallecs*.

Diseño experimental Capítulo I

En otoño de 2013 se planteó un experimento de dos años de duración en campos de cereales de invierno con gestión convencional que pretendía introducir prácticas agrícolas más sostenibles ambientalmente como son el laboreo mínimo y el control mecánico de la flora arvense. El objetivo general de este estudio es la evaluación de un sistema económicamente favorable para los agricultores convencionales, y a la vez menos perjudicial para el medio ambiente, mediante el estudio de distintos sistemas de laboreo del suelo y control de la flora arvense.

Para ello se seleccionaron seis campos de cereal con gestión convencional en el *Parc de l'Espai d'Interés Natural de Gallecs*. En cada campo se estableció dos sistemas de laboreo del suelo que diferían en la intensidad de la perturbación (vertedera con inversión de las capas del suelo vs. cincel sin inversión de las capas del suelo) y distintas prácticas para el control de la flora arvense (aplicación de herbicidas vs. control mecánico) y el no control de la flora arvense. En otoño 2013 y 2014 todos los campos fueron sembrados con trigo de invierno (*Triticum aestivum* L. var. Montcada). Cada campo se dividió por la mitad según el tipo de laboreo, una mitad fue labrada con el arado de vertedera (inversión de las capas del suelo) y la otra con el arado de cincel (no inversión de las capas del suelo). El laboreo con arado de vertedera se realizó a 25 cm de profundidad con inversión de las capas del suelo y una grada rotativa a 5 cm de profundidad para la preparación de la siembra. El arado de cincel se realizó también a una profundidad de 25 cm pero sin inversión de las capas del suelo y también la grada rotativa a 5 cm de profundidad para la preparación de la siembra. En cada una de las mitades de cada campo (cincel y vertedera) se establecieron tres parcelas de 6 m de ancho por 48 m de largo, divididas en cuatro subparcelas de 12 m de largo cada uno (Figura 4). En la primera de las parcelas se realizó el control de la flora arvense mediante la aplicación de herbicida, en la segunda la flora arvense se controló mecánicamente mediante la grada de púas flexibles y en la tercera, no se realizó ningún control de la flora arvense (Figura 5). Las parcelas con aplicación de herbicidas se separaron del resto de las parcelas mediante una banda de 6 m de ancho para evitar el potencial efecto del herbicida por deriva. Durante los dos años de experimentación se evaluaron diversos parámetros agronómicos y ecológicos como son el rendimiento de los cultivos, la abundancia y la diversidad de la flora arvense. En el capítulo I se explica detalladamente las metodologías de evaluación de cada parámetro y la maquinaria y actividades agrícolas realizadas.

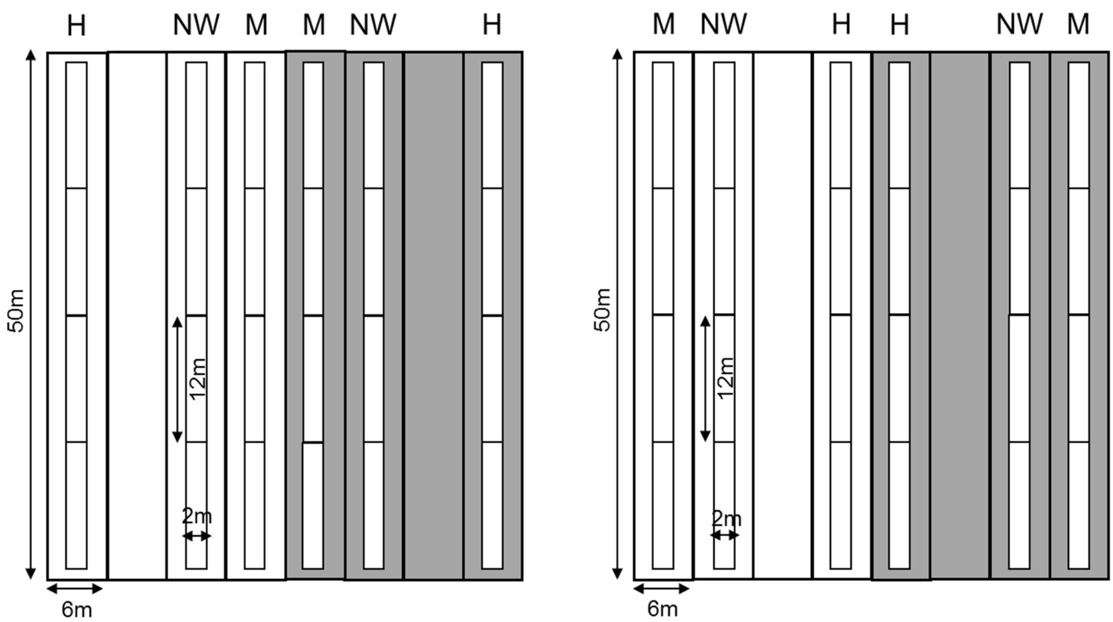

Figura 4. Diseño experimental del capítulo I. En gris las parcelas que fueron labradas con el arado de vertedera y en blanco las parcelas que fueron labradas con el arado de cincel. H: control químico de la flora arvense mediante la aplicación de herbicidas. M: control mecánico de la flora arvense con grada de púas flexibles. NW: parcelas donde no se realizó un control de la flora arvense. A la izquierda el diseño con las parcelas con herbicida en los extremos (3 campos), a la derecha el diseño con las parcelas con herbicida en el centro (3 campos).

Figura 5. Izquierda: control químico - aplicación de herbicida. Derecha: control mecánico - grada de púas flexible.

Diseño experimental Capítulo II, III y IV

En noviembre de 2011, el equipo de investigación "Ecología de los Sistemas Agrícolas" estableció un experimento a medio plazo dentro de *l'Espai Rural d' Interès Natural de Gallecs* que se enmarcaba dentro del proyecto europeo TILMAN-ORG. El objetivo general de este proyecto es el desarrollo de un sistema de producción de cultivos herbáceos extensivos sostenible mediante la introducción de técnicas de laboreo mínimo, combinada con el uso estratégico de los abonos verdes en una rotación de cultivos de secano ecológicos, que fuera compatible con el mantenimiento y la mejora de la calidad del suelo y los parámetros de productividad de los cultivos.

Para este experimento se seleccionó una parcela de 0,5 ha, gestionada bajo los principios de la agricultura ecológica desde 2005 con una rotación de cultivos de cereales y leguminosas destinados al consumo humano. El año previo al establecimiento del experimento el campo fue sembrado con *Vicia ervilia* (L.) Willd. En el momento del establecimiento del experimento se evaluaron las propiedades del suelo, la fracción mineral estaba compuesta en promedio por 43.3 ± 6.9 % arena, 26.9 ± 4.7 % limo y 29.7 ± 3.7 % arcilla; la textura del suelo se clasificó como franco-arcillosa (Soil Survey Staff, 1998); y el tipo de suelo como Haplic Luvisol (IUSS Working Group WRB, 2015). El contenido de materia orgánica media fue de 1.5 ± 0.1 % (Walkley-Black) y el pH (H_2O) de 8.1 ± 0.1.

Área de estudio y diseño experimental

El diseño experimental comprende tres factores: el sistema de laboreo (convencional (arado de vertedera) (P) vs. mínimo (arado de cincel) (C)), la fertilización (con fertilización (+F) vs. sin fertilización (-F)) y la incorporación de los abonos verdes entre cultivos (con abono verde (+G) vs. sin abono verde (+G)), que definieron 8 tratamientos con cuatro réplicas cada uno, sumando un total de 32 parcelas. Los tres factores y las cuatro repeticiones (bloques) se dispusieron en un diseño en bloques divididos, en el que los factores que intervienen están subordinados unos a otros. Empezando con el sistema de laboreo como factor principal distribuido en bandas verticales, la fertilización como factor secundario distribuido horizontalmente a través de las bandas verticales y el abono verde anidado dentro del factor principal (Figura 6). El tamaño de las parcelas fue de 12 m x 13 m, para permitir llevar a cabo las actividades agrícolas con maquinaria de tamaño regular. La rotación de cuatro años de cultivos comprendió: espelta (*Triticum spelta* L., 2011–2012), garbanzos (*Cicer arietinum* L., 2013) trigo (*Triticum aestivum* L., 2013-2014) y lentejas (*Lens culinaris* Medik., 2015).

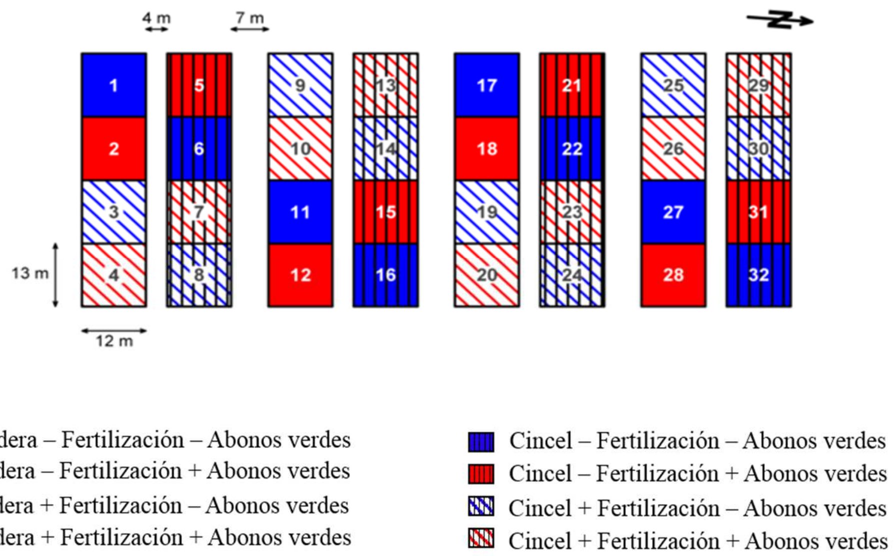

Figura 6. Diseño experimental con distribución de bloques divididos comprendido por tres factores: Sistema de laboreo: vertedera vs cincel; fertilización: + con fertilización, - sin fertilización; abonos verdes: + con abonos verdes, - sin abonos verdes. En total 32 parcelas de 13 × 12 m repartidas en cuatro bloques.

El laboreo convencional se realizó con un arado de vertedera (inversión de las capas del suelo a 25 cm de profundidad) y una grada rotativa a 5 cm de profundidad para la preparación de la siembra. Para el laboreo mínimo se utilizó el arado de cincel (sin inversión de las capas del suelo, a 25 cm de profundidad) y también la grada rotativa a 5 cm de profundidad para la preparación de la siembra (Figura 7). El tratamiento de fertilización consistió en estiércol de ganado vacuno semi-compostado, procedente de una granja próxima al área de estudio, y se aplicó cada año antes de la siembra de cada cultivo y se incorporó mediante los sistemas respectivos de laboreo del suelo. La cantidad de estiércol varió en función del cultivo, para el cultivo de espelta se aplicaron 134,6 kg N ha^{-1}, para el cultivo de garbanzos se aplicaron 40,04 kg N ha^{-1}, para el trigo 138,28 kg N ha^{-1} y finalmente para el cultivo de lentejas se aplicaron 62,36 kg N ha^{-1}. En septiembre 2012 y 2014 los abonos verdes fueron sembrados en las 16 parcelas correspondientes y comprendieron una mezcla de avena (*Avena sativa* L.), mostaza (*Sinapis alba* L.), yeros (*Vicia ervilia* (L.) Willd.), y veza (*Vicia sativa* L.). A finales de marzo del año siguiente, los abonos verdes fueron incorporados al suelo mediante el arado de discos. El manejo de la flora arvense varió según el cultivo y el año. El primer año no se pudo realizar el control debido a condiciones meteorológicas adversas. Durante el cultivo de garbanzos se realizó el control de la flora arvense utilizando un cultivador adaptado para pasar entre líneas del cultivo. En el cultivo de trigo se realizó el control mediante una grada de púas flexible y finalmente durante el cultivo de lenteja dada la alta infestación de hierbas que competían con el cultivo se realizó un control manual.

Figura 7. Izquierda: arado de cincel. Derecha: arado de vertedera.

Este experimento de medio plazo comprende los últimos tres capítulos de la tesis debido a la complejidad y a los diferentes aspectos evaluados. Durante los cuatro años de experimentación se evaluaron diversos parámetros agronómicos y ecológicos como son el crecimiento y rendimiento de los cultivos, la abundancia y la diversidad de la flora arvense, los parámetros de calidad y fertilidad del suelo (densidad aparente, contenido de carbono y nitrógeno del suelo, biomasa microbiana y lombrices de tierra) y el contenido de carbono y nitrógeno de los cultivos (Figura 8). En cada capítulo se explica detalladamente las metodologías de evaluación de cada parámetro.

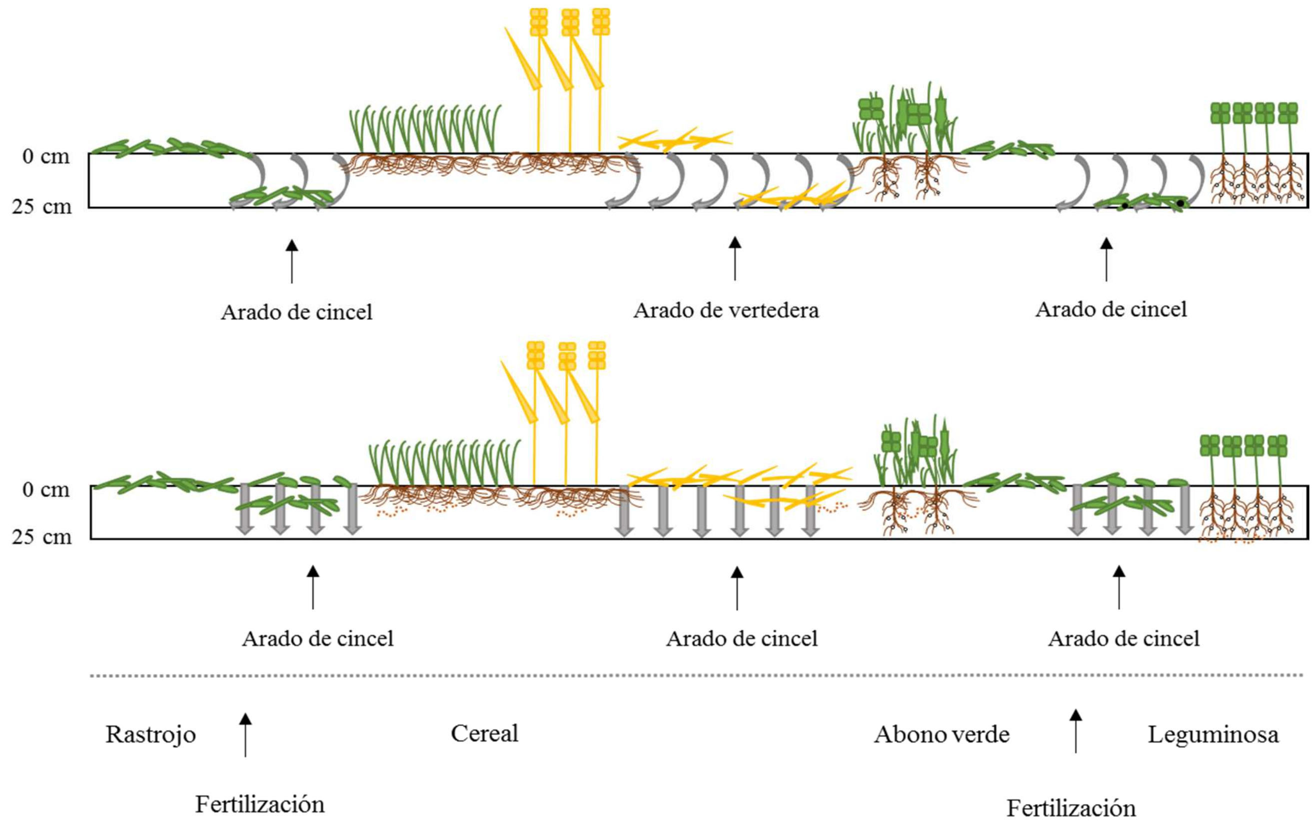

Figura 8. Esquema de la rotación de cereal y leguminosa correspondiente al experimento de los capítulos II, III y IV, con dos sistemas de laboreo distintos. El primer esquema (arriba) representa la rotación con arado de vertedera (inversión de capas de suelo) y el segundo esquema (abajo) representa la rotación con arado de cincel (sin inversión del suelo). Los esquemas parten de la incorporación de rastrojos del cultivo anterior, seguido por la fertilización (en las parcelas correspondientes) y el laboreo del suelo para el establecimiento del cultivo de cereal. Después de la cosecha del cereal y la incorporación del rastrojo, los cultivos de cobertura son sembrados y posteriormente se incorporan al suelo (en las parcelas correspondientes), finalmente se realiza la fertilización y el laboreo para el cultivo de leguminosa.

Capítulo I

Chapter I

Tillage and weed management options in cereal fields in the Mediterranean region

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Opciones de laboreo del suelo y el control de la flora arvense en cultivos de cereales en la región mediterránea

Resumen

El laboreo intensivo del suelo mediante el arado de vertedera, que implica la inversión de las capas del suelo y la modificación de su estructura, acelera la descomposición de la materia orgánica, la compactación del suelo y la consecuente degradación del mismo. Recientemente, se ha promovido el uso de prácticas de la agricultura de conservación para reducir la intensidad de laboreo. Estas prácticas pueden mejorar la fertilidad del suelo, incrementar la biodiversidad y minimizar la erosión. Sin embargo, una de las preocupaciones de los agricultores al reducir la intensidad del laboreo del suelo es el incremento de la abundancia de la flora arvense. Por ello, la incorporación de las prácticas de conservación de laboreo del suelo requiere el adecuado control de la flora arvense. El uso de métodos alternativos a los herbicidas, como el control mecánico mediante el uso de la grada de púas flexibles puede ser tan eficaz como el control químico y evita el impacto negativo sobre el medio ambiente. Este experimento a corto plazo evalúa el efecto del uso del arado de vertedera comparado con el arado de cincel, y el control mecánico respecto al control químico sobre el rendimiento del cultivo trigo de invierno, y la abundancia y diversidad de la flora arvense en la región mediterránea. El tipo de laboreo fue el principal factor que afectó el rendimiento del cereal. El rendimiento del cereal fue significativamente mayor en las parcelas con arado de vertedera que en las parcelas con arado de cincel, mientras que la abundancia y riqueza de flora arvense fue mayor en las parcelas con arado de cincel. El uso de herbicidas disminuyó drásticamente la densidad y la riqueza de la flora arvense en comparación con el control mecánico y las parcelas sin ningún tipo de control. Durante el primer año del experimento, el rendimiento del cereal fue mayor en las parcelas que se aplicó herbicida y la biomasa de flora arvense disminuyó. Sin embargo, el tipo de control de la flora arvense no afectó significativamente el rendimiento del cereal ni la biomasa de la flora arvense durante el segundo año. El estudio pone de manifiesto que la decisión de controlar la flora arvense en post-emergencia debe ser evaluado cada año con el objetivo de tener en cuenta las pérdidas de rendimiento asociadas a la presencia de la flora arvense y los costes de los tratamientos y el posible daño al cultivo, especialmente

en áreas de secano, donde los rendimientos son inferiores a los de las regiones templadas, y donde la producción de los cultivos puede estar limitada por las condiciones climáticas.

Tillage and weed management options in cereal fields in the Mediterranean region

Abstract

Intensive soil tillage practices, such as the use of a mouldboard plough, cause soil inversion, destroy the soil structure and cause accelerated descomposition of the organic matter of the soil, leading to compaction and, eventually, soil loss. Recently, the use of soil conservation practices to reduce soil tillage intensity has been promoted. These practices can improve soil fertility, increase biodiversity and reduce soil erosion. However, the increase in weed infestation is one of the main concerns when implementing reduced tillage, and the control of weeds can be crucial. The use of alternative methods to chemical weed control, i.e., mechanical weed control, can produce similar results but avoids the environmental impacts of herbicides. In this short-term trial in the Mediterranean region, we compared the effect of using a chisel plough compared to a mouldboard plough and the effect of mechanical weeding compared to herbicides on winter wheat grain yield and on weed abundance and diversity. The type of tillage showed the largest effects on cereal yields; mouldboard ploughing resulted in greater cereal yields compared to chisel ploughing. Weed abundance and species richness were higher in plots with chisel ploughing than in plots with mouldboard ploughing. The use of herbicides drastically decreased weed density and species richness compared to mechanical weed control and no weed control. Chemical weed control favoured cereal yields in the first year, but it did not have a significant effect on cereal yields or on weed biomass in the second year. The use of weed control methods should be case-specific to balance the corresponding reduction in crop yields with the cost of the treatment and the potential damage to the crop, especially in drylands, where crop yields are lower than those in temperate regions; thus, crop production can be limited by the climatic conditions.

Key words: chisel plough, mouldboard plough, mechanical weeding, weed abundance

Highlights:

- Mouldboard ploughing favours cereal yields compared to chisel ploughing
- Mechanical weed control can maintain weed abundance and diversity
- Cereal yields can be affected by mechanical weed control if the conditions are not favourable

Introduction

Soil conservation is one of the major issues that need to be addressed in agricultural systems. Recent studies have estimated a total of 130 million ha affected by soil erosion in the European Union; almost 20 % of European soils are subjected to degradation (European Environment Agency, 2012), thus raising awareness of the importance of preserving soil quality and fertility and preventing soil loss (Holland, 2004; Kassam et al., 2012). Many soils are currently not managed in a sustainable way. For many European farmers, soil tillage in arable crops consists of inverting soil structure using tools such as mouldboard ploughs (Plaza et al., 2011). Mouldboard ploughing can modify the soil structure and promote soil organic matter degradation, causing compaction and eventually leading to soil loss; mouldboard ploughing can also substantially reduce soil biodiversity and many of the soil-mediated ecosystem functions that provide and regulate environmental services (Holland, 2004; Montgomery, 2007).

Most agricultural soils in the dry climates of the Mediterranean region have low organic matter contents, with poor soil aggregate structures. The predominant intensive tillage worsens this situation (Lahmar and Ruellan, 2007). The analysis of a large set of Mediterranean agricultural soils found low organic matter contents attributed to soil degradation as a result of intensive ploughing over several centuries (Romanyà and Rovira, 2011).

The Food and Agriculture Organization of the United Nations and the Common Agricultural Policy in the European Union both promote soil conservation techniques, such as no-till and reduced soil tillage. It is argued that these conservation techniques provide potential benefits by improving soil fertility and increasing biodiversity, as well as by reducing soil erosion, energy consumption and the emissions of greenhouse gases (Berner et al., 2008; Gruber and Claupein, 2009; Holland, 2004). Reducing tillage intensity can entail changes in crop functioning, mainly affecting the dynamics of nutrient availability, crop emergence and growth, and competition with weeds (Légère et al., 2008). Moreover, it can affect the distribution and viability of weed seeds in the soil profile, which in turn can influence seedling emergence and survival, and the survival of vegetative structures of perennial species (Bullied et al., 2003). Non-inversion tillage e.g., chisel ploughing, increases the amount of seeds in the upper soil layer and modifies the micro-topography, the light, water and temperature conditions in the soil surface layer, which may influence the emergence of weed seedlings according to their type and the climatic conditions promoting weed infestation (Gruber and Claupein, 2009; Peigné et al., 2007).

Weed infestation is one of the main concerns of farmers who use reduced tillage practices (Peigné et al., 2015), and weed management has been demonstrated to be crucial for a successful crop production (Armengot et al., 2012; Plaza et al., 2011). Application of herbicides has been effective for reducing yield losses and minimizing weed infestations. However, continued application of herbicides has caused the appearance of herbicide-resistant biotypes and other negative side effects on the environment (Boutin et al., 2002). Together with a generalized intensification of agricultural practices, the application of herbicides has reduced weed abundance and diversity and has changed the long-term composition of weed communities (Chamorro et al., 2016; Marshall et al., 2003). These changes can exert unexpected consequences on agroecosystems, as weeds are important components supporting agroecosystem services. Weeds provide ecological services, such as pest control, and can maintain the stability and balance of these systems (Marshall et al., 2003).

There is growing interest in alternative methods such as mechanical weed control (Armengot et al., 2012), particularly in areas with environmental and ecological values. Weed control via harrowing with long-flex tines is one of the most widely used mechanical methods for weed control in organically grown cereals. It disturbs the soil surface mildly, so weed seedlings are uprooted and covered by soil. This may not completely kill the weeds, but the damage caused may be enough to slow down their growth and reduce competition with the crop (Pardo et al., 2008). Mechanical weeding can prevent weeds from being a limiting factor in crop productivity while maintaining high levels of weed diversity (Armengot et al., 2012).

There is a lack of studies in the Mediterranean region on alternative practices in conventional agriculture to reduce intensive soil tillage and herbicide application in contrast to central and north European regions. Mediterranean agricultural systems need to cope with multiple environmental constraints and threats such as negative annual water balance, a short and variable rainy season and soil degradation (Kassam et al., 2012). Therefore, in 2013, we established a short-term experiment in six dryland cereal fields. The objective of the two-year trial was to assess the suitability of environmentally friendly agricultural practices. To this end, we evaluated the effect of chisel ploughing compared to mouldboard ploughing and the effect of mechanical weeding compared to herbicides on winter wheat grain yield and weed abundance and diversity. The hypotheses of this experiment were that a) reduced tillage does not affect crop yields in comparison with soil inversion tillage, b) the application of mechanical weed control can smother weeds, thus reducing the competition with crops and simultaneously preserve weed diversity, and c) mechanical weed control will be equally effective under both tillage systems.

Materials and methods

Study area and experimental design

The study was conducted in Gallecs, a peri-urban agricultural area of 753 ha near Barcelona. The climate is Mediterranean, with a mean annual temperature of 14.9°C and a mean annual precipitation of 647 mm. The total rainfall and mean temperature in the study years during the crop season (from December to June) was 198 mm and 13.1°C, respectively, in the first year and 160 mm and 15.7°C, respectively, in the second year. A two-year field experiment was established in autumn 2013 in six conventionally managed cereal fields with similar soil conditions and agronomic practices. The soils have been traditionally managed with intensive tillage that consisted of mouldboard ploughing with soil inversion. The type of soil was Haplic Luvisol (IUSS Working Group WRB, 2015), and the average mineral fraction of the soils in the study area consisted of 54.82 % sand, 24.34 % loam and 20.84 % clay, giving a sandy-loam texture (Soil Survey Staff, 1998), and the average soil organic carbon was 0.75 %.

The experimental design to analyse the effect of tillage and weed control on weeds and grain yield was a split plot with two factors: tillage (reduced tillage versus conventional tillage) and weed control with three levels (no control, herbicide and mechanical weed control), replicated six times (fields). Conventional tillage (P) consisted of a mouldboard plough (soil inversion) and a rotary harrow, and the reduced tillage (Ch) consisted of chisel plough (no soil inversion) and a rotary harrow (Table 1).

In each field, two adjacent areas of 48 m × 50 m were delimited and randomly assigned to mouldboard plough or chisel. Within each area, four adjacent plots with dimensions of 50 m long and 6 m wide were delimited to assess the weed control factor. In one plot, weeds were not controlled (NW); in the other two plots, weeds were controlled by herbicides (H) and mechanically with long-flex tines (M) (Figure 1). The fourth plot was used as a buffer plot between the herbicide plot and the others to avoid the drift effect. In three of the fields, the herbicide plots were placed together in the middle of the delimited area, and in the other three, the herbicide plots were placed at the borders of the area following two different designs (Figure 1).

All fields were sown both years with winter wheat (*Triticum aestivum* L. var. Montcada), and mineral fertilizer was applied after sowing (Table 1), according to local farming practices. No differences in the cereal establishment between the type of tillage were found in any of the years (mouldboard vs chisel: 0.27 ± 0.24, $p = 0.26$ in 2014, and mouldboard vs chisel: -0.07 ± 0.12, $p = 0.56$ in 2015). However, the establishment of cereal was significantly lower in the second year (2015 vs 2014: -1.06 ± 0.31, $p = 0.001$).

Table 1. Date of field operations, sowing characteristics, and fertilisation inputs during the two crop periods of the experiment.

	2013-2014	**2014-2015**
Sowing		
Crop	*Triticum aestivum* L. var Montcada	*Triticum aestivum L.* var Montcada
Sowing date	12 December 2013	20 December 2014
Sowing density	220 kg ha^{-1}	220 kg ha^{-1}
Distance between rows	12 cm	12 cm
Tillage		
Conventional tillage		
Mouldboard plough EG 85-240-8, Kverneland Soil inversion at depth 25 cm	7 November 2013	12 December 2014
Rotary harrow. HR3003D. Kuhn depth 5 cm	12 December 2013	20 December 2014
Reduced tillage		
Chisel KCCC 1187 - A00, Kverneland No soil inversion, depth 25 cm	11 November 2013	12 December 2014
Rotary harrow. HR3003D. Kuhn depth 5 cm	12 December 2013	20 December 2014
Fertilisation		
Mineral fertilizer NPK 15:15:15	9 December 2013	13 December 2014
Dose	340 kg ha^{-1}/ 51 N kg ha^{-1}	340 kg ha^{-1}/ 51 N kg ha^{-1}
Weed control		
Herbicide		
Ioxynil (12% octanoate) + Mecoprop (36% butoxyethyl ester) Nufarm Spain, S.A.	27 February 2014	20 March 2015
Dose	3 L ha^{-1}	3 L ha^{-1}
Mechanical weed control		
tractor driven long-flex tines harrows Herse-6M, Pichon	5 March 2014	2 April 2015
Harvest		
M 4075 H Top Liner, Deutz-Fahr Wide: 5.40 m	22 June 2014	30 June 2015

Weed and crop assessment

The inner part of each plot was divided in four subplots of 12 m × 2 m for weed assessment (Figure 1). Before and after weed control, four frames of 25 cm × 30 cm were randomly placed in each subplot (Figure 2), and all weed species were identified and their density registered; the nomenclature follows the method of de Bolòs et al.

(2005). One month before crop harvest, weed biomass was evaluated within a frame of 1 m × 1 m that was randomly placed in each subplot. The total aboveground biomass of weeds was collected and oven-dried at 60 °C for 48 h before weighting. In each plot, crop yield was assessed in a 5.40 m (width of the harvester) × 50 m (length of the plots) area by a combine harvester (Table 1).

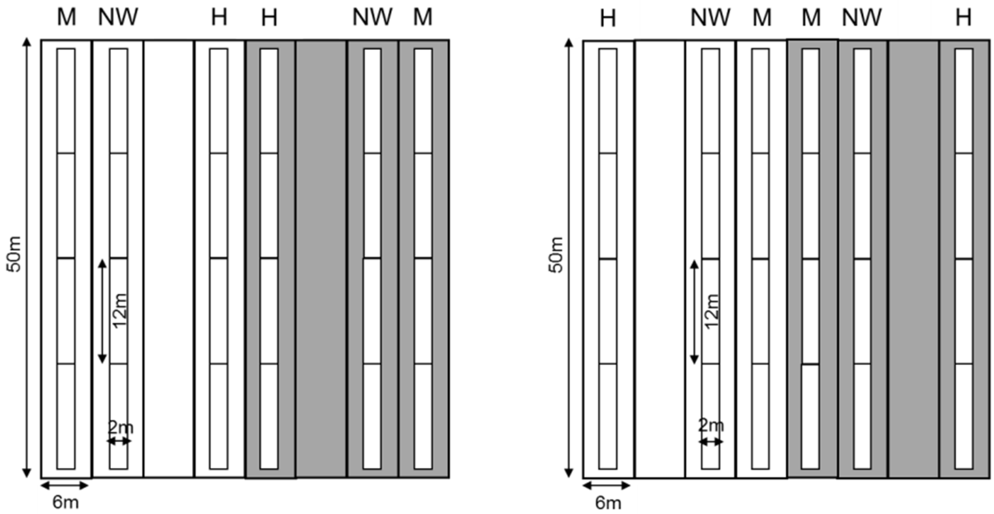

Figure 1. Schemes showing the experimental designs implemented in the study fields. Plots with chisel ploughing (Ch) in white background and plots with mouldboard ploughing (P) in grey background. Within each tillage type, three different weed control strategies were applied: H: herbicide control. M: mechanical weeding. NW: no weed control. The design on the left was established in three fields whereas the design on the right was applied on the remaining. The wider distance between the herbicide plots and the rest was to prevent the drift of herbicide on the other plots.

Statistical analysis

The effects of the type of tillage, weed control and their interactions on weed density, species richness before and after weed control, weed biomass before crop harvest and crop yield were evaluated through linear mixed-effects models. An additional analysis was performed to evaluate the effect of both factors on the eight most abundant weed species recorded in both years using generalized linear mixed-effects models with a Poisson error term for count data. Analyses including data from both years were performed, but since the weather conditions experienced each year were very different and because there was a significant interaction between year and experimental factors, each year was analysed separately.

Figure 2. Square of 25 × 30 cm for weed density assessment.

Tillage and weed control were included as fixed factors, and fields and plots, as random factors; for the analyses of crop yield, plots were not included in the model because there was a single measure per field and treatment. Mouldboard ploughing was compared with chisel ploughing (P vs Ch) for the factor type of tillage. Weed controlled plots were compared to non-weed controlled plots (H vs NW and M vs NW). The normality of residuals was verified by the Shapiro-Wilk test, and the homoscedasticity using the Bartlett's test. Data were square root transformed when necessary to meet the normality and homoscedasticity requirements.

In addition, we conducted a permutational multivariate analysis of variance, using distance matrices, to analyse how tillage and weed control affected species composition. This analysis allows partitioning distance matrices among sources of variation and fitting linear models to distance matrices. We used the Bray-Curtis dissimilarity indexes to measure differences in community composition between plots. All of the analyses were performed in R version 3.2.2 (R Development Core Team, 2015) with the packages lme4 (Bates et al., 2011) for generalized and linear mixed-effects models fitting, and lmerTest (Kuznetsova et al., 2015) for testing the significance of the effect of factors in linear mixed-effects models. The vegan package (Oksanen et al., 2013) was used for the community composition analysis.

Results

Effect of tillage on weed abundance, species richness and crop yield

The type of tillage did not affect weed density either before or after weed control in the first year of the trial. In the second year, plots with mouldboard ploughing had a significantly lower weed density than plots subjected to chisel ploughing, both before and after weed control (Figure 3, Table 2). Species richness followed the same pattern as density. Weed biomass was lower under mouldboard ploughing both in 2014 and 2015. Yields were significantly higher (on average 17.7 % in 2014 and 17.2 % in 2015) in plots subjected to mouldboard ploughing than in plots subjected to chisel ploughing (Figure 3, Table 2).

Effect of weed control on weed abundance, species richness and crop yield

In both years, weed density and species richness after weed control were significantly lower in the plots with herbicide application than in the plots with mechanical weed control and in the plots without weed control (Figure 3, Table 2). No significant differences were found in weed density and species richness between plots with mechanical weed control and plots without control. Considering that there were no differences in weed density and species richness among plots before weed control (Table 2), the observed effects of the weed control were due to its direct effect and not to previous differences on weed density and species richness.

In 2014, weed biomass was significantly lower in plots with herbicide application, but no differences were found between plots with mechanical weed control and plots without control (Table 2). In 2015, weed biomass did not differ between plots with and without weed control. In 2014, crop yields were higher in plots with herbicide than in plots with mechanical weed control and plots without control (Figure3, Table 2). Furthermore, crop yield in plots with mechanical weed control was lower than in plots without control. However, no differences in crop yields between the type of weed control were found in 2015 (Figure 3, Table 2). Overall, yields were significantly lower in the second year of the trial than in the first year (estimate: -727.58 ± 58.88, $p<0.001$; mean of all plots 1890 ± 327 kg ha^{-1} in 2014, and 1162 ± 201 kg ha^{-1} in 2015; Figure 3).

There were no significant interactions between tillage and weed control on weed abundance, weed species richness and crop yields (data not shown).

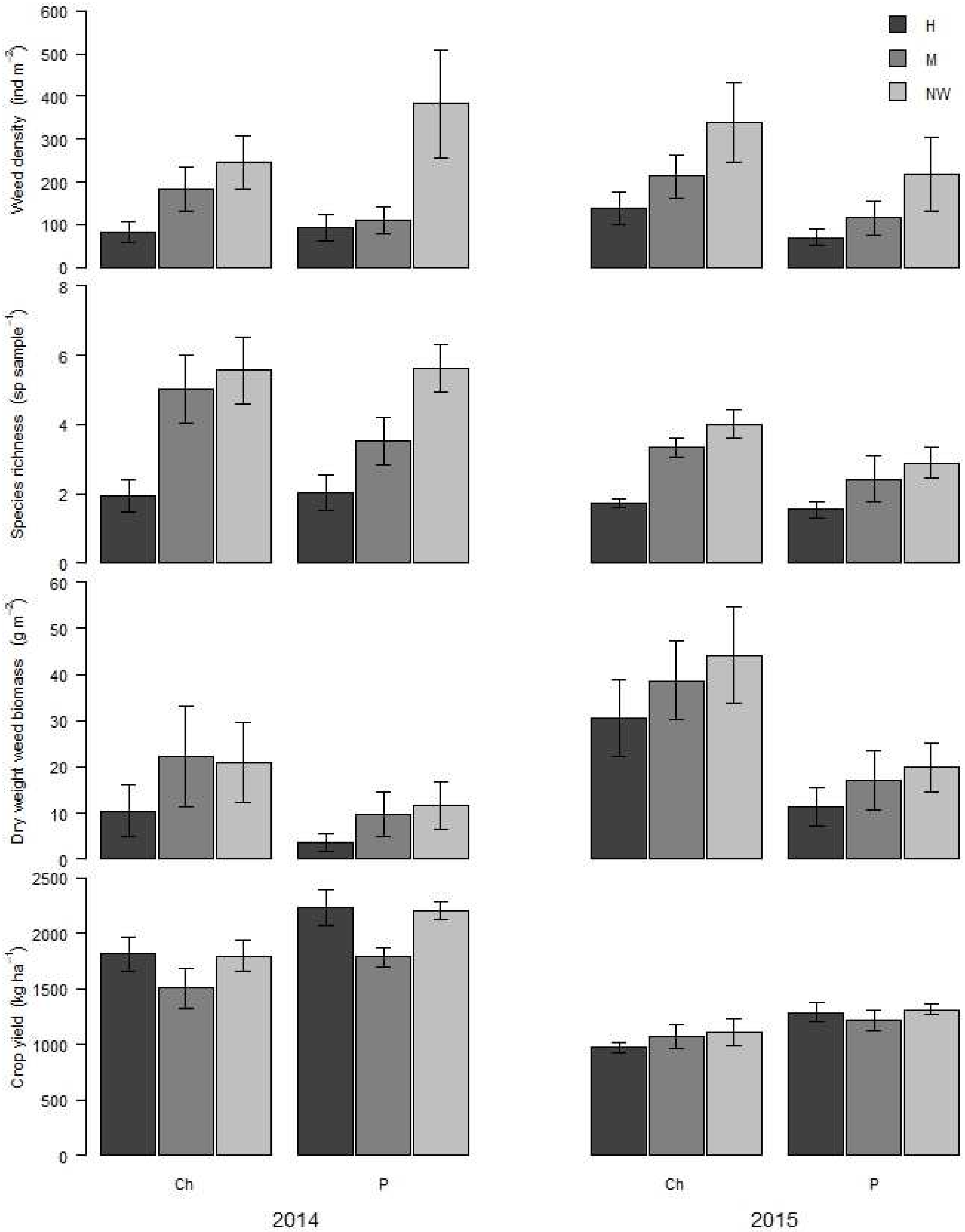

Figure 3. Mean (± standard error) weed density and weed species richness after weed control, weed biomass before crop harvest and winter wheat crop yield in plots with chisel (Ch) and mouldboard ploughing (P) with chemical (H) and mechanical (M) weed control and without control (NW).

Table 2. Coefficients (estimate ± standard error) from the linear mixed models testing the effect of the type of tillage and weed control on weed density and species richness before and after weed control, weed biomass before crop harvest and crop yields.

	Year	Intercept	Tillage	Weed control	
			P vs Ch	NW vs H	NW vs M
Weed density (before control) §	2014	266.59 ± 77.85*	35.92 ± 18.96^{NS}	-8.89 ± 26.81^{NS}	10.57 ± 26.81^{NS}
	2015	250.57 ± 68.21***	-62.54 ± 28.69*	72.79 ± 40.58^{NS}	-37.51 ± 40.58^{NS}
Weed density (after control) §	2014	12.14 ± 1.71***	0.19 ± 0.58 ^{NS}	3.45 ± 0.82***	0.88 ± 0.82^{NS}
	2015	11.92 ± 1.10***	-1.81 ± 0.65*	2.72 ± 0.91**	0.44 ± 0.91^{NS}
Weed richness (before control)	2014	467.52 ± 0.76**	-0.09 ± 0.12^{NS}	0.27 ± 0.18^{NS}	-0.03 ± 0.18^{NS}
	2015	381.50 ± 0.53***	-0.37 ± 0.15*	0.31 ± 0.21^{NS}	-0.14 ± 0.21^{NS}
Weed richness (after control)	2014	3.94 ± 0.61**	-0.22 ± 0.19^{NS}	1.96 ± 0.27***	-0.32 ± 0.27 ^{NS}
	2015	2.66 ± 0.30***	-0.36 ± 0.11**	1.02 ± 0.16***	-0.22 ± 0.16 ^{NS}
Weed biomass (after control) §	2014	3.09 ± 0.67**	-0.57 ± 0.14***	0.93 ± 0.19***	-0.39 ± 0.19^{NS}
	2015	4.79 ± 0.45***	-1.08 ± 0.24***	0.64 ± 0.34^{NS}	-0.11 ± 0.34^{NS}
Yields	2014	1870.37 ± 105.31***	181.07 ± 38.50***	-132.57 ± 55.69*	243.06 ± 55.6***
	2015	1158.95 ± 51.98***	108.54 ± 30.22**	29.99 ± 43.71 ^{NS}	22.10 ± 43.71^{NS}

For the factor type of tillage: Mouldboard ploughing (P) vs Chisel (Ch), for the factor weed control: no control (NW) vs herbicide (H) and no control (NW) vs mechanical weeding (M). §: Square root transformation was applied to accomplish homoscedasticity and normality of residuals. Significance levels according to the following codes: *** p < 0.001. ** p < 0.01. * p < 0.05. ^{NS} not significant).

Weed community composition

Of the 34 identified weed species during the two years of the trial, 8 species represented 85 % of all individuals. These are common arable weed species in cereal fields of the Mediterranean region: *Ammi majus* L., *Anagallis arvensis* L., *Convolvulus arvensis* L., *Chenopodium album* L., *Kickxia spuria* (L.) Dumort, *Polygonum aviculare* L., and *Polygonum convolvulus* L. Most species are annual weeds, with the exception of *Convolvulus arvensis*. The permutational multivariate analysis of variance showed that weed community composition was significantly affected by tillage and weed control. However, the r^2 values of these factors only explain 15 % of the variance (Table 3, Figure 3).

The effect of tillage on the weed abundance was different for annual and perennial species. The density of *C. arvensis* was significantly higher in plots subjected to chisel ploughing than plots subjected to mouldboard ploughing (Table 4). In contrast, annual weeds were not significantly affected by the type of tillage, except populations of *P. convolvulus*, which showed higher density in plots subjected to mouldboard ploughing. Overall, lower densities of these species were found in plots with herbicide than in plots without weed control (Table 4). *Amni majus, A. arvensis, C. album, C. arvensis, K. spuria* and *P. convolvulus* showed significantly lower densities when herbicide was applied compared to no weed control (Table 4). However, no effect of mechanical weed control compared to no weed control was found on the species densities. There were no significant interactions between tillage and weed control for any of the species (data not shown).

Table 3. Results from the permutational analysis of variance on species composition of the six fields conducted during both years of the trial.

	2014		2015	
	SS	r^2	SS	r^2
Tillage	0.460	0.046**	0.380	0.047*
Weed control	1.031	0.105***	1.065	0.131***
Tillage × Weed control	0.219	0.022	0.185	0.023
Total	9.824		8.071	

Sums of squares (*SS*), partial R-squared (r^2) and levels of significance (*** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$) of the different sources of variation considered, based on 1000 permutations.

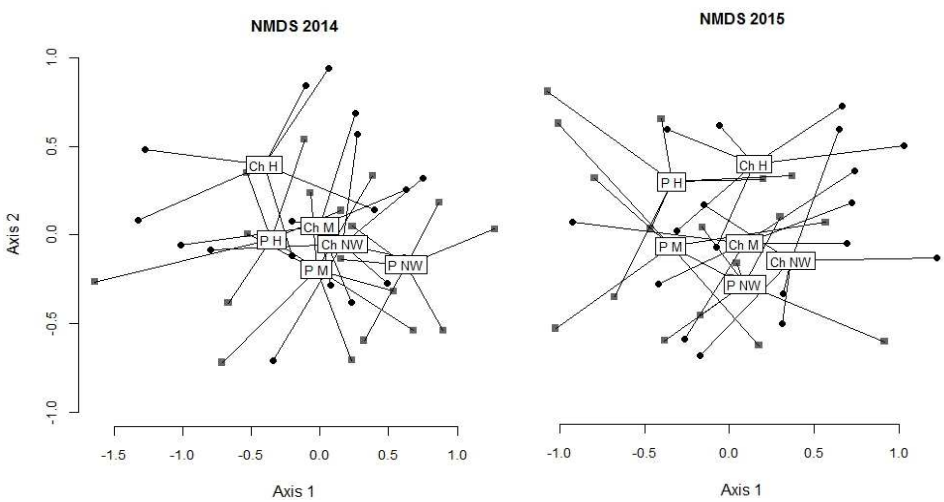

Figure 3. Ordination based on Nonmetric Multidimensional Analysis (NMDS) with the abundance of all species based on floristic similarities of: P: mouldboard ploughing, Ch: chisel, H: herbicide, M: mechanical weed control, NW: no weed control. (6 fields x 6 plots = 36).

Overall, most of the annual species showed a significant decrease in abundance the second year, with the exception of *P. aviculare*, *P. convolvulus* and *C. arvensis*, which populations increased in the second year (Table 4). *Polygonum aviculare* increased in plots with chisel ploughing compared to plots with mouldboard ploughing. In contrast, *P. convolvulus* increased in plots with mouldboard ploughing. *Convolvulus arvensis* experienced a significant increase in all plots (total 453 ind m^{-2} in 2014 to 891 ind m^{-2} in 2015), but no significant interaction was found regarding the different treatments (Table 4).

Discussion

Effect of tillage on weed abundance, species richness and community composition and cereal yields

In agreement with previous studies, we have found that weed abundance and species richness resulted higher in plots with chisel ploughing than with mouldboard ploughing (Peigné et al., 2007). This result can be related to the fact that weed propagules remain more superficial under reduced tillage (Gruber and Claupein, 2009). Murphy et al. (2006) reported that the adoption of reduced tillage in a 5-year experiment also resulted in increased weed diversity. This effect can be of special interest in light of the current decline of species diversity in agroecosystems. Reduced tillage can promote a slightly greater diversification of germination ecological niches and opportunities, resulting in higher weed species richness (Plaza et al., 2011).

However, the increase of weed infestation caused by reduced tillage could affect crop yield. Several studies have indicated that reduced tillage increases weed infestation in the long-term, especially of perennial weeds, because of its lesser effects on underground vegetative reproducing organs compared to inversion tillage (Gruber and Claupein, 2009; Peigné et al., 2007). In our study, we found an increase of 96% in the density of the perennial C. arvensis from the first to the second year of the trial. Convolvulus arvensis is an important weed in annual crops, under both non-inversion and inversion tillage, because of its relative tolerance to tillage, and it is one of the most troublesome species for farmers (Gruber and Claupein, 2009). Nevertheless, these changes in abundance of species in relation to tillage do not affect all species the same way. Thus, the increase in populations of *P. convolvulus* in plots with mouldboard ploughing might be related to its germination and emergence requirements (Armengot et al., 2012; Dorado and López-Fando, 2006).

In this trial, tillage exerted the strongest effects on cereal yields; mouldboard ploughing benefits cereal yields compared to plots with chisel ploughing. This pattern might be seen as a consequence of changes in weed pressure, as weed abundance was lower in plots with mouldboard ploughing, thus reducing weed-crop interaction. Woźniak and Soroka (2014) also indicated that reduced tillage afforded poorer conditions compared to the conventional tillage for the growth and development of plants, which resulted in poor tillering of *Triticale* and low yield. Furthermore, the transition period from conventional tillage to conservation tillage tends to be particularly prone to compaction, leading to impeded drainage, restrict crop emergence and poorer root growth (Peigné et al., 2007).

Reduced tillage can also cause lower yields owing to nutrient availability. Some studies in temperate climates have found a decrease of available nitrogen for the crop under reduced tillage due to lower mineralization rates, especially in organic fields (Berner et al., 2008; Peigné et al., 2007). However, in our study, this effect might not be as important, since mineral fertilizer was adequately applied in both tillage types. The slower soil nutrient release with reduced tillage would only be an issue in the case of a shortage of nutrients, e.g., at the end of the cropping season. A better understanding of the relative importance of fertilizer inputs and of soil nutrients in crop yields could clarify the effect of both weeds and nutrients on crop yields. Moreover, in a long-term experiment, Madarász et al. (2016) showed that during the first three years of transition from conventional tillage, yields decreased under reduced tillage. However, during the last seven years of their experiment, yields were higher with reduced tillage than with mouldboard ploughing.

Effect of weed control on weed abundance, species richness and community composition and cereal yields

Our results indicate that chemical weed control drastically decreases weed density and species richness compared to mechanical weed control and no weed control.

Table 4. Coefficients (estimate ± standard error) from the generalized linear mixed-effects models testing the effect of the type of tillage, weed control and the year of the trial on the 8 most abundant weed species found in the trial after weed control.

	P vs Ch	NW vs H	NW vs M	Year	P vs Ch × Year	NW vs H × Year	NW vs M × Year
Ammi majus L.	-0.05 ± 0.35 NS	1.25 ± 0.57*	-0.09 ± 0.48NS	-0.82 ± 0.05***	0.09 ± 0.06NS	0.90 ± 0.10***	-1.01 ± 0.10***
Anagallis arvensis L.	0.23 ± 0.22 NS	1.71 ± 0.32***	-0.09 ± 0.31NS	-1.26 ± 0.04***	-0.76 ± 0.06***	1.10 ± 0.12***	-0.43 ± 0.08***
Avena sterilis L.	-0.26 ± 0.28NS	0.62 ± 0.40NS	0.04 ± 0.39NS	-0.38 ± 0.39***	-0.44 ± 0.05***	0.25 ± 0.07***	0.61 ± 0.08***
Chenopodium album L.	-0.27 ± 0.14NS	0.74 ± 0.19***	-0.29 ± 0.19NS	-0.17 ± 0.03***	-2.23 ± 5.63NS	-7.60 ± 10.66NS	3.54 ± 5.33NS
[1]*Convolvulus arvensis* L.	-1.23 ± 0.33***	1.40 ± 0.47**	-0.43 ± 0.45NS	0.62 ± 0.05***	0.41 ± 16.78NS	13.74 ± 39.32NS	-6.46 ± 19.66NS
Kicxia spuria (L.) Dumort.	-0.37 ± 0.34NS	1.32 ± 0.49**	0.01 ± 0.48 NS	-1.26 ± 0.09***	-3.05 ± 10.09NS	-2.96 ± 10.08NS	6.43 ± 20.18NS
Polygonum aviculare L.	-0.18 ± 0.12NS	-0.09 ± 0.17NS	0.30 ± 0.17 NS	0.64 ± 0.02***	-0.17 ± 0.02***	-0.04 ± 0.02NS	0.10 ± 0.03***
Polygonum convolvulus L.	0.41 ± 0.18*	1.34 ± 0.26***	-0.05 ± 0.25NS	0.23 ± 0.02***	0.44 ± 0.03***	-0.04 ± 0.05NS	0.14 ± 0.04***

P: mouldboard ploughing, Ch: chisel, NW: no weed control, H: herbicide, M: mechanical weed control. [1]Perennial weed. Significance levels according to the following codes: *** p < 0.001. ** p < 0.01. * p < 0.05. NS not significant).

Sans et al. (2011) suggest that to preserve the ecosystems services and biodiversity, the aim of weed control should be not the total elimination of weeds but the reduction of competition with crops to obtain economically acceptable yields.

Even though weed density and species richness were not reduced after control in plots with mechanical weed control, we did not detect an increase of weeds in the second year, neither under no control; in fact, weed abundance was not higher in these plots compared to the ones with herbicide application in the second year. Farmers usually apply herbicides routinely because they are afraid that weed infestation will be higher the following year (Bàrberi, 2002; Peigné et al., 2015), but in this experiment, we did not find this effect. Furthermore, during the second year, the type of weed control did not affect weed biomass. The use of weed control methods should be a balance between the crop loss and the cost of the treatment and the potential damage to the crop due to the phytotoxicity of herbicides and the mechanical damage by harrows (Armengot et al., 2012). Although, most of the species densities were reduced by herbicides, the weed community composition analyses showed that other factors might be more important in shaping the assemblage of weed communities.

Chemical weed control favoured cereal yields in the first year, but it did not have a significant effect on cereal yields in the second year, neither an effect on weed biomass. Crop-weed competition depends to a large extent on the weather conditions; Campiglia et al. (2015) indicated that an excess of rainfall and soil water availability in late spring could cause an increase in weed proliferation, thus decreasing grain yield performance. Weed biomass was significant higher during the second year than during the first, and yields were significant lower during the second year. Weeds are often recognized as a major constraint for crop production because they use part of the resources that are essential for crop growth, and in many situations, they lead to higher economical losses (Armengot et al., 2012).

A plausible explanation for the lower yields in mechanical weed control in the first year of this experiment could be the damage of the crop caused by harrowing with long-flex tines, added to the fact that weather conditions were very dry after performing mechanical weed control. Mechanical weed control could maintain similar crop yields as herbicide and at the same time not affect weeds if it is carried out in a timely fashion in relation to weed and crop growth stage and in relation to weather conditions. Some studies have reported that it is necessary to have more and deeper passes of harrowing to achieve similar weed control levels with harrowing as with the application of herbicide, but then crop damage can also be increased (Pardo et al., 2008). There are several factors that can be very important when harrowing: phenological stages of weed and crop, soil type and moisture, weather conditions and even the method of tillage (Bàrberi, 2002). In contrast with Bàrberi et al. (2000), who reported that the use of different types of tillage affected the effectiveness of weed harrowing, the lack of dependency between the type of tillage and weed control in this experiment may allow for future study and optimization of these practices separately.

In the Mediterranean region, the soil moisture and weather conditions can be key factors for a correct application of mechanical weeding without damaging crops (Pardo et al., 2008). Nevertheless, grain yields are more sensitive to year-to-year variations in temperatures and to the amount of rainfall during the cropping season than to other factors, and a shortage of water availability combined with high temperatures in spring can significantly decrease grain yield. Campliglia et al. (2015) reported the highest wheat yields when rainfall was homogeneously distributed throughout the wheat-cropping period.

Conclusions

This short-term experiment has shown that mouldboard ploughing increased cereal yields compared to reduced tillage, while simultaneously reducing both weed abundance and weed diversity in the cereal fields. Conversely, reduced tillage such as chisel ploughing can maintain weed abundance and diversity. No consistent pattern relative to the use of reduced tillage and mechanical weed control in dryland conventional fields has been found in previous studies, but our results suggest that care must be taken with weed infestation in reduced tillage, especially with perennial weeds, which can increase seriously over a single crop cycle. The use of herbicides dramatically decreases weed growth compared to mechanical weed control, but no effect of weed control was found during the second year of the experiment. In dryland areas, where crop yields are lower compared to template regions, the use of weed control methods should be case-specific to balance crop loss with the cost of the treatment and the potential damage to the crop due to the phytotoxicity of herbicides and the mechanical damage by harrows.

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References

Armengot, L., José-María, L., Chamorro, L., Sans, F.X., 2012. Weed harrowing in organically grown cereal crops avoids yield losses without reducing weed diversity. Agronomy for Sustainable Development. 33, 405–411. Doi:10.1007/s13593-012-0107-8

Bàrberi, P., 2002. Weed management in organic agriculture : are we addressing the right issues ? Weed Research. 42, 177–193. Doi:10.1046/j.1365-3180.2002.00277.x

Bàrberi, P., Silvestri, N., Peruzzi, A., Raffaelli, M., 2000. Finger-harrowing of durum wheat under different tillage systems. Biological agriculture & horticulture. 17, 285–303. Doi:10.1080/01448765.2000.9754850

Bates, D., Maechler, M., Bolker, B., 2011. lme4: Linear mixed-effects models using S4 classes

Berner, A., Hildermann, I., Fliesbach, A., Pfiffner, L., Niggli, U., Mäder, P., 2008. Crop yield and soil fertility response to reduced tillage under organic management. Soil and Tillage Research. 101, 89–96. Doi:10.1016/j.still.2008.07.012

Boutin, C., Jobin, B., Bélanger, L., Choinière, L., 2002. Plant diversity in three types of hedgerows adjacent to cropfields. Biodiversity & Conservation. 11, 1–25. Doi:10.1023/A:1014023326658

Bullied, W.J., Marginet, A.M., Van Acker, R.C., 2003. Conventional- and conservation-tillage systems influence emergence periodicity of annual weed species in canola. Weed Science. 51, 886–897. Doi:10.1614/P2002-117

Campiglia, E., Mancinelli, R., De Stefanis, E., Pucciarmati, S., Radicetti, E., 2015. The long-term effects of conventional and organic cropping systems, tillage managements and weather conditions on yield and grain quality of durum wheat (*Triticum durum* Desf.) in the Mediterranean environment of Central Italy. Feld Crop Research. 176, 34–44. Doi:10.1016/j.fcr.2015.02.021

Chamorro, L., Masalles, R.M., Sans, F.X., 2016. Arable weed decline in Northeast Spain: Does organic farming recover functional biodiversity? Agriculture, Ecosystems and Environment. 223, 1–9. Doi:10.1016/j.agee.2015.11.027

de Bolòs, O. de, Vigo, J., Masalles, R.M., Ninot, J.M., 2005. Flora Manual dels Països Catalans. Ed. Pòrtic. Barcelona

Dorado, J., López-Fando, C., 2006. The effect of tillage system and use of a paraplow on weed flora in a semiarid soil from central Spain. Weed Research. 46, 424–431. Doi: 10.1111/j.1365-3180.2006.00526.x

European Environment Agency, 2012. Soil erosion. Copenhagen, Denmark

Gruber, S., Claupein, W., 2009. Effect of tillage intensity on weed infestation in organic farming. Soil & Tillage Research. 105, 104–111. Doi:10.1016/j.still.2009.06.001

Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agriculture, Ecosystems and Environment. 103, 1–25. Doi:10.1016/j.agee.2003.12.018

IUSS Working Group WRB, 2015. World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106 FAO, Rome. Doi:10.1017/S0014479706394902

Kassam, A., Friedrich, T., Derpsch, R., Lahmar, R., Mrabet, R., Basch, G., González-Sánchez, E.J., Serraj, R., 2012. Conservation agriculture in the dry Mediterranean climate. Field Crop Research. 132, 7–17. Doi:10.1016/j.fcr.2012.02.023

Kuznetsova, A., Brockhoff, P.B., & Christensen, R.H.B., 2015. Package “lmerTest”. R package version, 2

Lahmar, R., Ruellan, A., 2007. Dégradation des sols et stratégies coopératives en Méditerranée: la pression sur les ressources naturelles et les stratégies de développement durable. Cahiers Agricultures. 16, 318–323. Doi:10.1684/agr.2007.0119

Légère, A., Stevenson, F.C., Ziadi, N., 2008. Contrasting Responses of Weed Communities and Crops to 12 Years of tillage and Fertilization Treatments. Weed Technology. 22, 309–317. Doi:10.1614/WT-07-124.1

Madarász, B., Juhos, K., Ruszkiczay-Rüdiger, Z., Benke, S., Jakab, G., Szalai, Z., 2016. Conservation tillage vs. conventional tillage: long-term effects on yields in continental, sub-humid Central Europe, Hungary. International Journal of Agricultural Sustainability. 14, 408–427. Doi:10.1080/14735903.2016.1150022

Marshall, E.J.P., Brown, V.K., Boatman, N.D., Lutman, P.J.W., Squire, G.R., Ward, L.K., 2003. The role of weeds in supporting biological diversity within crop fields. Weed Research. 43, 77–89. Doi:10.1046/j.1365-3180.2003.00326.x

Montgomery, D.R., 2007. Soil erosion and agricultural sustainability. Proc. Natl. Acad. Sci. 104, 13268–13272. Doi:10.1073/pnas.0611508104

Murphy, S.D., Clements, D.R., Belaoussoff, S., Kevan, P.G., Swanton, C.J., 2006. Promotion of weed species diversity and reduction of weed seedbanks with conservation tillage and crop rotation. Weed Science. 54, 69–77. Doi:10.1614/WS-04-125R1.1

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O’Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., others, 2013. Vegan. Community Ecology Package Project. Community ecology package, version2

Pardo, G., Cirujeda, A., Aibar, J., Cavero, J., Zaragoza, C., 2008. Weed harrowing in winter cereal under semi-arid conditions. Spanish Journal of Agricultural Research. 6, 661–670. Doi:10.5424/sjar/2008064-358

Peigné, J., Ball, B.C., Roger-Estrade, J., David, C., 2007. Is conservation tillage suitable for organic farming? A review. Soil Use and Management. 23, 129–144. Doi:10.1111/j.1475-2743.2006.00082.x

Peigné, J., Casagrande, M., Payet, V., David, C., Sans, F.X., Blanco-Moreno, J.M., Cooper, J., Gascoyne, K., Antichi, D., Bàrberi, P., Bigongiali, F., Surböck, A., Kranzler, A., Beeckman, A., Willekens, K., Luik, A., Matt, D., Grosse, M., Heß, J., Clerc, M., Dierauer, H., Mäder, P., 2015. How organic farmers practice conservation agriculture in Europe. Renewable Agriculture and Food Systems. 31, 1–14. Doi:10.1017/S1742170514000477

Plaza, E.H., Kozak, M., Navarrete, L., González-Andújar, J.L., 2011. Tillage system did not affect weed diversity in a 23-year experiment in Mediterranean dryland. Agriculture, Ecosystems and Environ. 140, 102–105. Doi:10.1016/j.agee.2010.11.016

R Development Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Romanyà, J., Rovira, P., 2011. An appraisal of soil organic C content in Mediterranean agricultural soils. Soil Use and Management. 27, 321–332. Doi:10.1111/j.1475-2743.2011.00346.x

Sans, F.X., Berner, A., Armengot, L., Mäder, P., 2011. Tillage effects on weed communities in an organic winter wheat-sunflower-spelt cropping sequence. Weed Research. 51, 413–421. Doi:10.1111/j.1365-3180.2011.00859.x

Soil Survey Staff, 1998. Keys to soil taxonomy, Soil Conservation Service, United States Department of Agriculture

Woźniak, A., Soroka, M., 2014. Effects of a 3-year reduced tillage on the yield and quality of grain and weed infestation of spring triticale (Triticosecale Wittmack). International Journal of Plant Production. 8. Doi:10.22069/ijpp.2014.15260

Capítulo II

Chapter II

Crop yield, weed infestation and soil fertility responses to contrasted ploughing intensity and manure additions in a Mediterranean organic crop rotation

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Efecto del sistema de laboreo y la aplicación de enmiendas orgánicas sobre el rendimiento de los cultivos, la flora arvense y la calidad del suelo en una rotación de cultivos orgánicos mediterráneos

Resumen

Los suelos son la base de la producción de alimentos y desempeñan un papel clave en los sistemas agrícolas, sin embargo, la mayoría de los suelos agrícolas son propensos a la degradación, debido principalmente al manejo intensivo del suelo. La agricultura de conservación y la agricultura ecológica son dos estrategias complementarias destinadas a mejorar la calidad del suelo y la fertilidad de los sistemas herbáceos. Las prácticas de la agricultura de conservación implican reducir la intensidad del laboreo, mantener la cubierta vegetal del suelo todo el año y diversificar e incrementar la rotación de cultivos. La agricultura ecológica se basa en el reciclaje de nutrientes, mediante la aportación de enmiendas orgánicas como estiércol y abonos verdes y la prohibición del uso de insumos externos (químicos). Los sistemas basados en el laboreo mínimo pueden conservar la fertilidad del suelo y prevenir la erosión, sin embargo, la mayor preocupación de los agricultores es el aumento de la abundancia de la flora arvense y la limitada disponibilidad de nitrógeno (N) especialmente, al comienzo del período de crecimiento del cultivo. Por lo tanto, la adecuada gestión de la flora arvense y la fertilización son esenciales para la producción de los cultivos y para mantener la reserva de carbono orgánico en el suelo (SOC). En este trabajo se estudiaron los efectos del tipo de laboreo (inversión de las capas de suelo con arado de vertedera vs. no inversión de las capas de suelo con arado de cincel); fertilización (con estiércol semicompostado vs, sin fertilización); y los abonos verdes (cultivo de cobertura incorporado en el suelo como abono verde vs sin abono verde) sobre los indicadores de calidad del suelo (SOC, N, densidad aparente y biomasa microbiana del suelo -C_{mic} y N_{mic}-), la abundancia de flora arvense y el rendimiento de los cultivos en una rotación de cuatro años de espelta, garbanzo, trigo de invierno y lentejas en la región mediterránea. De manera general, el tipo de laboreo del suelo y los abonos verdes no afectaron el rendimiento de los cultivos ni la biomasa de la flora arvense. Sin embargo, el arado de vertedera afecto

negativamente la biomasa del cultivo de lentejas y favoreció la abundancia de la flora arvense durante el último año de la rotación. La fertilización fue el factor más importante en este experimento debido a que aumentó el rendimiento de los cereales, el contenido de SOC, N y la biomasa microbiana del suelo (C_{mic} y N_{mic}). Sin embargo, la fertilización no favoreció los cultivos de garbanzo y lenteja a causa de la competencia de la flora arvense que limitó el crecimiento de estos cultivos. En general, el SOC y la reserva de carbono disminuyeron a lo largo de los cuatro años, mientras que el contenido de N aumentó en todas las parcelas, y de manera más acusada en las fertilizadas con estiércol. Los resultados indican que el uso del arado de cincel estratifica la distribución del SOC y del N a lo largo del perfil del suelo. El C_{mic} y, en consecuencia, la relación C_{mic}/SOC aumentó a causa de las formas lábiles del fertilizante aplicado que incrementó la descomposición de la materia orgánica. Sin embargo, la aplicación de materia orgánica más estable podría mejorar la calidad del suelo, el manteniendo de la materia orgánica y el rendimiento de los cultivos en sistemas ecológicos.

Crop yield, weed infestation and soil fertility responses to contrasted ploughing intensity and manure additions in a Mediterranean organic crop rotation

Abstract

Soils represent the basis of food production and play a key role in agricultural systems, however most arable soils are prone to degradation, mainly caused by intensive soil management. Conservation agriculture and organic farming are two alternative strategies aiming at improving soil quality and fertility in arable cropping systems. Conservation agriculture practices imply to reduce tillage intensity, maintain soil cover all year round and diversify crop rotations. Organic farming focus on nutrient recycling, using farmyard manure and green manures inputs and prohibiting the use of chemical inputs. Reduced tillage systems can conserve soil fertility and prevent erosion, but farmers have concerns of weed infestation and of limited nitrogen (N) availability mainly at the beginning of the growing season. Therefore, appropriate weed management and fertilization can be essential for a successful crop production, and can increase soil organic carbon (SOC) sequestration in the soil. In addition, maximizing soil cover and diversified crop rotations are essential components of sustainable systems design. We studied the effects of tillage type (soil layer inversion with mouldboard ploughing vs. no soil layer inversion with chisel ploughing); fertilization (with farmyard manure vs, no fertilization); and green manure (a mixed cover crop incorporated into the soil as green manure vs. no green manure) on soil parameters (SOC, N, bulk density, carbon stocks, and soil microbial biomass C_{mic} and N_{mic}), weed abundance and crop yields in a four year rotation of spelt, chickpea, winter wheat and lentil in the Mediterranean region (Catalonia, Spain). Tillage and green manures did not affect crop yields or weed biomass, although during the last year of the experiment, plots with mouldboard ploughing resulted in less weed biomass and higher lentil biomass. The fertilization was the most important factor, it increased cereal yields, SOC, N and soil microbial biomass (C_{mic} and N_{mic}) content of the soil. However, fertilization did not favour chickpea and lentil crops because weed competition limited legume crop growth. Overall, there was a loss of SOC and an overall reduction of carbon stocks over the four years of the trial in the soil. N content increased in all plots, and this was enhanced by fertilization. The use of chisel plough stratified the distribution of SOC and the N in the surface layers (0-10 cm). The C_{mic} and in consequence the C_{mic}/SOC ratio was also increased in fertilized treatments because the labile organic matter forms applied in manures enhanced organic matter decomposition. The application of more stabilized organic matter may be a better practice for the maintenance and enhancement of soil quality and the build-up of soil organic matter in the soil and the maintenance of crop yields in organic farming systems.

Keywords: chisel plough; carbon stock; amendments; microbial biomass; cover crop

Highlights:

- Reduced tillage can maintain similar cereal yields compared to conventional tillage

- Farmyard manure is the major driver of crop yields in dryland organic farming systems
- Farmyard manure increases cereal yields, but increases legume crop vulnerability to weed competition
- Soil organic carbon and soil microbial biomass increase with organic fertilization
- Reduced tillage causes stratification on the soil profile in relation to the soil fertility indicators
- The use of more stabilised manure inputs can maintain/enhance the build-up of soil organic matter

Introduction

Sustainable agriculture aims to maintain crop productivity over the long term, protecting the potential of natural resources and preventing degradation of soils and of water quality (Food and Agriculture Organization of the United Nations, 2013). Soils play a key role in agricultural systems since they represent the basis of food production (Fließbach et al., 2007). Despite soils are not renewable resources over a human timescale, most arable soils are prone to degradation, mainly caused by intensive soil use (Gadermaier et al., 2011). Almost 20 % of European soils are subject to degradation (European Environment Agency, 2012) and this has brought to attention the importance of preserving soil quality and fertility and preventing soil losses (Holland, 2004; Kassam et al., 2012).

Conservation agriculture and organic farming are two alternative strategies aiming at improving soil quality and fertility in arable cropping systems. While conservation agriculture relies on crop rotation, cover crops and reduced or no tillage, the practices of organic farming focus on nutrient recycling through the use of farmyard manures and green manures (organic fertilizers). Integrating conservation agriculture into organic farming would be an environmentally sound strategy, although, it is still not very common among organic farmers (Gadermaier et al., 2011).

Arable soils usually have low organic matter content (Fließbach et al., 2007, Romanyà and Rovira, 2011), since intensive soil tillage such as mouldboard ploughing with soil inversion can modify the soil structure and promote soil organic matter degradation leading to compaction and eventually to soil loss. Intensive soil tillage can also reduce substantially soil biodiversity and many of the soil-mediated ecosystem functions that provide and regulate environmental services (Holland, 2004; Montgomery, 2007). Evidence from long-term experiments suggests that soil organic matter losses can be reversed with soil management practices that minimize soil disturbance and optimize plant yield (Post et al., 2001). Soil management regimes based on no-till and reduced tillage are highly suited to conserve soil fertility and prevent erosion (Berner et al., 2008; Gadermaier et al., 2011). Some authors indicated that soil organic carbon (SOC) content, microbial activity and soil structure are often enhanced in the upper soil layer and that the soil fertility can be strongly improved after several years of reduced tillage (Mäder and Berner, 2011; Peigné et al., 2013).

However, there are major concerns about the adoption of no-tillage or reduced tillage in organic arable cropping systems such as the increase of weed infestation and the limited availability of N mainly at the beginning of the growing season (Gadermaier et al., 2011; Peigné et al., 2007; Sans et al., 2011). Moreover, organic matter increases in long term no-tillage experiments of the Mediterranean area have been shown to be rather low.

Non-inversion soil tillage, e.g. chisel plough, can concentrate weed seeds in the upper soil layer, increasing weed seedling emergence and in consequence increasing weed infestation (Gruber and Claupein, 2009; Shahzad et al., 2016). Weed infestation is one of the main concerns of farmers against implementing reduced tillage, which calls in for an appropriate weed management for a successful crop production (Armengot et al., 2012; Plaza et al., 2011). Mechanical weed control e.g. long flex tine harrow is an effective post-emergence weed control method (Armengot et al., 2012), although it has to be performed properly and timely in relation to weed and crop growth stage and in relation to weather conditions (Pardo et al., 2008).

Additionally, organic farms rely on nutrient transformation processes. Therefore, nitrogen inputs are crucial for their productivity (Fließbach et al., 2007). Nitrogen fertilization contributes to maintain soil nutrients that are depleted by crop production and thus sustain productivity, but it can also increase soil organic matter content (Alvarez, 2005). The use of organic fertilizers is considered an effective way of increasing SOC sequestration and supplying nutrients to crops (Krauss et al., 2010; Lal, 2009; Maltas et al., 2013). The management of nitrogen is also essential to optimize crop yield and quality in organic farming. This management must adapt to reduced tillage systems (Peigné et al., 2007), taking into account the effects of changes in soil conditions (e.g. soil water content, organic matter and temperature) and competition from weeds on the availability of nitrogen (N). However, there is little information on the dynamics of N after the introduction of conservation practices in organic arable cropping systems.

In addition to reduce tillage intensity and to fertilize adequately with composted manure, the maximum soil cover and diversified crop rotations are essential components of sustainable systems design (Cooper et al., 2016; Food and Agriculture Organization of the United Nations, 2013). The year-round soil cover aims at maximizing soil protection from extreme temperatures and minimizing soil erosion. However, in Mediterranean environments with hot and dry summer periods, maintaining ground cover can be difficult, as these periods are generally very restrictive for plant growth. An option to increase ground cover during non-productive periods is the introduction of cover crops during autumn and winter before spring cash crops. These crops are grown specifically to provide ground cover and provide protection to the soil during a period that is more favourable for plant development, while protecting from the effects of heavy rainfall that tend to occur during autumn and winter in the Mediterranean area (Ward et al., 2012). A cover crop contributes to the accumulation of organic matter in the upper soil layer, increasing soil fertility, especially when legume cover crops are used, which provides N through the N fixation (Hobbs et al., 2008). Furthermore, cover crops can smother weeds and reduce weed infestation through competition and can prevent weed seed germination through shadowing (Hobbs et al., 2008). Cover crops can also reduce the development of diseases and pests and incorporate nutrients remaining in the soil into a biological metabolism cycle (Masilionyte et al., 2017).

Contrasting results were found regarding the effect of cover crops on cash crop yields in organic cropping systems. Masilionyte et al. (2017) showed that cover crops had a strong competitive ability against weeds. However, Plaza-Bonilla (2016) suggested that cash crops had lower water use efficiency when cover crops were used; this result indicates that inserting cover crops must be accompanied by a careful redesign of the cropping system to compensate for the possible consequences of competition for nutrients and water.

Moreover, a balance between inputs and outputs of nutrients is critical to ensure short-term productivity and long-term sustainability in these systems. Suitable crop rotations containing legumes are being fundamental to produce surpluses in the N budgets (Gadermaier et al., 2011). Taking into account that the residue from cover crops, as a green manure, rich in legume species is often mineralised very fast and nutrients can be released before the demands of the subsequent cash crop (Pang and Letey, 2000), it may be necessary to fertilize the crop with farmyard manure.

The assessment of sustainability in organic systems requires studying the crop performance along the crop rotation in relation to soil fertility, and some key indicators of soil quality such as the SOC, the N content and the bulk density of the soil (Berner et al., 2008; Peigné et al., 2007). Furthermore, farming practices can cause changes in the carbon stocks of the soil, which is related to the SOC and bulk density and can be also a key indicator of soil quality. Several studies have shown that organic farming can enhance soil carbon stocks in the topsoil after several years if a suitable arable cropping system is designed, with organic matter recycling and forage legumes in the crop rotation and an adequate application of organic fertilizers (Cooper et al., 2016; Gattinger et al., 2012). Moreover, reducing tillage intensity in organic farming systems can increase the SOC in topsoil, improve soil physical and biological quality and could lead to reduced carbon losses or even increased soil carbon storage in the soil (Cooper et al., 2016; Gattinger et al., 2012).

In addition, soil microbiological activity is of primary importance in organic farming as nutrient supply is mainly dependent on the degradation of soil organic matter by soil micro-organisms (Vian et al., 2009). Soil microbial biomass can be a better indicator of changes in soil management at shorter term since soil microbial biomass changes are faster and greater compared to SOC and N (Fließbach et al., 2007).

Few experiments have been conducted, integrating conservation agriculture practices under organic farming systems and most of them were performed in temperate climates (Berner et al., 2008; Krauss et al., 2010; Peigné et al., 2007; Pekrun et al., 2003). Some authors have studied the application of conservation practices in conventional systems in Mediterranean climates (Kassam et al., 2012; López-Garrido et al., 2014; Ward et al., 2012), although there is a lack of long term studies of implementation of conservation practices in organic systems. The incorporation of these systems in the Mediterranean region is a challenge. The low organic matter content with poor soil aggregate structure and the climatic constraints such as the dry and hot summer with high evapotranspiration, which in consequence can cause water stress, limits plant growth during this season (Kassam et al., 2012; Romanyà and Rovira, 2011).

In 2011 a mid-term experiment was established aiming at testing the use of reduced tillage combined with the strategic use of farmyard and green manures in organic arable crop rotations. The objective of this paper is to study the effects of tillage type (soil layer inversion with mouldboard plough vs. no soil layer inversion with chisel plough); fertilization (with composted farmyard manure vs. no fertilization); and green manure (a cover crop incorporated into the soil as green manure vs. no green manure) on soil parameters (SOC, N, bulk density, carbon stocks, and soil microbial biomass C_{mic} and N_{mic}), weed abundance and crop yields in a four-year rotation of spelt, chickpea, winter wheat and lentil.

We hypothesized that a) reduced tillage will increase, or at least maintain, soil quality parameters such as SOC, N, bulk density; carbon stocks; b) fertilization with farmyard manures will increase crop yields; c) the use of green manures will reduce weed infestation; d) the fertilization and green manure will increase soil fertility and soil microbial biomass.

Materials and methods

Site conditions

In November of 2011, a midterm field experiment was established in Gallecs (41°33'31.9"N 2°11'59.5"E), a peri-urban agricultural area of 753 ha situated 15 km north of Barcelona (Catalonia, Spain). It has a Mediterranean climate; the mean annual temperature and precipitation are 14.9 °C and 647 mm, respectively. At the beginning of the experiment, soil properties of the field were evaluated. On average, the mineral fraction consisted of 43.3 ± 6.9 % sand, 26.9 ± 4.7 % loam and 29.7 ± 3.7 % clay; the texture was classified as loamy-clay (Soil Survey Staff, 1998); the soil type was a Haplic Luvisol (IUSS Working Group WRB, 2015), the average soil organic matter was 1.5 ± 0.1 % (Walkley-Black) and the pH (H_2O) was 8.1 ± 0.1.

Field experiment

The trial consisted of a four-year crop rotation in a strip-split-block design of three factors (with two levels each): tillage system (mouldboard ploughing (P) vs. chisel (C)) fertilization (composted farmyard manure (+F) vs. no fertilizer (-F)) and green manures (with green manures (+G) vs. no green manures (-G)). The factors were arranged with tillage treatments laid out in strips, fertilization was applied in perpendicular strips across the experiment, and the tillage strips were split into subplots for the green manure treatment. In total 32 plots measuring 13 m × 12 m were established, comprising four replicates of each treatment (Figure 1).

Two tillage systems were used: a mouldboard plough (P) (soil inversion at 25 cm depth) plus a rotary harrow (5 cm depth); and a chisel plough (C) (no soil inversion at 25 cm depth) plus a rotary harrow (same as for mouldboard plough) (Figure 3). The fertilization treatment (+F) consisted of partially composted farmyard manure, composed of cattle manure and plant residues, obtained without managing and controlling the process, by gradually accumulating the material that was seasonally available, according to the normal practice used in the area.

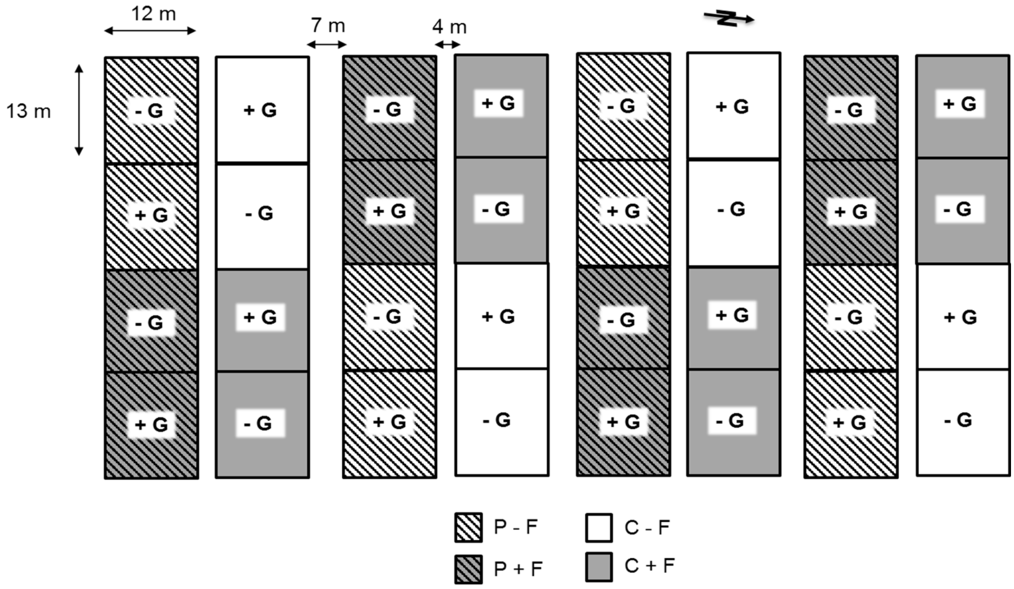

Figure 1. Experimental design in a strip-split-block with three factors of two levels each. P, mouldboard ploughing; C, chisel ploughing; F +, fertilization with farmyard manure, F -, not fertilized: G +, with green manure, G -, no green manure. Each treatment is replicated four times, summarizing a total of 32 plots.

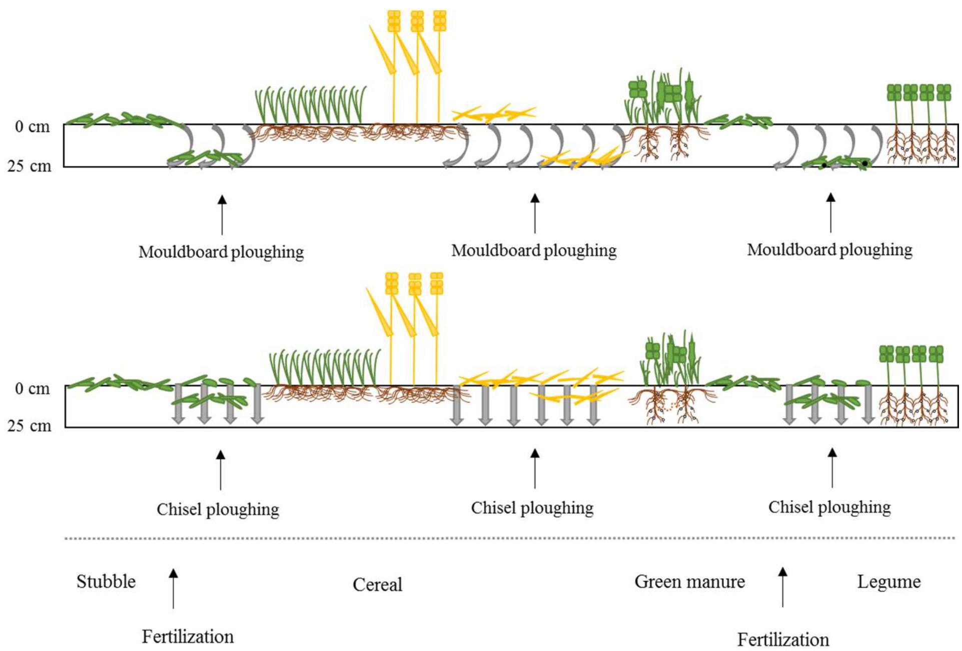

Figure 2. Cereal-legume crop rotation schemes of the experiment with two different tillage systems. The first scheme (top) represents the rotation with mouldboard ploughing (inversion of soil layers) and the second scheme (bottom) represents the rotation with chisel ploughing (no soil inversion). The schemes start from the stubble incorporation of the previous crop, followed by the fertilization (in the corresponding plots) and the soil tillage for the cereal crop season. After cereal harvest and the stubble incorporation, the cover crops are sown and later incorporated into the soil (in the corresponding plots), before the fertilization and tillage for the legume crop season.

It was applied every year before sowing the main crop. The total amount of manure applied differed in relation to the nutritional demands of each crop (Table 1). The organic fertilizers were mixed in the soil by means of a chisel or mouldboard plough in accordance with the tillage treatment. In September 2012 and 2014, cover crops (+G) were sown in the corresponding 16 plots. It consisted of a mixture of oat (*Avena sativa L.*), white mustard (*Sinapis alba* L.), bitter vetch (*Vicia ervilia* (L.) Willd.) and common vetch (*Vicia sativa* L.) (Table 1). At the end of March of the following year, cover crops were incorporated into the soil as green manures, by disc harrowing.

Weeds were not controlled during the first year of the crop rotation, due to an extremely prolonged rainy period that prevented the mechanical post-emergence weeding. In the second year of the rotation, weeds were controlled with an inter-row cultivator adapted to pass between the seeding rows of chickpea. The third year of the rotation, weeds were controlled with a flex-tine harrow during wheat crop season. Finally, the last year of the rotation, lentil established poorly because of drought, and were outcompeted by weeds despite the manual removal of individuals of lamb's quarters (*Chenopodium album*), which was the most important weed during lentils' growth (Table 1).

Weed and crop assessment

Crop density was evaluated every year once crop plants were well-established. The individuals were counted in a sample of 0.5 m long, comprising two crop lines in four replicates in each plot.

Before crop harvest, four permanent frames of 1 m × 1 m were randomly established, one in each quarter of the plot, to assess weed and crop aboveground biomass. The total aboveground biomass of weeds and crop were harvested in each 1 m × 1 m frame and was oven-dried at 60 °C for 48 h. Grain crop yield was assessed in the inner 9 m × 8 m of each plot by a plot combine each year (except for lentils) (Figure 3). The straw of the crops was not removed from the field; it was incorporated with the stubble into the soil by disc harrowing. The spelt straw was chopped by a hammer straw chopper before it was incorporated. Aboveground biomass of green manure and weeds were evaluated as well during the green manure period.

Table 1. Date of field operations, sowing characteristics, and fertilization inputs for each crop of the rotation. The type and brand of agricultural equipment are also indicated.

Year of rotation	1st	2nd	3rd	4th
Sowing	Spelt	Chickpea	Winter wheat	Lentil
Amazone D09- 30				
Date of sowing	December 14th, 2011	April 13th, 2013	December 16th, 2013	March 31st, 2015
Sowing density	195 kg ha^{-1}	30 kg ha^{-1}	220 kg ha^{-1}	180 kg ha^{-1}
Spacing between rows	12 cm	75 cm	12 cm	12 cm
Tillage				
Conventional tillage				
Mouldboard ploughing, depth 25 cm EG 85-240-8, Kverneland	December 12th, 2011	March 28th, 2013	December 10th, 2013	March 20th, 2015
Rotative, depth 5 cm HR3003D, Kuhn	December 14th, 2011	April 13th, 2013	December 16th, 2013	March 30th, 2015
Reduced tillage				
Chisel, depth 25 cm KCCC 1187 - A00, Kverneland	December 14th, 2011	March 28th, 2013	November 12th, 2013	March 20th, 2015
Rotative, depth 5 cm HR3003D, Kuhn	December 14th, 2011	April 13th, 2013	December 16th, 2013	March 30th, 2015
Fertilization				
Composted cow farmyard manure	December 12th, 2011	March 28th, 2013	November 12th, 2013	March 19th, 2015
N_t kg.ha^{-1}	134.60	40.04	138.28	62.36
Weed control	No control	May 30th, 2013	March 4th, 2014	June 2nd, 2015
Machinery for weed control		Inter-row cultivator	Flex-tine harrow Herse-6M, PICHON	Hand weeding
Harvest				
Plot combine Elite, Wintersteiger, Inc.	July, 12th 2012	July 31st, 2013	August 12th, 2014	-
Deuthz fhar				
Disc harrowing Norma RLBH 32, RAU	September 18th, 2012	October 26th, 2013	September 9th, 2014	September 20th, 2015
Green manure	October 17th, 2012	-	September 22th, 2014	-
Sowing density kg. ha^{-1}	45.8 oat 1.5 mustard 61 bitter vetch 39.7 common vetch	-	45.8 oat 1.5 mustard 61 bitter vetch 39.7 common vetch	-

Figure 3. Harvest by a plot combine.

Soil sampling and analyses of SOC, N, bulk density and carbon stocks

In November 2011 and 2015 the soil was studied at four depths: from 0 to 10 cm, from 10 to 20 cm, from 20 to 30 cm and from 30 to 40 cm. The first two depths were sampled in all plots, whereas the two deepest soil layers were sampled only in plots with farmyard manure and green manure with mouldboard ploughing and with chisel ploughing (P +F + GM and C + F + GM). To study soil bulk density, 3 soil cores of 6.2 cm diameter and 10 cm deep were extracted in each soil layer. Soil samples were oven-dried at 90-100 °C for 48 h. Soil bulk density was calculated according to the formula: Bulk density (g cm^3) = dry soil weight (g) / core volume (cm^3).

To study total soil organic carbon (SOC) and total nitrogen content (N), 20 soil cores of 2.5 cm of diameter were systematically extracted every 2 meters of distance in each plot. Each set of 20 cores extracted at each plot and depth constituted a sample. Soil samples were kept in plastic bags, properly labelled, in a fridge at 4 °C until analysis. Samples were air dried and sieved on a 2 mm mesh. A minimum amount of 50 g dried soil was prepared for SOC and N analysis and the rest was separated for the soil microbial analyses (see below section 2.5.). Total carbon and total nitrogen were analysed through dry combustion with a LECO© Truspec CHNS analyser (Bremner, 1996). The Walkley-Black procedure/ISO 14235 was finally chosen to indirectly estimate the soil organic carbon (SOC) due to the high proportion of carbonates.

Based on the soil bulk density and SOC, carbon stocks were calculated according to the formula (Lee et al., 2009): Soil carbon stock (g m^{-2}) = soil carbon content (mg g^{-1}) × depth of soil layer (m) × area (m^2) × bulk density (g cm^{-3}) × 10^6.

Soil microbial biomass analyses

All soil microbial analyses were carried out on moist soil samples adjusted to a water content corresponding to 40–50% of maximum water retention capacity. Soil microbial biomass (C_{mic} and N_{mic}) were estimated by chloroform fumigation extraction (CFE) in accordance with Vance et al. (1987). CFE was done in triplicate on 20 g (dry matter) subsamples that were extracted with 80 ml of a 0.5M K_2SO_4 solution. Total organic carbon (SOC) in soil extracts was determined by infrared spectrometry after combustion at 850ºC. Total nitrogen (N) was measured subsequently in the same sample by chemoluminescence. The soil microbial biomass was then calculated according to the formula (Joergensen, 1995): C_{mic} (µg g^{-1} oven dry soil) = EC/k_{EC}, where EC = (SOC in fumigated samples - SOC in control samples) and $k_{EC} = 0.45$ (Joergensen, 1996). N_{mic} (µg g^{-1} oven dry soil) = EN/k_{EN}, where EN = (N extracted from fumigated samples – N extracted from control samples) and k_{EN} = 0.40 (Joergensen and Mueller, 1996).

Statistical analyses

The individual and combined effects of the type of tillage (P vs. C), fertilization (+F vs. -F) and green manure (+G vs. -G) on crop yields (spelt, chickpea, winter wheat), lentil aboveground biomass and weed aboveground biomass were evaluated using linear mixed effects models. For spelt crop the factor green manure was not analysed because it was implemented after it. Tillage, fertilization and green manure were used as fixed factors and the block was introduced as a random factor. The normality of data was verified by the Shapiro-Wilk test and homoscedasticity was assessed using the Barlett's test. To meet the normality and homoscedasticity requirements, we used logarithmic or square root transformation on data when necessary. The same statistical procedure was followed to analyse the effects of tillage, fertilization, green manure and depth of the soil layers and the interaction between the factors on soil parameters: SOC, N, soil bulk density, carbon stocks, and soil microbial biomass (C_{mic} and N_{mic}). The changes on soil quality indicators over the 4-year rotation have been also studied, comparing soil samplings carried out twice during the experiment ($\Delta = t_f - t_i$). The first analysis was performed at the beginning of the trial, representing the initial status of the soil (t_i) and the second analysis was performed at the end of the experiment (t_f). All the analyses were performed in R version 3.2.2 (R Development Core Team and R Core Team, 2015) with the package lme4 (Bates et al., 2011) for linear mixed effects model fitting.

Results

Crop yields and weed biomass

No differences in the density (individuals/m^2) of the established crops were found between treatments in the first two years (spelt and chickpea), although the establishment of winter wheat and lentil differed according to the type of tillage and the presence or not of green manure the previous year. Wheat establishment was significantly higher in plots with mouldboard ploughing and no green manure compared to chisel (T (P vs C) × G (+G vs -G): estimate -1.24 ± 0.47, $p = 0.009$). More plants of lentil emerged in plots with no green manure in general, and in plots with green manure, crop emergence was significantly higher in plots with mouldboard ploughing ((T (P vs C) × G (+G vs -G): estimate 1.56 ± 0.55, $p = 0.04$).

In the four-year crop rotation of cereals and legumes, cereal yields were significantly higher compared to legumes (legumes vs cereals*:* estimate -0.16 ± 0.002, $p < 0.001$). The winter wheat crop had the highest yields (3200 ± 280.08 kg ha^{-1}) followed by spelt (2328 ± 100.51 kg ha^{-1}) and chickpea (384 ± 65.38 kg ha^{-1}). Lentil did not produce grain because extended drought dramatically affected both flowering and fruiting. Cereal yields were significantly higher in plots with fertilization; both the spelt and winter wheat yields were higher in plots with farmyard manure (Table 2 and Figure 4). Legumes did not follow the same trend, the chickpea yield and lentil biomass did not vary in relation to fertilization. Regarding the effects of the type of tillage and the incorporation of cover crops as green manure, crop yields did not vary significantly, with the exception of lentil biomass. The lentil biomass was significantly higher in plots using mouldboard ploughing (Table 2 and Figure 4).

The effect of tillage on aboveground weed biomass varied over time. While no significant differences were found in the first two crops in the rotation, the aboveground weed biomass was significantly lower in plots tilled with mouldboard ploughing than in those with chisel ploughing during winter wheat and lentil crop. The incorporation of the cover crop as green manure did not affect weed biomass during crops of winter wheat and chickpea. However, in the fourth year (during the lentil crop), weed biomass was significantly higher in plots where cover crops were incorporated into the soil prior to lentil seeding. No statistically significant interaction between factors were found, with the exception of a significant lower weed biomass in plots with fertilization and mouldboard ploughing in the spelt crop (Table 2).

Green manure biomass did not differ between treatments in 2013 nor 2015. The analysis of the effect of the green manure on weed abundance and on the crop yield of the subsequent crop reflects that cover crop was effective in controlling weeds during its growing season, but it did not last over the following year. The effect of green manures on the control of weed biomass was statistically significant (+G vs -G: $p < 0.001$ in 2013 and 2015).

The weed biomass was introduced in the models as a covariate to evaluate the effect of weeds on grain yields. Results showed that it did not affect spelt and winter wheat grain yield (slope for the effect of weed biomass on spelt yield: 1.60 ± 4.17, $p = 0.7$ and slope for the effect of weed biomass on winter wheat yield: -6.54 ± 26.99, $p = 0.8$). On the contrary, chickpea yield and lentil biomass resulted negatively affected by weed biomass (chickpea slope for the effect of weed biomass on chickpea yield: -3.03 ± 0.66, $p < 0.001$ and slope for the effect of weed biomass on lentil biomass: -0.43 ± 0.13, $p = 0.003$).

Changes in SOC and N during the four years of the experiment

Overall, SOC decreased significantly (t_f vs t_i: estimate -0.184 ± 0.033, $p < 0.001$) in all treatments over the 4-years rotation of the experiment, with the exception of plots with chisel plough and fertilization between 0 to 10 cm deep. On the contrary N content increased (t_f vs t_i: estimate 0.02391 ± 0.002, $p = 2.00E-16$) (Table 3 and Figure 5). The highest SOC losses occurred at superficial soil layers (0 to 10 cm) of those plots without fertilization. SOC decreases were significantly higher at deeper soil layers (10 to 20 cm) of plots with chisel plough (C) without inversion of soil layers than of plots with soil layers inversion using mouldboard ploughing (P) (Table 4). Although no significant interaction was found between the type of tillage and fertilization, our results showed that SOC content at 0 to 10 cm was maintained over the 4-years rotation in plots with chisel and fertilization (Table 3 and Figure 5).

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Regarding the changes in N content, the highest increases occurred in plots with fertilization (Table 3). The type of tillage also affected ΔN; plots with chisel ploughing had higher increase than plots with mouldboard ploughing (Table 4 and Figure 5). However, this significant increase in N content occurred at the top soil layer of plots with chisel and fertilization, as indicated by the significant interaction between fertilization, tillage and soil layer (Table 4). No significant differences were found on ΔSOC and ΔN over the 4-year rotation of the trial according to the presence of green manures.

Overall the C:N ratio of the soil decreased by 32% after the four years (t_f vs t_i: estimate -0.314 ± 0.032, $p < 0.001$) and there was a significant interaction between tillage and fertilization, indicating a higher C:N ratio in plots with fertilization and reduced tillage compared to plots with mouldboard ploughing, irrespective of the soil depth layer (Table 4).

Table 2. Results of the linear mixed effects models of the effect of fertilization (F: +, fertilization with farmyard manure; F: - ,not fertilized), tillage system (T:P:, mouldboard ploughing; T:C:, chisel ploughing), green manure (G: +, with green manure,; G: -, no green manure) and the interaction between factors (F, T and G) on the different variables measured (crop yields of spelt, chickpea and winter wheat, crop biomass of lentil, and weed biomass during each cropping season).

	F: + vs -	T: P vs C	G: + vs -	F (+ vs -) × T (P vs C)	F (+ vs -) × G (+ vs -)	T(P vs C) × G(+ vs -)	F × T × G
Crop yields (kg ha^{-1})							
Spelt	163.06 ± 38.25*	6.72 ± 58.29^{NS}	23.28 ± 22.48^{NS}	-26.44 ± 22.48^{NS}	-2.25 ± 22.48^{NS}	13.95 ± 22.48^{NS}	16.92 ± 22.48^{NS}
Chickpea	-61.47 ± 29.10^{NS}	-0.12 ± 30.46^{NS}	5.35 ± 15.76^{NS}	9.34 ± 15.76^{NS}	-29.57 ± 15.76^{NS}	-9.06 ± 15.76^{NS}	-1.06 ± 15.76^{NS}
Wheat	484.96 ± 121.61*	36.82 ± 120.04^{NS}	28.92 ± 68.36^{NS}	-45.32 ± 68.36^{NS}	-8.45 ± 68.36^{NS}	-4.80 ± 68.36^{NS}	-1.42 ± 68.36^{NS}
Lentil (biomass g m-^{2})	-7.33 ± 5.13^{NS}	12.251 ± 4.70*	2.47 ± 3.87^{NS}	-2.16 ± 3.87^{NS}	-4.18 ± 3.87^{NS}	5.29 ± 3.87^{NS}	-4.91 ± 3.87^{NS}
Weed biomass (g m-^{2})							
Spelt	-0.16 ± 0.04**	-0.26 ± 0.12^{NS}	-0.07 ± 0.04^{NS}	-0.24 ± 0.04***	0.001 ± 0.04^{NS}	-0.01 ± 0.04^{NS}	-0.05 ± 0.04^{NS}
Chickpea	12.46 ± 5.47^{NS}	-2.46 ± 7.53^{NS}	1.92 ± 3.40^{NS}	-2.77 ± 3.40^{NS}	3.67 ± 3.40^{NS}	1.09 ± 3.40^{NS}	2.19 ± 3.40^{NS}
Wheat	-0.31 ± 0.08*	-0.28 ± 0.08*	-0.03 ± 0.07^{NS}	0.11 ± 0.07^{NS}	0.10 ± 0.07^{NS}	0.03 ± 0.07^{NS}	-0.04 ± 0.07^{NS}
Lentil	32.45 ± 7.75**	-12.79 ± 4.25**	11.55 ± 4.25*	-6.88 ± 4.25^{NS}	6.98 ± 4.25^{NS}	-3.95 ± 4.25^{NS}	0.81 ± 4.25^{NS}

The values are estimated differences, standard errors and their significance levels, which are indicated according to the following codes: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, ^{NS} not significant.

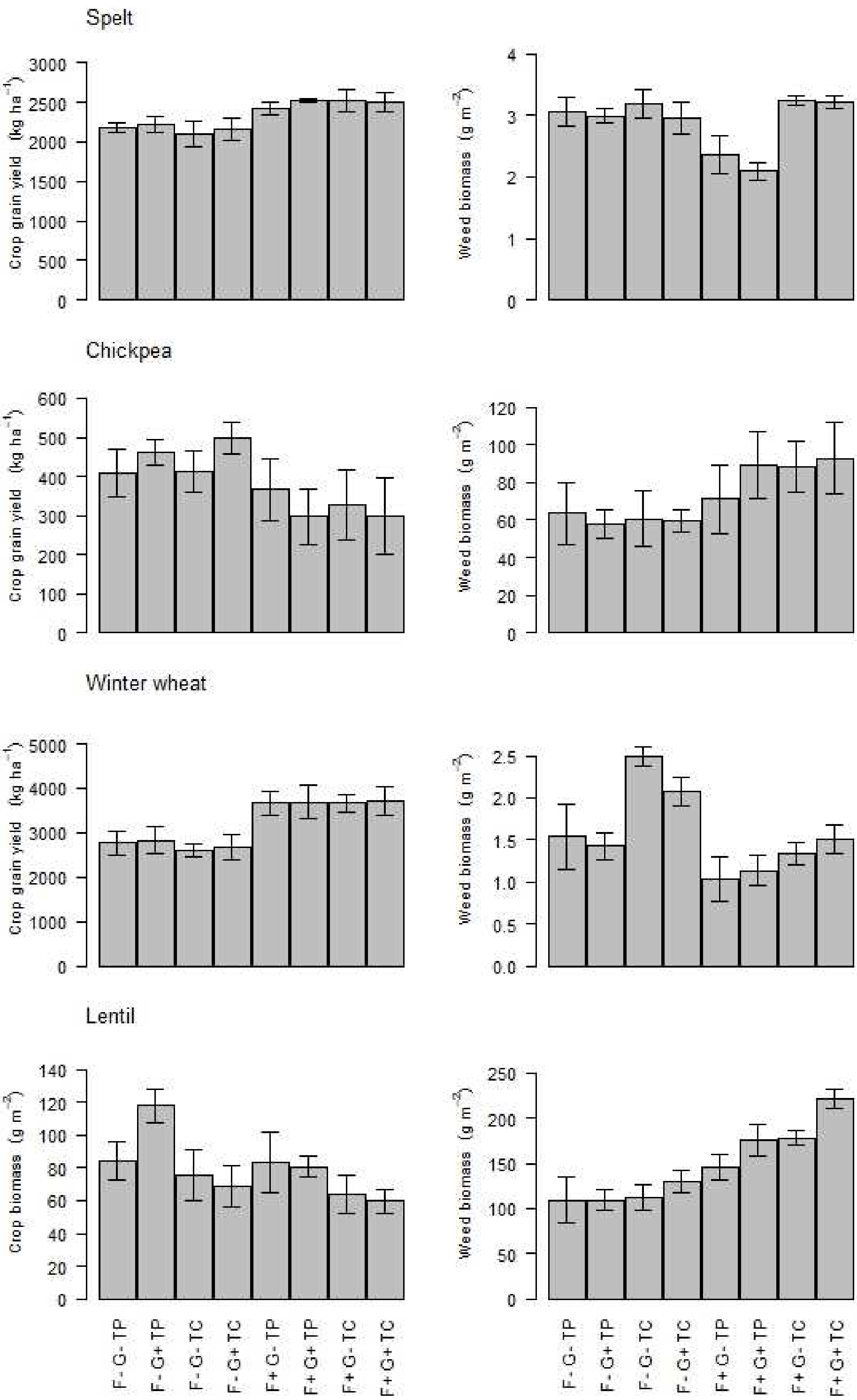

Figure 4. Mean (± SE standard error of differences) crop yields of spelt, chickpea, winter wheat and crop biomass of lentil (left) and the corresponding aboveground weed biomass (right) in each treatment: fertilization (F - , not fertilized; F +, fertilization with farmyard manure); green manure (G -, no green manure; G + sowed with green manure); tillage systems (T P, mouldboard ploughing; T C, chisel ploughing). Because lentil crop was not harvested, aboveground lentil biomass was evaluated. Note the different Y-axis scales of crops.

Table 3. Mean (± SE) of SOC and N at two soil depths (0 -10 cm and 10 – 20 cm) in each treatment: fertilization (F: +, fertilization with farmyard manure; F: -, not fertilized); tillage system (T: P, mouldboard ploughing; T: C, chisel ploughing); green manure (G: +, sowed with green manure; G: -, no green manure,) in the first and last year of the trial (t_i and t_f).

	0-10 cm		**10-20 cm**		**0-10cm**		**10-20 cm**	
	SOC t_i	**SOC** t_f	**SOC** t_i	**SOC** t_f	**N** t_i	**N** t_f	**N** t_i	**N** t_f
F: +	1.170 ± 0.090	1.118 ±0.06 0	1.110 ±0.090	0.970 ± 0.060	0.124 ± 0.005	0.163 ±0.006	0.113 ± 0.006	0.148 ± 0.006
F: -	1.150 ± 0.130	0.827 ± 0.060	1.090 ±0.120	0.800 ± 0.040	0.119 ± 0.007	0.127 ± 0.005	0.112 ± 0.005	0.123 ± 0.005
T: P	1.140 ± 0.100	0.904 ±0.036	1.090 ±0.100	0.900 ± 0.030	0.120 ± 0.005	0.139 ± 0.003	0.110 ± 0.005	0.137 ± 0.005
T: C	1.180 ± 0.120	1.042 ± 0.080	1.110 ±0.120	0.860 ± 0.070	0.121 ± 0.006	0.152 ± 0.008	0.114 ± 0.006	0.134 ± 0.006
G: +	1.180 ± 0.120	0.97 ± 0.060	1.120 ±0.120	0.880 ± 0.050	0.121 ± 0.006	0.144 ± 0.005	0.114 ± 0.006	0.136 ± 0.006
G: -	1.140 ± 0.100	0.975 ± 0.050	1.080 ±0.100	0.890 ± 0.040	0.122 ± 0.006	0.146 ± 0.006	0.110 ± 0.005	0.135 ± 0.005

Table 4. Results of the linear mixed effects models of the effect of the different experimental factors, plus depth and year and their interactions on the ΔSOC, ΔN, ΔC:N. F: +, fertilization with farmyard manure; F: -, not fertilized; T: P, mouldboard ploughing; T: C, chisel ploughing; G: +, sowed with green manure; G: -, no green manure.

	ΔSOC	**ΔN**	**ΔC:N**
F: + vs -	0.137 ± 0.075^{NS}	0.015 ± 0.002***	0.264 ± 0.675^{NS}
T: P vs C	-0.049 ± 0.060^{NS}	-0.008 ±0.001***	0.085 ± 0.579^{NS}
G: + vs -	-0.021 ± 0.020^{NS}	-0.001 ±0.001^{NS}	-0.169 ± 0.188^{NS}
F (+ vs -) × T (P vs C)	0.009 ± 0.020^{NS}	-0.006 ±0.001**	0.452 ± 0.188*
F (+ vs -) × G (+ vs -)	-0.007 ± 0.020^{NS}	-0.002 ±0.001^{NS}	0.082 ± 0.188^{NS}
T (P vs C) × G (+ vs -)	-0.022 ± 0.020^{NS}	-0.002 ±0.001 ^{NS}	0.007 ± 0.189^{NS}
Depth 10-20 vs 0-10 cm	-0.025 ±0.029^{NS}	-0.001 ±0.002^{NS}	-0.461 ± 0.267^{NS}
F (+ vs -) × depth 10-20 vs 0-10 cm	-0.060 ±0.029*	-0.003 ±0.002^{NS}	-0.297 ± 0.267^{NS}
T: P vs C × depth 10-20 vs 0-10 cm	0.084 ±0.029**	0.012 ±0.002***	-0.021 ± 0.267^{NS}
G: + vs – × depth 10-20 vs 0-10 cm 10-20 cm	-0.002 ±0.029^{NS}	-0.002 ±0.002^{NS}	0.180 ± 0.267^{NS}
F (+ vs -) × T (P vs C) × depth 10-20 vs 0-10 cm	0.0578 ±0.29^{NS}	0.006 ±0.002*	0.190 ± 0.267^{NS}

The values are estimated differences in marginal means, standard errors and their significance levels, which are indicated according to the following codes: *** p < 0.001, ** p < 0.01, * p < 0.05, NS not significant. ΔSOC and ΔN correspond to the differences between the t_f and the $t_{i.}$

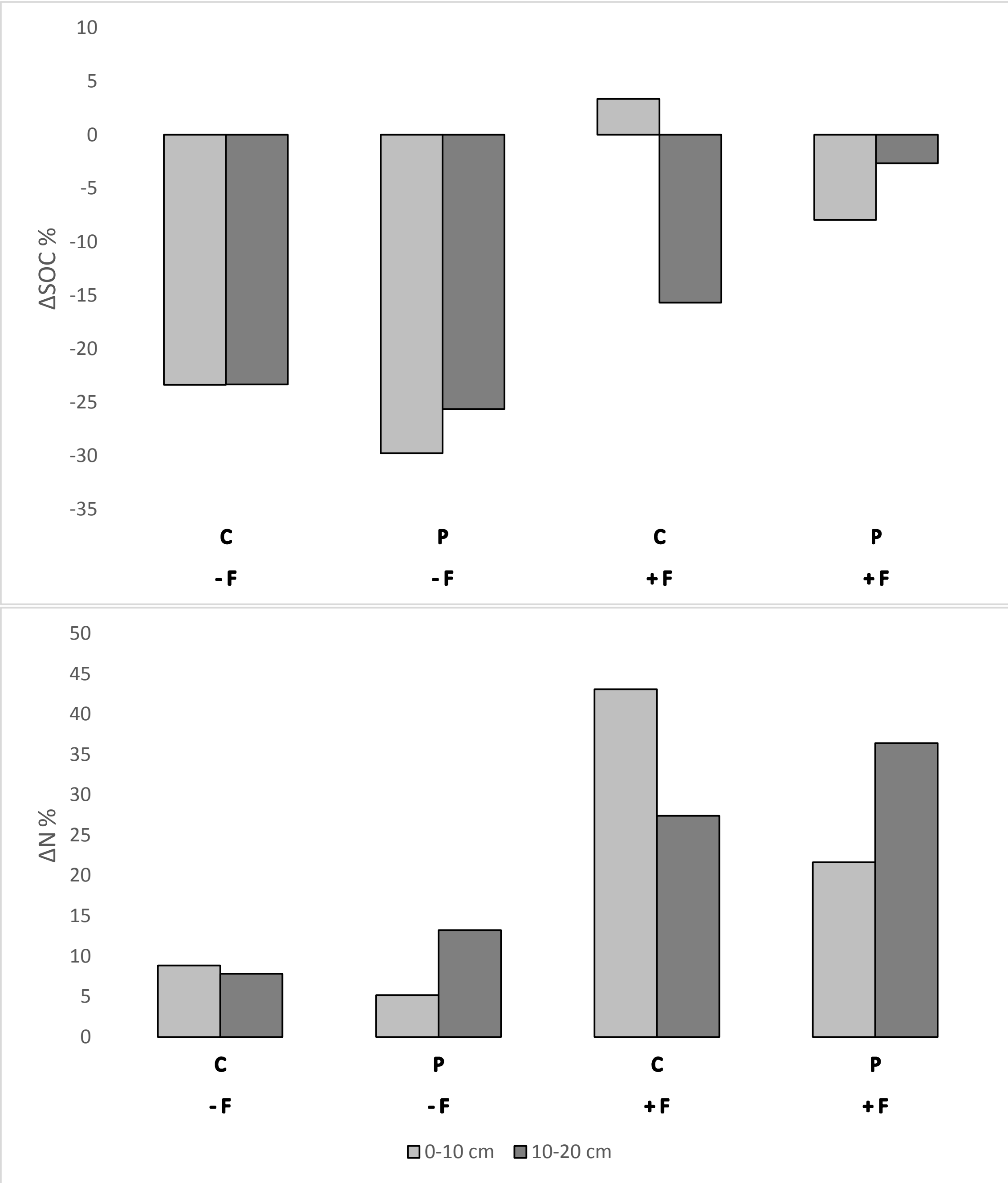

Figure 5 Changes in SOC and N contents, at two soil depths (0 to 10 cm and 10 to 20 cm), in plots under different treatments over the 4-year rotation of the experiment. Fertilization (F: -, not fertilized; F: +, fertilization with farmyard manure); tillage system T: (P, mouldboard ploughing; C, chisel ploughing). ΔSOC and ΔN represent the differences between the t_f and the t_i.

Bulk density and carbon stocks after four years of reduced tillage and organic inputs

After four years of the experiment, soil bulk density did not vary significantly in relation to the different experimental factors. Deeper soil layers had a higher bulk density than surface layers, but this pattern was not associated with the type of tillage neither to the organic fertilizer inputs such as composted farmyard manure and green manure (Table 5).
Carbon stocks, assessed from the SOC content and the soil bulk density of soil samples at different soil layers, were significantly higher in plots fertilized with composted farmyard manure. Moreover, they were higher at deeper soil layers from 10 to 20 cm (Table 5), although this is mainly associated with higher bulk density. Furthermore, there was a significant interaction with the type of tillage and green manures; thus, higher carbon stocks were detected in plots with chisel and green manure. The effect of the treatments at different soil layers showed some significant results as well. Carbon stocks were higher at deeper soil layers in plots with fertilization, and the plots with mouldboard ploughing presented lower carbon stocks at superficial soil layers (Table 5).
The diachronic analyses of carbon stocks over 4-year rotation at four different soil layers (0 to 10 cm, 10 to 20 cm, 20 to 30 cm and 30 to 40 cm) in relation to the tillage (P +F + GM and C + F + GM), reflects that carbon stocks were significant lower in deeper soil layers (soil layer 20 to 30 cm vs superficial soil layers: estimate -2270.18 ± 61, $p < 0.001$; and 30 to 40 cm vs. superficial soil layers: estimate -7490.42 ± 62, $p < 0.001$). Overall carbon stocks decreased after four years, irrespective of the soil layer (estimate -3295.06 ±1227.87, $p = 0.01$), and the negative effect of soil layer inversion using mouldboard ploughing was only statistically significant on the two upper soil layers (0 to 10 and 10 to 20 cm).

Changes in soil microbial biomass

Soil microbial biomass (assessed as the C_{mic} and N_{mic}) was significantly higher in plots with farmyard manure (Table 6 and 7). Furthermore, soil microbial biomass was lower at deeper soil layers and the significant interaction with fertilization reflects differences in C_{mic} and N_{mic} in fertilized and no fertilized plots (Table 7).

Table 5. Results of the linear mixed effects models of the effect of the different factors: fertilization (F: -, not fertilized; F: +, fertilization with farmyard manure); tillage system (T: P, mouldboard ploughing; T: C, chisel ploughing); green manure (G: -, no green manure, G: +, sowed with green manure); depth (1, 0 – 10 cm; 2, 10 – 20cm); and the interaction between them (F, T, G and depth) on soil bulk density and carbon stocks after the four years of trial (t_f).

	Bulk density (t_f)	**Carbon stocks (t_f)**
F: + vs -	-0.0038 ± 0.017^{NS}	1064.77 ± 295.35*
T: P vs C	-0.007 ± 0.015^{NS}	415.15 ± 334.56^{NS}
G: + vs -	-0.003 ± 0.015^{NS}	-149.21 ± 203.76^{NS}
F (+ vs -) × T (P vs C)	0.012 ± 0.015^{NS}	18.22 ± 203.76^{NS}
F (+ vs -) × G (+ vs -)	0.008 ± 0.015^{NS}	130.66 ± 203.76^{NS}
T (P vs C) × G (+ vs -)	0.003 ± 0.015^{NS}	597.26 ± 203.76**
Depth: 2 vs 1	0.259 ± 0.022***	1234.81 ± 269.12***
F (+ vs -) × depth 2 vs 1	-0.017 ± 0.022^{NS}	716.31 ± 269.12*
T: P vs C × depth 2 vs 1	0.021 ± 0.022^{NS}	-1287.24 ± 269.12***
G: + vs – × depth 2 vs 1	-0.010 ± 0.022^{NS}	96.99 ± 269.12^{NS}

The values are estimated differences in marginal means, standard errors and their significance levels, which are indicated according to the following codes: *** p < 0.001, ** p < 0.01, * p < 0.05, NS not significant.

Superficial soil layers showed greater differences between fertilized and no fertilized plots in C_{mic} and N_{mic}, compared to soil layers at 10 to 20 cm. Plots with mouldboard ploughing showed similar C_{mic} at 0-10 cm depth and at 10 to 20 cm depth, conversely plots with chisel showed significant higher microbial biomass at superficial soil layers compared to the deeper ones (Table 6 and Figure 6). The highest C_{mic} resulted in superficial layers (0 to 10 cm) in plots with farmyard manure and chisel ploughing (Figure 6). N_{mic} did not vary significantly between soil layers in interaction with tillage, and C_{mic} and N_{mic} were not significantly affected by the presence or not of green manure (Table 6 and 7).

The comparison of C_{mic} and N_{mic} between superficial and deeper soil layers in relation to the tillage (plots P +F + GM vs. plots C + F + GM) reflects that both C_{mic} and N_{mic} were lower in deeper soil layers (20 to 30 cm C_{mic}: estimate -107.57 ± 7.88, p<0.001, N_{mic}: estimate -1.55 ± 0.43, p<0.001 and 30 to 40 cm C_{mic}: estimate -224.66 ± 7.70, p<0.001, N_{mic}: estimate -1.95 ± 0.46, p<0.001), although no significant differences were found in relation to tillage (data not shown).

Table 6. Mean (± SE) of soil microbial biomass (C_{mic} and N_{mic}) and the ratio (C_{mic}/SOC) in the last year of the trial (t_f) at two soil depths (1: 0 -10 cm and 2: 10 – 20 cm) in each treatment: fertilization (F: +, fertilization with farmyard manure; F: -, not fertilized); tillage system (T: P, mouldboard ploughing; T: C chisel ploughing); green manure (G: +, sown with green manure; G: - no green manure).

System	**C_{mic} (µ g^{-1}) (t_f)**		**N_{mic} (µ g^{-1}) (t_f)**		**C_{mic}/SOC (%)(t_f)**	
Depth	**1**	**2**	**1**	**2**	**1**	**2**
F: +	297.13 ± 15.66	250.48 ± 11.66	31.81 ± 7.00	25.27 ± 4.13	2.64 ± 0.31	2.57 ± 0.36
F: -	234.73 ± 13.59	210.48 ± 12.65	19.70 ± 3.51	24.37 ± 4.75	2.88 ± 0.41	2.60 ± 0.24
T: P	243.34 ±12.93	234.41 ± 11.00	26.37 ± 4.62	27.60 ± 4.86	2.72 ± 0.31	2.61 ± 0.26
T: C	288.53 ± 16.31	226.56 ± 13.31	25.14 ± 5.89	22.04 ± 4.02	2.80 ± 0.41	2.56 ± 0.33
G: +	269.04 ± 14.50	231.69 ± 11.85	26.33 ± 5.85	25.44 ± 5.66	2.80 ± 0.39	2.61 ± 0.32
G: -	262.82 ± 14.74	229.27 ± 12.45	25.18 ± 4.65	24.19 ± 3.22	2.72 ± 0.34	2.55 ± 0.28

Table 7. Results of the linear mixed effects models of the effect of the different factors: fertilization (F +, fertilization with farmyard manure; F: -, not fertilized); tillage system (T: P, mouldboard ploughing; T: C, chisel ploughing); green manure (G: +, sown with green manure; G: - no green manure; depth (1, 0 – 10 cm; 2, 10 – 20cm); and the interaction between them (F, T, G, and depth) on C_{mic}, N_{mic} and C_{mic}/SOC in the last year of the trial (t_f).

System	**C_{mic} (t_f)**	**N_{mic} (t_f)**	**C_{mic}/SOC (t_f)**
F: + vs -	30.77 ± 3.56***	5.91 ± 2.71*	-0.10 ± 0.21^{NS}
T: P vs C	-21.66 ± 14.93^{NS}	0.74 ± 2.25^{NS}	-0.07 ± 0.05^{NS}
G: + vs -	3.02 ± 4.02^{NS}	0.58 ± 2.25^{NS}	0.03 ± 0.03^{NS}
Depth: 2 vs 1	-35.69 ± 3.77***	-1.06 ± 2.05^{NS}	-0.15 ± 0.07*
F: + vs - × depth: 2 vs 1	-11.57 ± 3.78**	-5.04 ± 2.05*	0.11 ± 0.07^{NS}
T: P vs C × depth: 2 vs 1	26.38 ± 3.78***	1.40 ± 2.04^{NS}	0.07 ± 0.07^{NS}
F: + vs - × T: P vs C	-3.13 ± 3.42^{NS}	-1.41 ± 2.25^{NS}	0.11 ± 0.05*
F: + vs - × T: P vs C × depth: 2 vs 1	-6.04 ± 6.23^{NS}	-0.25 ± 2.05^{NS}	-0.27 ± 0.07***

Significance levels are indicated according to the following codes: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ^{NS} not significant. Interactions with the factor green manures were not significant.

The differences in C_{mic} and N_{mic} between the first and last year of the trial (ΔC_{mic} and ΔN_{mic}) did not vary in relation to the individual factors (tillage, fertilization and green manures). C_{mic} increased overall after the four years of the trial (t_f vs t_i: estimate 21.43 ± 6.27, p<0.001) but this result was due to the plots with fertilization. Significant interactions were found between fertilization, tillage and soil depth, indicating higher increases of C_{mic} in plots with chisel plough (T: P vs C: estimate -33.95 ± 6.30, p<0.001) and fertilization (F: + vs -: estimate 37.09 ± 6.25, p<0.001) at 0 to 10 cm and the opposite at 10 to 20 cm depth (depth: 2 vs 1: estimate -30.32 ± 6.42, p<0.001). In contrast, plots with mouldboard ploughing did not show significant changes on C_{mic} at different soil depths. The N_{mic} decreased, in general, in all plots after the four years of the trial (t_f vs t_i: estimate -1.97 ± 0.30, p<0.001), but the highest losses of N_{mic} were at superficial soil layers (depth 2 vs 1: estimate -1.03 ± 0.30, p<0.001). Also, there was a significant interaction between the year and the type of tillage, indicating lower N_{mic} values in the last year in plots with chisel (T: P vs C: estimate 0.69 ± 0.3, p = 0.02), this was associated to the superficial layers, although not significant interactions were found (data not shown).

The C_{mic}/SOC ratio increased in all plots after the four years of experimentation (t_f vs t_i: estimate - 0.91 ± 0.14, p<0.001). Furthermore, the C_{mic}/SOC after the four years of the experiment varied significantly with soil depth and this factor also interact significantly with the fertilization and the type of tillage (Table 7). We found the highest ratio at the superficial layers in plots with chisel plough and no fertilization compared to plots with chisel plough and no fertilization at deeper soil layers (Table 7 and Figure 6).

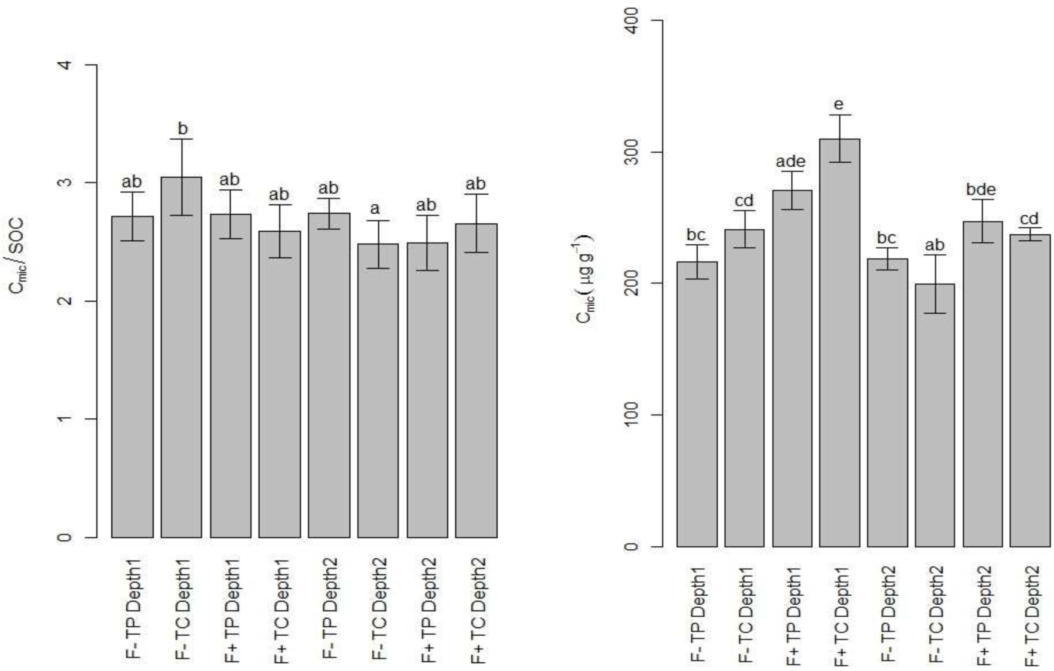

Figure 6. Soil microbial biomass C_{mic} (left) and C_{mic}/SOC ratio (right) after four years of the trial (t_f) in the different treatments: fertilization (F + fertilization with farmyard manure, F: - not fertilized); tillage system (T: P mouldboard ploughing, T: C chisel ploughing); green manure (G: + sowed with green manure, G: - no green manure); depth (1: 0 – 10 cm, 2: 10 – 20cm). Bars with no letters in common are significantly different (Tukey HSD test, P < 0.05).

Discussion

Crop yields and weed biomass

The study of crop yields and weed biomass over four years in relation to fertilization (with farmyard manure vs. without it), tillage (reduced vs. conventional) and green manure (cover crops rich in legume species incorporated into the soil before seeding legume crop vs without them) reveals that fertilization is the most important factor affecting crop yields, particularly during the cereal cropping period. Organic systems rely upon the use of organic fertilizers and amendments that typically release nutrients (especially N) at a slower rate compared with mineral fertilizers (Bàrberi, 2002). The significant enhancement of spelt and winter wheat grain yields with the farmyard manure highlights that fertilization is crucial to maintain an adequate production. Nitrogen inputs are critical to the productivity of these systems and the application of farmyard manure seems to be effective to maintain cereal yields and the sustainability of these systems (Fließbach et al., 2007; Maltas et al., 2013). Conversely, grain yield of chickpea and aboveground biomass of lentil were not increased by fertilization. This result could be explained by the fact that, in general, legumes do not need supplemental N fertilization (Clayton et al., 2003) because they can obtain a significant proportion of its N by symbiotic nitrogen fixation (Walley et al., 2005).

The type of tillage had no significant effects on grain yields of cereals (spelt and winter wheat) and chickpea, although many studies from temperate regions reveal lower crop yield in systems with no soil layer inversion by chiselling (Cooper et al., 2016) because of a combination of a shortage of nutrients and the competition by weeds (Mäder and Berner, 2011; Peigné et al., 2013). The availability of nutrients for crops did not seem to be affected by reduced tillage in our trial. Recently, López-Garrido et al. (2014) also obtained similar yields in reduced and conventional tillage under Mediterranean conditions. The lower biomass of lentil in plots with reduced tillage can be explained by the higher weed biomass under these conditions and the shortage of water which could have affected lentil growth. As Peigné et al. (2013) pointed out the success of reduced tillage in organic farming depends on crop management, soil type, and climatic conditions. The fact that some long-term studies showed better results with reduced tillage is because of more experience in managing the system and better soil structure after some years (Tørresen et al., 2003). Krauss et al. (2010) found that long-term effects of reduced tillage positively influenced crop performance; reduced tillage can improve soil structure, retains soil moisture and reduces water stress under dry conditions and in consequence enhances crop yields.

The positive effect of fertilization and of mouldboard ploughing in controlling weeds in spelt and winter wheat highlights the importance of both factors in enhancing the competitive ability of crops. Weed abundance did not affect significantly spelt and winter wheat grain yield, reflecting that the crop was able to suppress the growth of weeds to a point where their effect on crop growth is negligible. Despite, no post-emergence weed control was carried out during the spelt crop, the weed abundance was low, indicating the higher competitiveness of spelt against weeds. On the contrary, when weed biomass was introduced as a covariate on chickpea yields and lentil crop biomass, it resulted statistically significant reflecting that weeds competed with legume crops. The growth of weeds was significantly enhanced by fertilization during legume crops and, consequently, it significantly reduced the growth of chickpea and lentil. Some studies indicated that lentil is very vulnerable to weed competition because of its short stature, slow establishment, and limited vegetative growth (Ahmadi et al., 2016). Additionally, the high amount of weed biomass in chickpea and lentil irrespective of the treatment can be related to weakness of post-emergence control. Our results indicate that improving the weed management in chickpea and lentil crop is critical to the feasibility of such crops under the organic farming because of their high susceptibility to weed competition. In this study the weed control effect of the mouldboard ploughing over chisel started to be noticed at the end of the experiment, coinciding with higher lentil crop biomass in plots with mouldboard ploughing.

The analysis of the effect of the cover crop on weed abundance and on the crop yield of the subsequent crop reflects that cover crop was effective in controlling weeds during its growing season, but it did not last over the following year. Moreover, weed abundance was enhanced in plots with green manure during the lentil crop. This negative effect could be explained by the surplus fertilization coupled with the extremely weak growth of lentil related to drought, which dramatically reduced the competitive ability of the crop. While we expected a positive or neutral effect of cover crops on the control of weed abundance during the subsequent crop, our results highlights that inserting cover crops must be carefully evaluated to compensate for the possible consequences of competition for nutrients and water over the drought years (Plaza-Bonilla et al., 2016).

How did SOC, carbon stocks and N change in the experiment?

The amount of SOC stored in the soil is determined by the balance of two biotic processes: production of organic matter by plants and decomposition of organic matter by soil organisms. Each of these processes is strongly controlled by physical, chemical, and biological factors (Guo and Gifford, 2002). In organic arable cropping systems the intensity of soil disturbance, the farmyard manure and green manure fertilization are overriding factors of the amounts of SOC and N and of the pattern of distribution along the soil profile. Some authors have indicated that SOC is enhanced by farming practices from conservation agriculture after several years (Mäder and Berner, 2011; Peigné et al., 2013); however, other studies were unable to demonstrate such a positive effect (Berner et al., 2008) but this result was found over a short term experiment. Overall, our study shows that the implementation of soil tillage at 25 cm depth, whether with mouldboard plough or with a chisel plough in Mediterranean low input farming systems, may reduce SOC content and in consequence soil carbon stocks.

Furthermore, our results are in accordance with other studies which indicate that reduced tillage can cause stratification on SOC content (Berner et al., 2008) and that the amount and management of aboveground crop residues can affect the degree of stratification. The amount of SOC was significantly higher in the upper soil layers in plots ploughed with chisel, and especially in fertilized plots. The use of reduced tillage stratified SOC and in consequence soil carbon stocks (Hernanz et al., 2002; Cooper et al., 2016). Gadermaier et al. (2011) showed that SOC, microbial biomass and soluble soil nutrients after six years of rotation resulted in a stratification under reduced tillage, as in our experiment. On the contrary, in plots with mouldboard ploughing SOC was significantly lower in the topsoil and higher at deeper soil layers. Furthermore, it is important to point out that soil inversion can reduce carbon stocks in the topsoil, and this process is aggravated by the lack of fertilization.

Crop productivity is one of the main drivers of carbon stocks in arable systems. Both carbon stocks and crop productivity may be enhanced by crop fertilization practices (Johnston et al., 2009). Our results showed that a suitable fertilization is crucial to maintain SOC level and carbon stocks in low productivity organic systems. However, in our crop rotations, organic fertilization increased crop productivity only in cereal crops suggesting nutrient limitation for crop productivity in our soils. However, these positive effects of fertilization may be offset by decreased productivity in legume crops which are typically much less productive than that of cereals. Moreover, it is also noticeable the low productivity of these systems in which e.g. cereal grain yield even in fertilized plots is less than half than that of conventional systems in the region (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, 2009). Thus, plant productivity in our rotation was in all cases much lower that of neighbouring cereal monocultures. This may explain SOC losses throughout the soil profile that may be partly compensated by the addition of farmyard manure. It is interesting to note that such losses were lower in plots with chisel compared to in plots with mouldboard ploughing. Alvarez (2005) reviewed several experiments on conservation tillage and N additions under different climatic conditions and reported that the increases in SOC are produced when nitrogen fertilizer and conservation tillage are used for long periods (mora than 6 years). Other authors have pointed out that the use of organic amendments does not always contribute to SOC accrual. The use of fresh, unstabilized materials may induce the mineralization of native SOC and thus decrease SOC and carbon stocks (Molina-Herrera and Romanyà, 2015; Romanyà et al., 2012). Indeed, organic matter that has already undergone some level of decomposition such as the composted farmyard manure is more efficient in building up SOC in the soil than fresh organic matter (Romanyà et al., 2012).

Although cover crops can increase soil organic matter when they are incorporated into the soil as green manure, Masilionyte et al. (2017) found a significant decrease in SOC when legume cover crops are introduced in the rotation. This pattern suggests that the incorporation of crop residues with low C:N ratio into the soil can accelerate SOC decomposition. However, other studies show positive responses on SOC levels in response to legumes (Beedy et al., 2010). The lack of response of green manure in our experiment may have been due to the general low plant productivity in the experimental area.

N content was clearly enhanced after the 4-year rotation in all plots. Our results show that N increase was significantly higher with the incorporation of farmyard manure, and the highest increase occurred in plots with reduced tillage. Increased N levels after adding manures have also been reported by other authors. On the contrary, we did not find any significant effect of the incorporation of green manures on the increase of the N, reflecting that the effect of the inputs of farmyard manure was more important. Slight increases of N amount in unfertilized plots can be attributed to N fixation and or to atmospheric deposition (Pang and Letey, 2000).

Changes in soil microbial biomass and N availability

Reduced tillage causes a stratification of soil microbial biomass along the soil profile, and this is in accordance with the total SOC content. According with previous studies our results on microbial biomass (C_{mic} and N_{mic}) indicate that shifting from conventional to reduced tillage modifies crop residue distribution in the soil profile and environmental conditions for soil micro-organisms, and in consequence the distribution of the soil microbial life in the soil profile. In arable systems managed with no tillage and reduced tillage, C_{mic} tend to be greater in the upper layers, and decrease with depth, while it is more homogeneously distributed with traditional mouldboard ploughing (Vian et al., 2009). Vian et al. (2009) indicate that during the transition period from ploughing with soil layer inversion to no soil layer inversion, the soil structure, the SOC and the soil microbial biomass distribution within the 0-30 cm soil layer gradually change before reaching a new equilibrium. This transition generally lasts for up three years depending on the parent material, soil texture and climate. During this period a decrease in the turnover rate of the SOC and increased immobilization of nitrogen can occur, sometimes resulting in deficiencies of crop nutrients (Pekrun et al., 2003; Vian et al., 2009).

In our experiment the increase of C_{mic} after four years of trial can be mainly explained by the addition of manures. The addition of labile sources of SOC promotes the soil microbial activity and in consequence the increase of microbial biomass (Molina-Herrera and Romanyà, 2015; Fliessbach and Mader, 2000). Reduced tillage have been also found to increase microbial biomass in surface soils although its effects has been found to be much stronger when combined with fertilization. These increases however have not been related to increases in N availability. Indeed, some studies in temperate climates reported a decrease of N availability for the crop under reduced tillage, due to lower mineralization rates (Berner et al., 2008; Peigné et al., 2007).

The overtime decrease of N_{mic} coincided with a 32% decrease in the C:N ratio. Low C:N ratio indicates an increased degree of humification (Bayer et al., 2002). Humified organic matter strongly holds N in highly recalcitrant forms and is thus unavailable to soil microbial. In our experiment low N availability was indicated by the decreased Nmic. N mobilization in low available N soils may involve destabilization of soil organic matter and its subsequent mineralization (Clarholm et al., 2015). Thus, increased mineralization of stabilized soil organic matter in low N soil environments can increase N with crop rotations and green manures (Sun et al., 2016).

C_{mic}/SOC ratio can indicate that soil microbial efficiency of conversion of organic matter to microbial biomass and the stabilization of SOC by the soil mineral fractions (Sparling, 1992). In our mid-term trial, the loss of SOC in all plots coincides with the increase of the C_{mic}/SOC ratio indicating that the microorganisms are integrating a greater proportion of soil organic matter.

Conclusions

Farmyard manure is the main factor affecting crop yields and weed biomass, as well as governing soil fertility and quality. The organic fertilization is crucial to sustain cereal yields, but it can also exert a negative effect on legume crops, by increasing the competitive effects of weeds. Although farmers are concerned that reduced tillage could reduce the already low crop yields under organic farming by increased weed pressure and delayed nutrient mineralization, we have found that the concerns are unfounded. The tillage system does not have a consistent negative effect on yields, and the increased weed control of mouldboard plough does only take place on a mid-to-long term. The implementation of green manures in dryland areas requires a careful redesign of the cropping system. Although they could alleviate some fertility and weed control issues, we have not found positive effects on crop yields.

In the Mediterranean region of Spain, soils have low fertility and the fertilization might not have been enough to maintain SOC content. However, the application of more stabilized organic matter may be a better practice for the maintenance and enhancement of soil quality and the build-up of soil organic matter in the soil.

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References

Ahmadi, A.R., Shahbazi, S., Diyanat, M., 2016. Efficacy of Five Herbicides for Weed Control in Rain-Fed Lentil (Lens culinaris Medik.). Weed Technology. 30, 448–455. Doi: 10.1614/WT-D-15-00125.1

Angers, D.A., Chantigny, M.H., MacDonald, J.D., Rochette, P., Cote, D., 2010. Differential retention of carbon, nitrogen and phosphorus in grassland soil profiles with long-term manure application. Nutrient Cycling in Agroecosystems 86, 225–229. Doi:10.1007/s10705-009-9286-3

Alvarez, R., 2005. A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. Soil Use Management. 21, 38–52. Doi:10.1079/SUM2005291

Armengot, L., José-María, L., Chamorro, L., Sans, F.X., 2012. Weed harrowing in organically grown cereal crops avoids yield losses without reducing weed diversity. Agronomy for Sustainable Development. 33, 405–411. Doi:10.1007/s13593-012-0107-8

Bàrberi, P., 2002. Weed management in organic agriculture : are we addressing the right issues ? Weed Research. 42, 177–193. Doi:10.1046/j.1365-3180.2002.00277.x

Bates, D., Maechler, M., Bolker, B., 2011. lme4: Linear mixed-effects models using S4 classes

Bayer, C., Mielniczuk, J., Martin-Neto, L., Ernani, P. R. 2002. Stocks and humification degree of organic matter fractions as affected by no-tillage on a subtropical soil. Plant and soil, 238(1), 133-140. doi:10.1023/A:1014284329618

Berner, A., Hildermann, I., Fliesbach, A., Pfiffner, L., Niggli, U., Mäder, P., 2008. Crop yield and soil fertility response to reduced tillage under organic management. Soil & Tillage Research. 101, 89–96. Doi:10.1016/j.still.2008.07.012

Beedy, T.L., Snapp, S.S., Akinnifesi, F.K., Sileshi, G.W., 2010. Impact of *Gliricidia sepium* intercropping on soil organic matter fractions in a maize-based cropping system. Agriculture, Ecosystems & Environment 138, 139–146. Doi:10.1016/j.agee.2010.04.008

Bhogal, a., Nicholson, F. a., Chambers, B.J., 2009. Organic carbon additions: effects on soil bio-physical and physico-chemical properties. European Journal of Soil Science 60, 276–286. Doi:10.1111/j.1365-2389.2008.01105.x

Bremner, J.M., 1996. Nitrogen-total. Methods Soil Anal. Part 3 - Chemical Methods 1085–1121

Clarholm, M., Skyllberg, U., & Rosling, A. 2015. Organic acid induced release of nutrients from metal-stabilized soil organic matter - The unbutton model. Soil Biology and Biochemistry, 84, 168–176. Doi:10.1016/j.soilbio.2015.02.019

Clayton, G., Rice, W.A., Lupwayi, N.Z., Johnston, A.M., Lafond, G.P., Grant, C.A., Walley, F., 2003. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N2 fixation and nitrogen partitioning 84, 79–88. Doi:10.4141/P02-090

Cooper, J., Baranski, M., Stewart, G., Nobel-de Lange, M., Bàrberi, P., Fließbach, A., Peigné, J., Berner, A., Brock, C., Casagrande, M., Crowley, O., David, C., De Vliegher, A., Döring, T.F., Dupont, A., Entz, M., Grosse, M., Haase, T., Halde, C., Hammerl, V., Huiting, H., Leithold, G., Messmer, M., Schloter, M., Sukkel, W., van der Heijden, M.G.A., Willekens, K., Wittwer, R., Mäder, P., 2016. Shallow non-inversion tillage in organic farming maintains crop yields and increases soil C stocks: a meta-analysis. Agronomy for Sustainable Development. 36. Doi:10.1007/s13593-016-0354-1

European Environment Agency, 2012. Soil erosion. Copenhagen, Denmark.

Fliessbach, A., & Mäder, P. 2000. Microbial biomass and size-density fractions difer between soils of organic and conventional agricultural systems. Soil Biology and Biochemistry, 32, 757–768. Doi: 10.1016/S0038-0717(99)00197-2

Fließbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture Ecosystems & Environ,emt. 118, 273–284. Doi:10.1016/j.agee.2006.05.022

Food and Agriculture Organization of the United Nations, 2013. FAO Statistical Yearbook. World food and agriculture

Gadermaier, F., Berner, A., Fließbach, A., Friedel, J.K., Mäder, P., 2011. Impact of reduced tillage on soil organic carbon and nutrient budgets under organic farming.

Renewable Agriculture and Food Systems. 27, 68–80. Doi:10.1017/S1742170510000554

Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fliessbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., Scialabba, N.E.-H., Niggli, U., 2012. Enhanced top soil carbon stocks under organic farming. Proceedings of the National Academy of Sciences of the United States of America. 109, 18226–31. Doi:10.1073/pnas.1209429109

Gruber, S., Claupein, W., 2009. Effect of tillage intensity on weed infestation in organic farming. Soil & Tillage Research. 105, 104–111. Doi:10.1016/j.still.2009.06.001

Guo, L., Gifford, R., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change. Biology. 8, 345–360. Doi: 10.1046/j.1354-1013.2002.00486.x

Hernanz, J.L., López, R., Navarrete, L., Sánchez-Girón, V., 2002. Long-term effects of tillage systems and rotations on soil stuctural stability and orgaic carbon stratification in semiarid central Spain. Soil & Tillage Research 66, 129–141

Hernanz, J.L., Sanchez-Giron, V., Navarrete, L., 2009. Soil carbon sequestration and stratification in a cereal/leguminous crop rotation with three tillage systems in semiarid conditions. Agriculture, Ecosystems and Environment. 133, 114–122 Doi:10.1016/j.agee.2009.05.009

Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. Philosophical Transaction. The Royal Society Biological Sciences. 363, 543–555. Doi: 10.1098/rstb.2007.2169

Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agriculture Ecosystems and Environment. 103, 1–25. Doi:10.1016/j.agee.2003.12.018

IUSS Working Group WRB, 2015. World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106 FAO, Rome. Doi:10.1017/S0014479706394902

Joergensen, R. G., & Mueller, T. (1996). The fumigation-extraction method to estimate soil microbial biomass: calibration of the k EN value. Soil Biology and

Biochemistry, 28(1), 33-37

Joergensen, R. G. (1996). The fumigation-extraction method to estimate soil microbial biomass: calibration of the k EC value. Soil Biology and Biochemistry, 28(1), 25-31. Doi: 10.1016/0038-0717(95)00102-6

Kassam, A., Friedrich, T., Derpsch, R., Lahmar, R., Mrabet, R., Basch, G., González-Sánchez, E.J., Serraj, R., 2012. Conservation agriculture in the dry Mediterranean climate. F. Crop. Res. 132, 7–17. Doi:10.1016/j.fcr.2012.02.023

Krauss, M., Berner, A., Burger, D., Wiemken, A., Niggli, U., Mäder, P., 2010. Reduced tillage in temperate organic farming: implications for crop management and forage production. Soil Use and Management. 26, 12–20. Doi:10.1111/j.1475-2743.2009.00253.x

Laganiere, J., Angers, D.A., Pare, D., 2010. Carbon accumulation in agricultural soils after afforestation: a meta-analysis. Global Change Biology 16, 439–453. Doi:10.1111/j.1365-2486.2009.01930.x

Lal, R., 2009. Challenges and opportunities in soil organic matter research. European Journal of Soil Science. 60, 158–169. Doi:10.1111/j.1365-2389.2008.01114.x

Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock changes: simple bulk density corrections fail. Agriculture Ecosystems Environment. 134, 251–256. Doi:10.1016/j.agee.2009.07.006

López-Garrido, R., Madejón, E., León-Camacho, M., Girón, I., Moreno, F., Murillo, J.M., 2014. Reduced tillage as an alternative to no-tillage under Mediterranean conditions: A case study. Soil & Tillage Research. 140, 40–47. Doi:10.1016/j.still.2014.02.008

Mäder, P., Berner, A., 2011. Development of reduced tillage systems in organic farming in Renewable Agriculture and Food Systems. 27, 7–11. Doi:10.1017/S1742170511000470

Maltas, A., Charles, R., Jeangros, B., Sinaj, S., 2013. Effect of organic fertilizers and reduced-tillage on soil properties, crop nitrogen response and crop yield: Results of a 12-year experiment in Changins, Switzerland. Soil & Tillage Research. 126, 11–18. Doi:10.1016/j.still.2012.07.012

Masilionyte, L., Maiksteniene, S., Kriauciuniene, Z., Jablonskyte-Rasce, D., Zou, L., Sarauskis, E., 2017. Effect of cover crops in smothering weeds and volunteer plants in alternative farming systems. Crop Protection. 91, 74–81. Doi:10.1016/j.cropro.2016.09.016

Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, 2009, http://www.mapama.gob.es/es/agricultura/temas/producciones-agricolas/ (accesessed 20.05.2017)

Molina-Herrera, S., Romanyà, J., 2015. Synergistic and antagonistic interactions among organic amendments of contrasted stability, nutrient availability and soil organic matter in the regulation of C mineralization. European Journal of Soil Biology. 70, 118–125. Doi:10.1016/j.ejsobi.2015.09.001

Montgomery, D.R., 2007. Soil erosion and agricultural sustainability Proceedings of the National Academy of Sciences. 104, 13268–13272. Doi:10.1073/pnas.0611508104

Nett, L., Averesch, S., Ruppel, S., Ruehlmann, J., Feller, C., George, E., Fink, M., 2010. Does long-term farmyard manure fertilization affect short-term nitrogen mineralization from farmyard manure? Biology and Fertility of Soils 46, 159–167. Doi:10.1007/s00374-009-0416-5

Pang, X.P., Letey, J., 2000. Organic Farming : challenge of timing nitrogen availability to crop nitrogen requirements. Evaluation 64, 247–253. Doi:10.2136/sssaj2000.641247x

Pardo, G., Cirujeda, A., Aibar, J., Cavero, J., Zaragoza, C., 2008. Weed harrowing in winter cereal under semi-arid conditions. Spanish Journal of Agricultural Research. 6, 661–670. Doi:10.5424/sjar/2008064-358

Peigné, J., Ball, B.C., Roger-Estrade, J., David, C., 2007. Is conservation tillage suitable for organic farming? A review. Soil Use and Management. 23, 129–144. Doi:10.1111/j.1475-2743.2006.00082.x

Peigné, J., Vian, J.-F., Cannavacciuolo, M., Lefevre, V., Gautronneau, Y., Boizard, H., 2013. Assessment of soil structure in the transition layer between topsoil and subsoil using the profil cultural method. Soil & Tillage Research. 127, 13–25. Doi:10.1016/j.still.2012.05.014

Pekrun, C., Kaul, H.P., Claupein, W., 2003. Soil tillage for sustainable nutrient management. 4, 83-106

Plaza-Bonilla, D., Nolot, J.-M., Raffaillac, D., Justes, E., 2016. Innovative cropping systems to reduce N inputs and maintain wheat yields by inserting grain legumes and cover crops in southwestern France. European Journal of Agronomy. 2011, 1–11. Doi:10.1016/j.eja.2016.05.010

Plaza, E.H., Kozak, M., Navarrete, L., González-Andújar, J.L., 2011. Tillage system did not affect weed diversity in a 23-year experiment in Mediterranean dryland. Agriculture, Ecosystems and Environment. 140, 102–105. Doi:10.1016/j.agee.2010.11.016

Post, W., Izaurralde, R., Mann, L., Bliss, N., 2001. Monitoring and verifying changes of organic carbon in soil. Climate Change. 51, 73–99. Doi: 10.1007/978-94-017-3089-1_4

R Development Core Team, R Core Team, 2015. R: A Language and Environment for Statistical Computing

Romanyà, J., Arco, N., Solà-Morales, I., Armengot, L., Sans, F.X., 2012. Carbon and nitrogen stocks and nitrogen mineralization in organically managed soils amended with composted manures. Journal of Environmental Quality. 41, 1337. Doi:10.2134/jeq2011.0456

Romanyà, J., Rovira, P., 2011. An appraisal of soil organic C content in Mediterranean agricultural soils. Soil Use and Management. 27, 321–332. Doi:10.1111/j.1475-2743.2011.00346.x

Sans, F.X., Berner, A., Armengot, L., Mäder, P., 2011. Tillage effects on weed communities in an organic winter wheat-sunflower-spelt cropping sequence. Weed Research. 51, 413–421. Doi:10.1111/j.1365-3180.2011.00859.x

Shahzad, M., Farooq, M., Jabran, K., Hussain, M., 2016. Impact of different crop rotations and tillage systems on weed infestation and productivity of bread wheat. Crop Protection. 89, 161–169. Doi:10.1016/j.cropro.2016.07.019

Soil Survey Staff, 1998. Keys to soil taxonomy, Soil Conservation Service, United States Department of Agriculture

Sparling, G.P., 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Soil Research. 30, 195–207. Doi:10.1071/SR9920195

Sun, H., Koal, P., Liu, D., Gerl, G., Schroll, R., Gattinger, A., Joergensen, R., Munch, J, Charles, J. (2016). Soil microbial community and microbial residues respond positively to minimum tillage under organic farming in Southern Germany. Applied Soil Ecology, 108, 16–24. Doi: 10.1016/j.apsoil.2016.07.014

Tørresen, K.S., Skuterud, R., Tandsæther, H.J., Hagemo, M.B., 2003. Long-term experiments with reduced tillage in spring cereals. I. Effects on weed flora, weed seedbank and grain yield. Crop Protection. 22, 185–200. Doi:10.1016/S0261-2194(02)00145-x

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry. 19, 703–707

Vian, J.F., Peigne, J., Chaussod, R., Roger-Estrade, J., 2009. Effects of four tillage systems on soil structure and soil microbial biomass in organic farming. Soil Use and Management. 25, 1–10. Doi:10.1111/j.1475-2743.2008.00176.x

Walley, F.L., Kyei-Boahen, S., Hnatowich, G., Stevenson, C., 2005. Nitrogen and phosphorus fertility management for desi and kabuli chickpea. Canadian Journal of Plant Science. 85, 73–79. Doi: 10.4141/P04-039

Ward, P.R.R., Flower, K.C.C., Cordingley, N., Weeks, C., Micin, S.F.F., 2012. Soil water balance with cover crops and conservation agriculture in a Mediterranean climate. Field Crops Research. 132, 33–39. Doi:10.1016/j.fcr.2011.10.017

Capítulo III

Chapter III

Earthworm abundance response to conservation agriculture practices in organic arable farming under Mediterranean climate

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Influencia de la aplicación de prácticas de conservación sobre la abundancia de lombrices de tierra en cultivos herbáceos extensivos ecológicos en clima mediterráneo

Resumen

Las lombrices de tierra son uno de los grupos más importantes de la macrofauna edáfica y cumplen un papel muy importante en el funcionamiento de los ecosistemas agrícolas. La intensificación agrícola ha afectado estas poblaciones en los últimos años. No obstante, las prácticas de la agricultura de conservación como el laboreo mínimo, el uso de abonos verdes y la fertilización orgánica, pueden ser beneficiosas para las poblaciones de lombrices en sistemas agrícolas. Sin embargo, en la región mediterránea la abundancia de lombrices es reducida y no existen muchas referencias sobre su comportamiento en relación a la gestión agrícola. El objetivo de este estudio fue analizar los efectos individuales y colectivos del sistema de laboreo, la fertilización orgánica y los abonos verdes sobre la densidad y la biomasa de las lombrices en campos de cultivos herbáceos extensivos ecológicos de secano. El estudio se llevó a cabo en el marco de un experimento a medio plazo que consiste en una rotación de cuatro años de cereales y leguminosas, donde se evalúan tres factores: el sistema de laboreo (arado de vertedera con inversión de las capas del suelo versus arado de cincel sin inversión de las capas del suelo), la fertilización orgánica (estiércol semicompostado versus sin fertilizante) y la incorporación de abonos verdes antes del cultivo de leguminosas (con abonos verdes vs sin abonos verdes). Las lombrices de tierra se evaluaron en todas las parcelas mediante la extracción manual de todos los individuos en áreas de 33 cm × 33 cm, excavadas a una profundidad de 25 cm. En total se determinaron cinco especies de lombrices de tierra, pero la comunidad estaba dominada principalmente por dos ecotipos endógenos *Aporrectodea rosea* y *Allolobophora georgii*, y un ecotipo anécico *Aporrectodea trapezoides*. Las especies endógenas pueden verse beneficiadas por la inversión de las capas del suelo debido a la incorporación de materia orgánica, mientras que las especies anécicas pueden verse afectadas negativamente. Los resultados muestran que las parcelas con fertilización presentaron mayor densidad y biomasa de lombrices de tierra. Las parcelas que fueron labradas con arado de vertedera presentaron mayor abundancia de juveniles que las parcelas con arado de cincel. La biomasa de lombrices fue significativamente menor en las parcelas con abonos verdes y arado de cincel. Nuestros resultados indican que la combinación de fertilización y abonos verdes no fue la más óptima para las poblaciones de lombrices, pero la fertilización orgánica desempeñó un papel considerablemente más importante y aumentó su abundancia.

Earthworm abundance response to conservation agriculture practices in organic arable farming under Mediterranean climate

Abstract

Earthworms are one of the most important soil macrofaunal groups, and they play a major role in agricultural ecosystem functioning. Agricultural practices, such as reduced tillage, the use of green manures and organic fertilization, can be beneficial for earthworm populations in agricultural systems. However, under a Mediterranean climate, earthworms' abundance is reduced, and not much is known regarding their response to agricultural management. The aim of this study was to analyse the individual and collective effects of tillage type, organic fertilization, and green manures on the density and biomass of earthworms in organic arable dryland. The trial was conducted in a four-year crop rotation with a split-block design that combined tillage system (mouldboard ploughing vs. chisel), fertilization (composted farmyard manure vs. no fertilizer) and green manures (green manures vs. no green manures). Earthworms were assessed in each plot by the extraction of all individuals through hand sorting in soil areas of 33 cm × 33 cm that were excavated to a depth of 25 cm. Only five earthworm species were found in this trial, and the earthworm community was dominated by such endogeic ecotypes as *Aporrectodea rosea* and *Allolobophora georgii*, and the anecic ecotype *Aporrectodea trapezoides*. Endogeic species can benefit from soil inversion because of the incorporation of organic matter, but the anecic ones can be negatively affected by it. The results show that plots with farmyard manure had higher density and biomass of earthworms. We observed that the type of tillage significantly affected earthworm populations: plots that had been ploughed with mouldboard ploughing (soil inversion) the year prior to sampling presented more juveniles. The biomass of earthworms was significant lower in plots with green manures and chiselling. Our results indicated that the combination of chiselling and green manures was not optimal for earthworm populations, but organic fertilization played a considerably more important role and enhanced their abundances.

Keywords: organic farming, Mediterranean soils, green manures, earthworms, chisel

Introduction

Earthworms play a major role in ecosystem functioning because their burrowing and feeding activities modify the soil structure and several soil properties. In particular, earthworms increase soil macroporosity, relocate nutrients along the soil profile and form stable aggregates (Crittenden et al., 2014; Ernst and Emmerling, 2009; Metzke et al., 2007). The significant role of earthworms has been revealed by experiments in which they were eliminated in grass swards causing soil bulk density to increase, while organic matter, soil moisture and infiltration rate greatly decreased (Riley et al., 2008). Conversely, earthworm populations are influenced by soil moisture, organic matter, texture, pH and soil management (Crittenden et al., 2014). Soil tillage can modify the relative abundance of earthworm species and their community structure (Riley et al., 2008). Some studies concerning the impact of inversion tillage on the abundance of earthworm populations have found that the largest and most fragile earthworms (those with soft epitheliums) are most affected by intensive tillage, and species inhabiting the topsoil are at risk of being negatively affected by ploughing (Pelosi et al., 2014). The variability in burrowing and feeding behaviours can be important in determining the effects that tillage type can have on earthworms (Capowiez et al., 2009). According to Bouché (1972), earthworms can be divided into the following ecological groups based on soil habitats and feeding habits: (1) Epigeic species live and feed in the organic layers above the mineral soil surface. (2) Anecic species live in vertical burrows in mineral soil layers, but come to the surface to feed on leaf litter that they drag into their burrows (0–200 cm depth). (3) Endogeic species live in mineral soil layers and feed on soil organic matter. They make horizontal burrows through the soil that they sometimes reuse to feed and move around. Capowiez et al. (2009) and Ernst and Emmerling (2009) showed that soil layer inversion by mouldboard ploughing negatively affected the density of anecic earthworm species, while the density of endogeic species was enhanced.

The influences of other farming practices, such as crop rotation, crop residue management and fertilization, are also important for earthworm populations (Riley et al., 2008). Eriksen-Hamel et al. (2009) reported that the addition of crop residues to tilled soils could alleviate some of the negative impacts of tillage on earthworms, thus improving their growth and maintaining more stable populations. While many studies demonstrate the role of cover crops in decreasing soil erosion and improving weed control and soil fertility (Ward et al., 2012), there are few that investigate the effect of cover crops on earthworms. Farmyard manure is an organic amendment alternative to mineral fertilizers that can be beneficial for earthworm populations in arable fields (Andersen, 1979). Brown et al. (2004) reported that organic manures benefit earthworms both directly and indirectly by providing additional food resources and shelter (through the mulching effect), and stimulating plant growth and litter return.

Diversified crop rotation and green manures are used to manage weeds and pests, and the use of less intensive soil tillage (such as reduced tillage with no soil inversion) can reduce soil erosion, thus ensuring the sustainability of farming systems (Pelosi et al., 2014). Due to the potential beneficial effect of reduced tillage, green manures and organic fertilization on earthworms, a sensible hypothesis could be that the integration of conservation agriculture techniques into organic farming systems should increase their populations and diversity. For instance, Henneron et al. (2014) indicated that conservation agriculture and organic farming increased the density and biomass of all soil organisms. Several studies have found higher biodiversity in organically managed systems than in conventional systems (Scullion et al., 2002), Padmavathy and Poyyamoli (2013) reported higher earthworm populations in organically managed fields. Organic farming is fundamentally different than conventional systems due to the exclusion of synthetic pesticides and fertilizers. However, notably few studies provide results confirming that earthworm populations and diversity increase in arable cropping systems with a combination of conservation agriculture techniques and organic farming.

The aim of this study is to analyse the individual and collective effects of tillage type, organic fertilizer and green manures on the density and biomass of earthworms in organic arable cropping systems in the Mediterranean region. Indeed, there is a lack of studies of earthworm populations in Mediterranean agricultural areas. Monitoring earthworms in these areas can be challenging because environmental conditions strongly limit earthworm distribution. Frequently, earthworms are distributed in small patches because many species have narrow ecological requirements that are determined by the high spatial variability of soil and soil water regimes in many Mediterranean landscapes (Gutiérrez-López et al., 2016).

The hypotheses of this study are that (1) the application of farmyard manure as fertilizer will increase earthworm density and biomass; 2) mouldboard ploughing will decrease earthworm populations; 3) the incorporation of cover crops into the soil as green manures can increase earthworm density and biomass; and 4) the integration of conservation agriculture techniques into organic farming systems could help increase the abundance of earthworms in arable fields under a Mediterranean climate.

To answer these questions, we took advantage of a trial designed to evaluate the effects of tillage, fertilization and green manures on a Mediterranean rainfed crop rotation and measured the abundance of earthworm populations in relation to these factors.

Materials and Methods

Experimental site and design

In November of 2011, a midterm field experiment was established in Gallecs, a rural area of Catalonia, Spain. This location is a peri-urban agricultural area of 753 ha situated in the region of Vallès Oriental, 15 km North of Barcelona (41°33'31.9"N 2°11'59.5"E). It has a Mediterranean climate; the mean annual temperature and precipitation are 14.9 °C and 647 mm, respectively. At the beginning of the experiment, soil properties of the field were evaluated. On average, the mineral fraction consisted of 43.3 ± 6.9 % sand, 26.9 ± 4.7 % loam and 29.7 ± 3.7 % clay; the texture was classified as loamy-clay (Soil Survey Staff, 1998); and the soil type was a Haplic Luvisol (IUSS

Working Group WRB, 2015). At the beginning of the experiment the average soil organic matter was 1.5 ± 0.1 % (Walkley-Black) and the pH ($H2O$) was 8.1 ± 0.1.

The trial consisted of a four-year crop rotation in a split-block design of three factors: tillage system (mouldboard ploughing (P) vs. chisel (C)) fertilization (composted farmyard (+F) vs. no fertilizer (-F)) and green manures (with green manures (+G) vs. no green manures (-G)). In total 32 plots measuring 13 m × 12 m were established, comprising four replicates of each treatment. The field had been under organic management for five years prior to the trial, with a typical dryland Mediterranean crop rotation that alternated cereals and legumes for human consumption. The crop rotation of this trial consisted of spelt (*Triticum spelta* L., 2011–2012), chickpeas (*Cicer arietinum* L., 2013) winter wheat (Triticum aestivum L., 2013-2014) and lentils (*Lens culinaris* Medik. 2015).

Two tillage systems were used: a mouldboard plough (P) (soil inversion at 25 cm depth; EG 85-240-8, Kverneland) plus a rotary harrow (5 cm depth; HR3003D, Kuhn); and a chisel plough (C) (no soil inversion at 25 cm depth; KCCC 1187 - A00, Kverneland) plus a rotary harrow (same as for plough). The fertilization (+F) treatment utilized six-month-long composted cow farmyard manure sourced near the field. The farmyard manure was applied every year before sowing the main crop. The total amount of manure applied differed per the nutritional demands of each crop. The year before sampling, 40 Ton ha-1 (138.28 kg ha-1 N) of farmyard manure was applied before winter wheat was sown. The organic fertilizers were mixed in the soil by means of a chisel or mouldboard plough in accordance to the tillage treatment. In September 2012 and 2014, green manure (+G) was sown in the corresponding 16 plots. It consisted of a mixture of oat (*Avena sativa* L.), white mustard (*Sinapis alba* L.), bitter vetch (*Vicia ervilia* (L.) Willd.) and common vetch (*Vicia sativa* L.). At the end of March of the following year, green manure was incorporated into the soil by disc harrowing.

Earthworm sampling

In February 2015, after three years of crop rotation, earthworms were assessed during green manure or stubble (depending on the type of treatment). Three square sampling frames of 33 cm × 33 cm were placed 2 m from the edge of each plot, with two on the mid-line and one on the centre of a randomly chosen side and were manually excavated to a depth of 25 cm. All earthworm and cocoons were extracted by hand sorting and were preserved alive with moist soil at 4 °C until their fixation. In the laboratory, the earthworms were fixed using formalin (4 % formaldehyde) and preserved in alcohol (90 %) (Kuntz et al., 2013; Peigné et al., 2009). They were counted and sorted by adults (with a clitellum), juveniles (without a clitellum and tubercula pubertatis) and cocoons. Adults and juveniles were identified following Bouché (1972) and weighed (conserved weight in alcohol with gut contents).

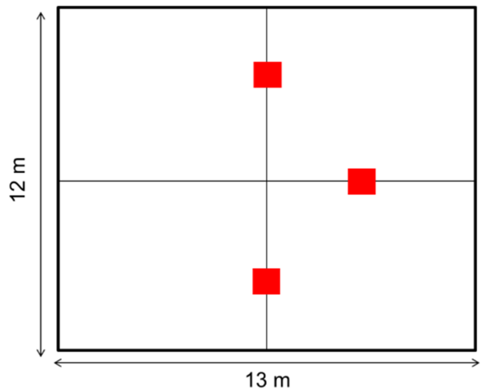

Figure 1. Square soil samples (33 cm × 33 cm × 25 cm) distribution in each plot.

Statistical analysis

The individual and combined effects of the type of tillage (P vs. C), fertilization (+F vs. -F) and green manure (+G vs. -G) on adult and juvenile earthworm density and biomass was evaluated using linear mixed-effects models. Tillage, fertilization and green manures were used as fixed factors and the block was introduced as a random factor (block for the fertilization treatment and block for the tillage treatment). The normality of data was verified by the Shapiro-Wilk test and homoscedasticity was assessed using the Barlett test. To meet the normality and homoscedasticity requirements we used logarithmic transformation on data when necessary. The differences in mean density and biomass among the treatments resulting from the combination of all three factors were verified using Tukey's HSD test. The same statistical procedure was followed while analysing the effect of tillage, fertilization and green manures on the densities of the main earthworm species. All the analyses were performed in R version 3.2.2 (R

Core Team, 2015) with the packages lme4 (Bates et al., 2011) for linear mixed effects model fitting and multcomp (Hothorn et al., 2008) for post hoc multiple comparisons.

Results

Overview of earthworm diversity

Overall, five earthworm species were found: *Aporrectodea trapezoides* (Duges, 1828), *Aporrectodea rosea* (Savigny, 1826), *Allolobophora georgii* (Michaelsen, 1890), *Octodrilus complanatus* (Duges 1828) and one unidentifiable specimen belonging to the family *Hormogastridae* (Michelsen, 1900). In this study, we have focused on the three most abundant species: A. rosea, A. georgii and A. trapezoides. The first two are endogeic ecotypes (Bouché, 1972), but the latter is quite variable in its behaviour. Some authors have considered it an endogeic ecotype that sometimes feeds on the surface (Lee, 1985) but is primarily considered to be anecic (Gutiérrez-López et al., 2016). Only one specimen of Octodrilus complanatus (anecic) and one unidentified Hormogastridae (endogeic) were observed. The most abundant species was A. rosea (mean 53 ± 12 ind m-2) followed by A. georgii (mean 30 ± 6 ind m-2), and A. trapezoides (mean 24 ± 8 ind m-2). However, the highest earthworm biomass was of A. trapezoides, (14.80 ± 1.94 g m-2), followed by A. rosea (7.56 ± 15.57 g m-2) and A. georgii (5.59 ± 1.93 g m-2).

Effect of organic fertilization, tillage and green manures on the density and biomass of earthworms

Fertilization was the main factor that influenced earthworm populations (Table 1). The density and biomass of earthworms were significantly enhanced by farmyard manure (Figure 1(I) and 1(II)). The densities of juveniles, adults and total earthworms were significantly higher in plots with farmyard manure (mean ± standard error of total density: +F= 585 ind ± 94 m-2; -F= 273 ± 47 ind m-2). This pattern is related to A. rosea and A. georgii, but not A. trapezoides (Table 1 and Table 2). Similarly, biomass was significantly enhanced by farmyard manure (mean ± standard error of total biomass: +F= 158.75 ± 36.78 g m-2; -F= 64.87 ± 17.81 g m-2). In contrast, total and adult densities were not affected by the type of tillage, but the density of juveniles was significantly higher in plots with mouldboard ploughing (Table 1). Total biomass of earthworms was not affected by the type of tillage. The presence of green manures did not affect the density of earthworms; however, significant differences were found for total and adult biomass (Table 1). The highest biomass was found in plots without green manures and chiselling (49.10 ± 10.01 g m-2). Furthermore, there was a significant interaction between tillage and green manure factors due to the significant decrease of total earthworm biomass in plots with chiselling and without green manures when no farmyard manure was incorporated (Table 1).

Effects of the combination of conservation agriculture practices and organic fertilization on the biomass and abundance of earthworms

Contrasting results were found regarding the combined effects of the factors on density and biomass of earthworms. The combination of the two techniques of conservation agriculture (C and +G) was not the best combination for earthworm populations, regardless of fertilization (Figure 2 (left)). Total biomasses were significantly lower in plots with green manure and chiselling in comparison to those managed without green manure; the incorporation of green manures seems to negatively affect the total biomass of earthworms in unfertilized plots managed with chisel (Figure 2 (right)).

Discussion

Overview of earthworm diversity

Our results show a low diversity of earthworms, with only four species identified in the study area. Studies in temperate climates had shown higher species richness (9-13 species) compared to our study in the Mediterranean region (Kuntz et al., 2013; Peigné et al., 2009). Furthermore, the earthworm community is dominated by only three species: two endogeic (*Aporrectodea rosea* and *Allolobophora georgii*) and one anecic ecotype (*Aporrectodea trapezoides*).

Arable cropping systems with annual rotation schemes, high rates of soil disturbance and habitat simplification likely contribute to low species richness.

Table 1. Results of the linear mixed effects models of the effect of fertilization (F: + fertilization with farmyard manure, F: - not fertilized; T: tillage system (P: mouldboard ploughing, C: chisel ploughing); G: + with green manure, G: - no green manure), and the interaction between factors (F, T and G) on the different variables measured. Total density and biomass refers to the sum of adults and juveniles. Logarithmic transformation was applied to meet normality and homoscedasticity requirements.

	F: + vs -	T: P vs C	G: + vs -	F×T	F×G	T×G
Total density	0.390**	0.076 NS	-0.021 NS	0.006 NS	0.034 NS	0.091 NS
Density adults	0.480***	-0.045 NS	-0.081 NS	0.028 NS	0.017 NS	0.046 NS
Density juveniles	0.305*	0.203**	0.042 NS	0.042 NS	0.103 NS	0.100 NS
A. rosea total density	19.984*	8.046 NS	3.890 NS	8.621 NS	5.965 NS	8.053 NS
A. georgii total density	0.0323*	0.009 NS	-0.050 NS	-0.019 NS	-0.097 NS	-0.074 NS
A. trapezoides total density	9.656 NS	-1.137 NS	-0.381 NS	-3.031 NS	0.762 NS	3.218 NS
Total biomass	0.489*	-0.091 NS	-0.151*	-0.068 NS	0.060 NS	0.160*
Biomass adults	0.582*	-0.166 NS	-0.232*	-0.028 NS	0.112 NS	0.150 NS
Biomass juveniles	1.209*	0.521 NS	0.121 NS	-0.378 NS	0.234 NS	0.334 NS
A. rosea total biomass	3.121*	0.215 NS	-0.221 NS	0.509 NS	-0.090 NS	0.903 NS
A. georgii total biomass	0.408*	-0.086 NS	-0.133 NS	0.006 NS	-0.049 NS	0.026 NS
A. trapezoides total biomass	7.134 NS	-2.834 NS	-1.222 NS	-2.234 NS	1.091 NS	2.347 NS

The values are estimated differences in log-transformed marginal means and their significance levels are indicated according to the following codes: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, NS not significant.

Table 2. Mean (± SE standard error of the differences) number of total individuals of *Aporrectodea rosea*, *Allolobophora georgii* and *Aporrectodea trapezoides*, and their sum per square meter: +F: fertilization with farmyard manure, -F: not fertilized, P: mouldboard ploughing, C: chisel, G+: with green manure and G-: no green manure.

Species	F+				F-			
	P		C		P		C	
	G+	G-	G+	G-	G+	G-	G+	G-
A. rosea	108.35 ± 33.13	71.95 ± 10.09	58.32 ± 11.24	55.30 ± 11.55	38.62 ± 4.17	27.25 ± 4.11	24.25 ± 9.51	43.92 ± 14.66
A. georgii	24.22 ± 1.24	53.05 ± 7.97	44.70 ± 14.77	34.85 ± 6.37	24.27 ± 7.21	19.70 ± 5.16	18.17 ± 2.76	19.70 ± 3.81
A. trapezoides	31.82 ± 8.97	27.27 ± 8.65	36.37 ± 13.67	39.40 ± 10.71	19.70 ± 6.84	12.90 ± 3.12	6.82 ± 4.35	18.20 ± 7.42
Total_abundance	164.40 ± 34.64	152.27 ± 13.71	139.40 ± 25.81	129.50 ± 20.71	82.57 ± 14.98	59.87 ± 6.82	49.22 ± 10.08	81.82 ± 15.50

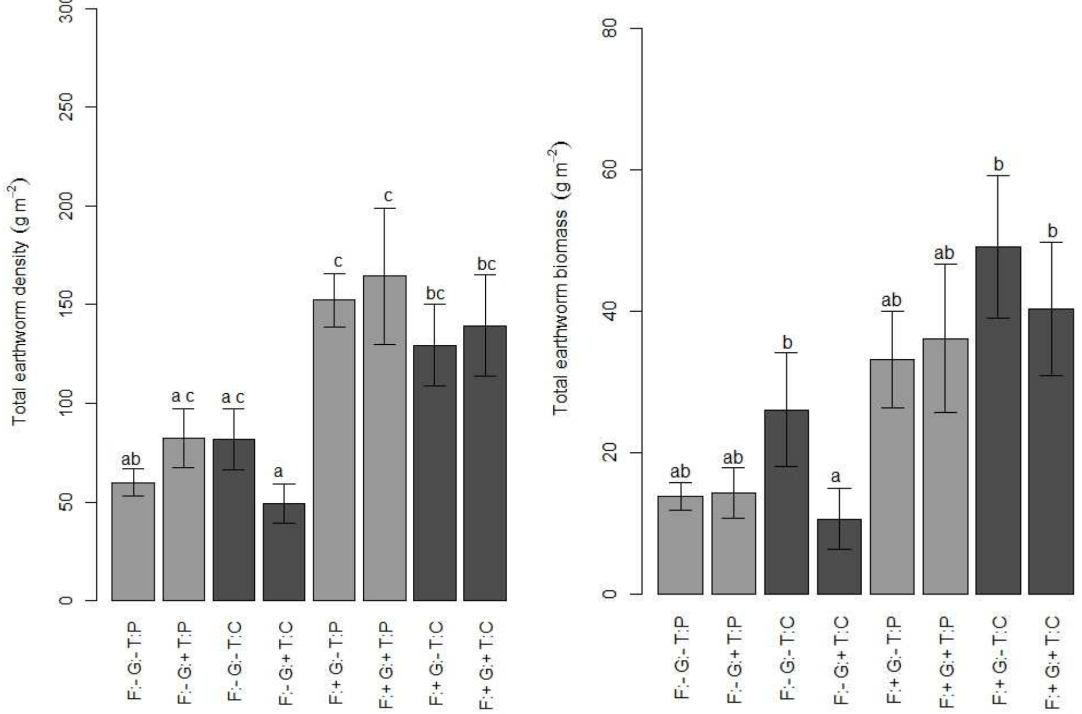

Figure 2. Mean (± SE standard error of differences) total number of individuals (left) and total biomass (right) in each treatment: F: - not fertilized, F: + fertilization with farmyard manure; G: - no green manure, G: + sowed with green manure; T: tillage system (P: mouldboard ploughing, C: chisel ploughing). Bars with no letters in common are significantly different (Tukey HSD test, $P < 0.05$).

Smith et al. (2008) reported low species richness in a study in Michigan, US, dominated by the genus Aporrectodea. These researchers relate the findings to the fact that this genus is relatively tolerant to agricultural activities, as it is able to persist deeper in the subsoil than other species.

Boström (1995) suggested that the large amount of organic matter ploughed under the soil, which served as food for the earthworms, together with the supply of cocoons allowed the endogeic earthworms to make a fast recovery. Capowiez et al. (2009) reported that *Aporrectodea caliginosa* increased with mouldboard ploughing due to an increase in food resources (buried organic matter) and a decrease in competition for food with anecic species. Anecic species, on the other hand, could be the most negatively affected by intensive and repeated soil disturbance because of direct physical damage and an indirect effect on food resources (burial of surface organic matter) and their habitat (destruction of burrows) (Capowiez et al., 2009).

Pelosi et al. (2014) suggested that anecic species are less abundant, or even absent, in ploughed fields. Though several studies have shown that ploughing reduced the number of the large-bodied anecic species (Chan, 2001; Ernst and Emmerling, 2009; Pelosi et al., 2014), our results suggested *A. trapezoides* were not significantly affected by the type of tillage. Kuntz et al. (2013) reported that the implementation of reduced tillage in an organically managed clay soil over a six-year crop rotation enhanced the density and biomass of earthworms while also influencing their community structure. In this four-year experiment, we did not find that the type of tillage significantly affected earthworm communities, perhaps indicating that the response of earthworm populations occurs over a longer time period.

<u>Effect of organic fertilization, tillage and green manures on the density and biomass of earthworms</u>

It is known that fertilizers have long-term benefits for earthworm populations through increased input of nutrients, organic matter and enhanced production of litter material, which ultimately result in more food for earthworms. Furthermore, farmyard manure is considered most suitable for earthworm population growth (Brown et al., 2004; Curry, 2004, 1976). Our study supports the claim that farmyard manure enhances earthworm growth and reproduction.

The effect of fertilization is significant for both density and biomass of adults and juveniles, while the effect of tillage is only significant for the density of juveniles. The high number of juveniles in plots using mouldboard ploughing can be explained by the enhancement of reproduction of earthworms in response to tillage disturbance. Several studies have shown that tillage with soil inversion can reduce earthworms by 70% both in numbers and biomass, but populations generally recover within a year if the disturbance is not repeated (Boström, 1995). Eriksen-Hamel et al. (2009) reported that ploughing reduced the earthworm population by 73-77%, but one year later, there were five times as many earthworms, and the biomass was similar to the pre-tillage level.

This could be explained because disturbance creates a pressure which reduces earthworm populations, but right after ploughing reproduction is increased. In this experiment, earthworm sampling was carried out six months after the soil was disturbed, probably leaving enough time for their recovery.

In this study, the highest biomass was found in plots without green manures, which is contrary to our initial hypothesis that the incorporation of organic matter from green manure should favour earthworms. However, few studies are available which analyse the effects of green manures and their interaction with tillage systems on earthworm populations, and thus our results are difficult to frame within existing evidence. Stroud et al. (2016) reported that *Lumbricus terrestris* L. was not enhanced by oilseed radish as cover crop in a long-term rotation. Valckx et al. (2011) also studied *Lumbricus terrestris* food and habitat preferences for cover crops and have found that rye grass was the preferred food resource, but no preference or repellence was found for mustard (*Sinapis alba*). However, they found an increasing trend for repellence against oats (*Avena sativa*) over time, suggesting that the allelopathic effect of oats may affect earthworms indirectly by changing habitat and food preference. Since we used oats and white mustard for the green manure mix, this could explain our results, although we cannot confirm that there is a repellent effect of green manures on earthworms. Furthermore, no studies were found regarding the food and habitat preferences of A. rosea, *A. georgii* or *A. trapezoides*. There is a need to study in more detail whether there are some allelopathic effects of oats or mustard as cover crops that may affect earthworms.

<u>Effects of the combination of conservation agriculture practices and organic fertilization on the biomass and abundance of earthworms</u>

Density and biomass of earthworms respond differently to the combination of experimental factors, but in no case conservation agriculture techniques were the best combination for earthworm populations. Nevertheless, it is important to note that similar density and biomass of earthworms resulted in plots with mouldboard ploughing and fertilization compared to plots with chiselling and no fertilization. Several studies have indicated that the use of conservation agriculture techniques increases populations of earthworms (Scullion et al., 2002). Our results showed that earthworms can have similar abundances with both tillage systems. Perhaps in the Mediterranean region, where extreme climate conditions -particularly summer drought- play an important role on agroecosystems functioning, the use of all conservation agriculture techniques does not contribute to a more sustainable cropping system. Organic fertilization seems to play an important role in these systems and could be a crucial factor to maintain and benefit earthworm populations in Mediterranean agricultural systems.

The highest density of earthworms was found in plots with mouldboard ploughing, fertilization and the presence of green manures. This can be explained due to a species-specific response. *Aporrectodea rosea*, which was the most abundant species, avoids compaction and reduced tillage seemed to create more compacted zones. Some authors have shown that minimum tillage and no-tillage result in more compacted soil than ploughing (Capowiez et al., 2009; Peigné et al., 2009). Compacted zones are created by wheel tracks, and in tilled plots those zones are fragmented into clods by the plough; but in reduced tillage plots only parts of the compacted zones are fragmented (Capowiez et al., 2009). In consequence, a soil structure with more macropores is obtained with mouldboard ploughing and, in the short term, earthworms are not able to improve soil macroporosity (Peigné et al., 2009). Furthermore, *A. rosea* lives mainly in the upper layers of the soil, eating soil organic matter. Therefore, the species could take advantage of increased availability of crop residues incorporated by inversion tillage (Chan, 2001).

The highest total biomass was found in plots tilled with chisel and fertilization, but without green manures. *A. trapezoides* had the highest biomass since it is the largest species and it was the only anecic ecotype found in this experiment. This makes it more likely that they are affected by soil inversion tillage since they go to the surface for food (Gutiérrez-López et al., 2016). Therefore, reduced soil tillage with non-inversion could benefit this species.

Conclusions

Farmyard manure demonstrated the strongest effects on the growth and reproduction of earthworm populations. The two tillage systems did not show significant differences in total density and biomass of earthworms, but they did show an important effect depending of the ecotype of earthworm. Endogeic species can benefit from soil inversion because of the incorporation of organic matter, but anecic species can be negatively affected by it. It is essential to study the effects of cover crops on earthworm populations and whether there are allelopathic effects of specific cover crops such as oats and mustard on earthworms. Furthermore, there is a need to study in more detail the biology of Aporrectodea *trapezoides*, *A. rosea* and *Allolobophora georgii*, the species dominant in our experiment, which are abundant in Mediterranean systems.

Our study did not show any positive effects of the combination of conservation agriculture techniques on earthworms. In our trial the combination of reduced tillage by chiselling and green manure did not enhance earthworm populations. It is important to understand how these different factors interact when designing a sustainable organic system under certain climate conditions, soil properties, and crop rotations.

This study shows the importance of analysing the combination of such conservation agriculture techniques as reduced tillage and green manures in organic arable crop rotations, and it adds relevant information for the Mediterranean region. However, long-term trials in a Mediterranean climate are needed to fully understand the ecology of different earthworm species, their interactions and relation with conservation agriculture techniques, as well as their potential roles in promoting more sustainable farming systems.

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References

Andersen, C., 1979. The influence of farmyard manure and slurry on the earthworm population (Lumbricidae) in arable soil Soil Biology as Related to Land Use Practices. Environmental Protection Agency, Washington DC, pp. 325–335

Bates, D., Maechler, M., Bolker, B., 2011. lme4: Linear mixed-effects models using S4 classes

Boström, U., 1995. Earthworm populations (Lumbricidae) in ploughed and undisturbed leys. Soil Tillage Res. 35, 125–133. Doi:10.1016/0167-1987(95)00489-0

Bouché, M.B., 1972. Lombriciens de France. Ecologie et systématique. INRA, Paris.

Brown, G.G., Edwards, C.A., Brussaard, L., 2004. How earthworms affect plant growth: burrowing into the mechanisms, in: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, FL, USA. Doi:10.1201/9781420039719.ch2

Capowiez, Y., Cadoux, S., Bouchant, P., Ruy, S., Roger-Estrade, J., Richard, G., Boizard, H., 2009. The effect of tillage type and cropping system on earthworm communities, macroporosity and water infiltration. Soil Tillage Res. 105, 209–216. Doi:10.1016/j.still.2009.09.002

Chan, K.Y., 2001. An overview of some tillage impacts on earthworm population abundance and diversity - implications for functioning in soils. Soil Tillage Res. 57, 179–191. Doi: /10.1016/S0167-1987(00)00173-2

Crittenden, S.J.J., Eswaramurthy, T., de Goede, R.G.. G.M., Brussaard, L., Pulleman, M.M.M., 2014. Effect of tillage on earthworms over short- and medium-term in conventional and organic farming. Appl. Soil Ecol. 83, 140–148. Doi:10.1016/j.apsoil.2014.03.001

Curry, J.P., 2004. Factors affecting the abundance of earthworms in soils, in: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press Boca Raton, Fla, USA, pp. 91–113.

Curry, J.P., 1976. Some effects of animal manures on earthworms in grassland. Pedobiologia (Jena). 16, 425–438

Eriksen-Hamel, N.S., Speratti, A.B., Whalen, J.K., Légère, A., Madramootoo, C.A., 2009. Earthworm populations and growth rates related to long-term crop residue and tillage management. Soil Tillage Res. 104, 311–316. Doi:10.1016/j.still.2009.04.006

Ernst, G., Emmerling, C., 2009. Impact of five different tillage systems on soil organic carbon content and the density, biomass, and community composition of earthworms after a ten year period. Eur. J. Soil Biol. 45, 247–251. Doi:10.1016/j.ejsobi.2009.02.002

Gutiérrez-López, M., Moreno, G., Trigo, D., Juárez, E., Jesús, J.B., Díaz Cosín, D.J., 2016. The efficiency of earthworm extraction methods is determined by species and soil properties in the Mediterranean communities of Central-Western Spain. Eur. J. Soil Biol. 73, 59–68. Doi:10.1016/j.ejsobi.2016.01.005

Henneron, L., Bernard, L., Hedde, M., Bertrand, M., Girardin, C., Blanchart, E., Pelosi, C., Villenave, C., Chenu, C., Bertrand, M., Girardin, C., Blanchart, E., 2014. Fourteen years of evidence for positive effects of conservation agriculture and organic farming on soil life. Agron. Sustain. Dev. Doi:10.1007/s13593-014-0215-8

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical J. 50, 346–363

IUSS Working Group WRB, 2015. World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106 FAO, Rome. Doi:10.1017/S0014479706394902

Kuntz, M., Berner, A., Gattinger, A., Scholberg, J.M., Mäder, P., Pfiffner, L., 2013. Influence of reduced tillage on earthworm and microbial communities under organic arable farming. Pedobiologia (Jena). 56, 251–260. Doi:10.1016/j.pedobi.2013.08.005

Lee, K.E., 1985. Earthworms: their ecology and relationships with soils and land use. Academic Press

Metzke, M., Potthoff, M., Quintern, M., Heß, J., Joergensen, R.G., 2007. Effect of reduced tillage systems on earthworm communities in a 6-year organic rotation. Eur. J. Soil Biol. 43, S209–S215. Doi:10.1016/j.ejsobi.2007.08.056

Padmavathy, A., Poyyamoli, G., 2013. Biodiversity comparison between paired organic and conventional fields in Puducherry, India. Pakistan J. Biol. Sci. 16, 1675–1686. Doi: 10.3923/pjbs.2013.1675.1686

Peigné, J., Cannavaciuolo, M., Gautronneau, Y., Aveline, A., Giteau, J.L., Cluzeau, D., 2009. Earthworm populations under different tillage systems in organic farming. Soil Tillage Res. 104, 207–214. Doi:10.1016/j.still.2009.02.011

Pelosi, C., Pey, B., Hedde, M., Caro, G., Capowiez, Y., Guernion, M., Peigné, J., Piron, D., Bertrand, M., Cluzeau, D., 2014. Reducing tillage in cultivated fields increases earthworm functional diversity. Appl. Soil Ecol. 83, 79–87. Doi: 10.1016/j.apsoil.2013.10.005.R Core Team, 2015. R: A Language and Environment for Statistical Computing

Riley, H., Pommeresche, R., Eltun, R., Hansen, S., Korsaeth, A., 2008. Soil structure, organic matter and earthworm activity in a comparison of cropping systems with contrasting tillage, rotations, fertilizer levels and manure use. Agric. Ecosyst. Environ. 124, 275–284. Doi:10.1016/j.agee.2007.11.002

Scullion, J., Neale, S., Philipps, L., 2002. Comparisons of earthworm populations and cast properties in conventional and organic arable rotations. Soil Use Manag. 18, 293–300. Doi:10.1079/SUM2002132

Smith, R.G., Mcswiney, C.P., Grandy, A.S., Suwanwaree, P., Snider, R.M., Robertson, G.P., 2008. Diversity and abundance of earthworms across an agricultural land-use intensity gradient. Soil Tillage Res. 100, 83–88. Doi:10.1016/j.still.2008.04.009

Soil Survey Staff, 1998. Keys to soil taxonomy, Soil Conservation Service, United States Department of Agriculture

Stroud, J.L., Irons, D.E., Watts, C.W., White, R.P., Mcgrath, S.P., Whitmore, A.P., 2016. Population collapse of *Lumbricus terrestris* in conventional arable cultivations and response to straw applications. Appl. Soil Ecol. 108, 72–75. Doi:10.1016/j.apsoil.2016.08.002

Valckx, J., Cordón, A., Govers, G., Hermy, M., Muys, B., 2011. Food and habitat preferences of the earthworm *Lumbricus terrestris* L. for cover crops. Pedobiologia (Jena). 54, S139–S144. Doi:10.1016/j.pedobi.2011.07.004

Ward, P.R.R., Flower, K.C.C., Cordingley, N., Weeks, C., Micin, S.F.F., 2012. Soil water balance with cover crops and conservation agriculture in a Mediterranean climate. Field Crops Research. 132, 33–39. Doi:10.1016/j.fcr.2011.10.017

Capítulo IV

Chapter IV

Nitrogen utilization in a mid-term cereal-legume rotation as a result of green manure, organic fertilization and tillage strategies

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Uso del nitrógeno en una rotación de cuatro años de cereales-leguminosas como respuesta a la aplicación de abonos verdes, fertilización orgánica y laboreo mínimo

Resumen

El creciente énfasis por el desarrollo de modelos agrícolas sostenibles desde el punto de vista medioambiental conlleva que se incremente el interés por la rotación de cultivos y el uso de fuentes de nitrógeno N orgánicas que afectan la disponibilidad de N y la eficiencia de su uso para mejorar o mantener la fertilidad del suelo y la productividad de los cultivos. El objetivo de este trabajo fue estudiar el efecto del laboreo mínimo, la aplicación de estiércol semicompostado y la incorporación de abonos verdes sobre el rendimiento de los cultivos y el uso del N, en una rotación de cuatro años de cultivos ecológicos de cereales y leguminosas e identificar la mejor combinación de las prácticas en términos del balance de N y los parámetros de la eficiencia del uso del N. Los tratamientos fueron evaluados mediante un diseño experimental de bloques divididos: arado de vertedera (P) vs. arado de cincel (C), fertilizante orgánico (+F) vs sin fertilizante (-F) y abonos verdes (+G) vs. sin abonos verdes (-G). El tipo de laboreo no afectó la absorción de N cuando se combinó con las diferentes fuentes de N. Los resultados reflejan que las leguminosas no necesitan la fertilización adicional de N. El excedente más elevado de N del suelo fue en parcelas con fertilización y abonos verdes con independencia del tipo de laboreo. El elevado exceso de N en estas parcelas puede ser problemático ya que puede dar lugar a pérdidas por lixiviación; por lo tanto, la práctica combinada de abonos verdes ricos en leguminosas y la fertilización deberían evitarse en las rotaciones de cereales-leguminosas.

Nitrogen utilization in a mid-term cereal-legume rotation as a result of green manure, organic fertilization and tillage strategies

Abstract

The growing emphasis on environmentally sustainable agriculture is expected to increase interest in complex rotations, organic nitrogen (N) sources, affecting N availability and N use efficiency, to build or maintain soil fertility and crop productivity. The aim of this research was to study the effect of reduced soil tillage, farmyard manure and green manure on yield and N use, in a 4-year cereal-legume rotation in organic farming, and to identify the best combination of these agro-ecological practices in terms of N balance and N efficiency parameters. The following treatments were compared in a strip-split-block design: conventional plough (P) *vs*. reduced chisel (C) tillage, organic fertilizer (F+) *vs*. no fertilizer (F-) and green manure (G+) *vs*. no green manure (G-). The tillage method did not affect N uptake when associated with various N sources. Results also suggested that legumes do not need supplemental N fertilization. The N surplus in the soil was the highest in plots combining (with both tillage approaches) green manure and farmyard manure, with the risk of losses by leaching, therefore, this combined practice should be avoided in cereal – legume rotations.

Keywords: organic farming; crop rotation; mid-term experiment; organic fertilizer; green manure; N balance

Introduction

Sustainable agriculture has fostered the interest in crop rotations and their effects on nitrogen (N) utilization efficiency, to promote profitable and efficient agriculture. In particular, different authors found that cereal-legume rotations are more productive than continuous cereal cropping and can more efficiently use the limited rainfall in a Mediterranean environment (Lòpez-Bellido et al., 2000; Pala et al., 2007)

In crop rotations on organic farms, yields depend both on the optimization of the N inputs and minimizing nutrient losses. According to Davis (2010), in properly designed rotational systems, cover crops can sustain cash crop production by reducing soil erosion and N leaching losses and by increasing soil organic matter when incorporated into the soil as green manure. In particular, the use of leguminous cover crops (pure or in mixture) may partially replace off-farm N fertilizers, improve soil N fertility and increase the yield of the subsequent crop, due to the N fixation in low C:N residues (Gilmour et al. 1998; Smukler et al., 2012). Cover crop N, however, is often mineralized before the root system of cash crop is fully developed, thus determining a lack of N release : uptake synchrony (Pang and Letey, 2000). To reduce N deficiency, it may be thus necessary to supplement crop fertility with off-farm organic compliant inputs, such as composted farmyard manure and crop residues.

Melero et al. (2011) found a significant interaction between crop rotation and tillage, pointing out the differential effect of the soil management system on the soil N status, since soil conservation tillage increased total N in the surface layer better than conventional tillage practices. In addition, according to Van Kessel and Hartley (2000), conservation tillage can stimulate N_2 fixation. Soils under the conservation tillage system, in fact, offer favourable conditions for symbiosis, such as reduced soil temperature and greater water availability, resulting from increased aggregate stability and high number of macropores (Souza et al., 2003).

Recent attempts have been made to assess the sustainability and crop performance of cropping systems in which cover crops and organic fertilization strategies were combined (Mazzoncini et al., 2011; Montemurro et al., 2013). However, there is still a substantial lack of knowledge on combined practices, particularly regarding the assessment of N efficiency in organic cropping systems. Therefore, the objective of this research was to study the effect of different agro-ecological practices (reduced soil tillage, organic fertilization and green manure) on crop productions and N use in a cereal-legume rotation in organic farming, under Mediterranean conditions. Since a growing number of agro-ecologists argued that the productivity of farmlands should be maximized with the increase of uptake efficiency of N inputs (Crews and Peoples, 2004), the aim was also to identify the best combination of the adopted agronomic measures in terms of N balance and N efficiency parameters.

Materials and methods

Site of study

In 2011, a mid-term (4-year) field experiment was established in the Rural Area of Gallecs that is a suburban agricultural land area in Catalonia (Spain), which is situated 15 km North of Barcelona (41°33'31.9"N 2°11'59.5"E, 90 m a.s.l.). This area has a Mediterranean climate and the mean annual temperature and precipitation are 14.6 ºC and 629.2 mm, respectively. The soil is loamy-clay, classified as a *Haplic Luvisol* according to the Soil Taxonomy definition (Soil Survey Staff, 1999).

On average, the main physical-chemical soil characteristics, determined at the beginning of the field experiment, are the following by weight: 43.3 ± 6.9 % sand, 26.9 ± 4.7 % loam and 29.7 ± 3.7 % clay. At the beginning of the experiment the average soil organic matter was 1.5 ± 0.1 % (Walkley-Black) and the pH (H_2O) was 8.1 ± 0.1. The field had been under organic management during five years before the trial, with typical Mediterranean crop rotations alternating cereals and legumes for human consumption.

Experimental setup and treatments

The field experiment consisted of an organic cereal-legume crop rotation in a strip-split-block design, with three factors (with two levels each) and four replications. The following treatments were compared: tillage system (conventional plough (P) *vs*. reduced chisel (C)), fertilization (organic fertilizer (F+) *vs.* no fertilizer (F-)) and green manures (with green manure (G+) *vs.* no green manure (G-)). The factors were arranged with tillage treatments laid out in vertical strips, fertilization horizontally stripped across the split-plot experiment, and the vertical strips were split into subplots with different green manure. The entire experiment was replicated 4 times.

The P tillage consisted of a mouldboard plough (soil layers inversion at 25 cm depth; EG 85-240-8, Kverneland) and a rotary harrow (5 cm depth; HR3003D, Kuhn) for seedbed preparation. For the C tillage system, a chisel plough (no soil layers inversion, about 25 cm depth; KCCC 1187 - A00, Kverneland) and a rotary harrow (5 cm depth) were used.

The fertilization treatment (F+) consisted of partially composted farmyard manure, composed of cattle manure and vegetable residues, obtained without managing and controlling the process, by gradually accumulating the material that was seasonally available, according to the normal practice used in the area. It was applied every year before sowing the main crop. The total amount of manure applied depended on each crop, ranging as follows: i) moderate (100-200 kg N/ha/yr) for cereals and ii) low (<100 kg N/ha/yr) for legumes. More in detail, the amounts per crop were: 134.60 kg N ha^{-1} for spelt, 138.28 kg N ha^{-1} for wheat; 40.04 kg N ha^{-1} for chickpea and 62.36 kg N ha^{-1} for lentil. The organic fertilizer was mixed in the soil by means of plough or chisel, according to the tillage treatment.

In September 2012 and 2014, cover crops were sown in the plots corresponding to green manure treatment (G+). A mixture of different crop species in different proportion and with appropriate sowing density was used: oat (*Avena sativa* L., 30.92%, 45.8 kg ha^{-1}), white mustard (*Sinapis alba* L., 1.01%, 1.5 kg ha^{-1}), bitter vetch (*Vicia ervilia* L. Willd., 41.23%, 61 kg ha^{-1}) and common vetch (*Vicia sativa* L., 26.80%, 39.7 kg ha^{-1}). At the end of March, green manure was incorporated into the soil by disc harrowing.

The four-year rotation was carried out with the following cash crops: spelt (*Triticum spelta* L., 2011–2012 season), chickpea (*Cicer arietinum* L., 2013) winter wheat (*Triticum aestivum* L., 2013-2014 season) and lentil (*Lens esculentum* L., 2015). The crops were cultivated on 32 plots each of 156 m^2 (13 m × 12 m), thus allowing the use of regular-sized farming equipment.

<u>Measurements</u>

1. Crop measurements

Four permanent frames of 1 m × 1 m were randomly established in each plot. The total aboveground biomass of the cash crops was collected before harvest in each frame and oven-dried at 60 °C for 48 h. All samplings were limited to the inner 9 m × 8 m of each plot. The plant N content (% N) was evaluated using the Dumas combustion method (Kalra, 1998), allowing the calculation of crop N uptake (N content × dry biomass; Kg ha^{-1}). Nitrogen use indices were also calculated as follows: i) N utilization efficiency index (NUE) as the ratio of yield to N uptake (kg kg^{-1}), and N uptake efficiency as the ratio of total plant N to total N supply, according to Montemurro (2010) and to Lòpez-Bellido and Lòpez-Bellido (2001) terminologies.

Cereals and chickpea grain yields (lentil didn't produce grain because extended drought dramatically affected flowering, therefore, no final product was obtained) were harvested by a plot combine (Elite, WINTERSTEIGER, Inc.) in the inner 9 m × 8 m of each plot. The straw of the crops was not removed from the field. The spelt straw was chopped by a straw chopper hammer (BB-P-240, Belafer) before it was incorporated into the soil by disc harrowing.

2. Soil sampling and analysis

At the start (November 2011) and at the end (November 2015) of the field experimental trial, 20 soil cores of 2.5 cm of diameter were systematically extracted at 0-20 cm soil depth (i.e., reduced depth, since it is a soil prone to deep compaction), every 2 meters of distance in each plot.

Each set of 20 cores extracted at each plot constituted a sample. Soil samples were air dried, ground to pass a 2-mm sieve, and then analyzed. Total nitrogen content was determined through dry combustion (Bremmer, 1996) with a LECO© Truspec CHNS analyser.

A surface balance for N (Watson et al., 2002) of the whole experiment, for each treatments combination, which is corresponding to the N surplus/deficit as reported by Montemurro et al. (2010), was calculated as follows:

$$N\ surplus/deficit = (N\ supply + (Final\ SMN - Initial\ SMN)) - N\ uptake \quad (1)$$

where N supply was: N from fertilizer + N fixed by green manure + N fixed by legume cash crop + N deposition (estimated as 18 kg N ha^{-1}). Moreover, N left into the soil after legume was determined, as the difference between N supply and N uptake.

Calculation of N-fixation values (BNF, biologically nitrogen fixation) was made based on the formula proposed by Høgh-Jensen et al. (2004), modified by Hansen et al. (2014):

*BNF = yield_DM * N content * Nfix * 1.55*
(2)

where:

yield_DM: t ha^{-1} of yield dry matter;

Nfix: % of aboveground nitrogen derived from the atmosphere according to Amossé et al. (2013);

1.55: coefficient obtained from (1 + $N_{root+stubble}$ + $N_{transsoil}$ + $N_{immobile}$), according to Hansen et al. (2014).

3. Statistical analysis

Data were analyzed using General Linear Models (GLM) procedure. Mean comparison was carried out according to the SNK at P ≤ 0.05 probability level. The selected analysis was performed by SAS/STAT software, release 9.3, 2012 (SAS Institute Inc., Cary, NC, USA), considering year as random and treatments as fixed factors.

Results

Temperature and rainfall during the experimental trial

As far as the climatic conditions during the rotation is concerned, over the period December 2011- July 2012, the total rainfall was 221 mm showing an increasing trend till to a peak in April and May (85 and 72 mm, respectively) followed by a notable decrease in June (5 mm; figure 1). Mean monthly temperature showed higher values in June and July (23 and 24 °C, respectively). Similarly, chickpea (in 2013) cycle had higher rainfall in April (84 mm, respectively) and the highest mean temperature in July (25 °C), whereas the total rainfall was 175 mm. The wheat cycle (2013-2014) was characterized by a more variable rainfall pattern, showing peaks in January (39 mm), May (51 mm) and August (52 mm) and a total rainfall of 294 mm over the period. As regards mean monthly temperature, the highest values were found in July and August (about 23 °C both). Finally, during lentil cropping (2015), total rainfall was very low from April to August (146 mm), reaching a peak in August (56 mm), when the crop was destroyed and incorporated into the soil because of no seed production. From April to June, the most critical period for lentils growth, the rainfall was 67.5% lower than the mean last 30 years. The temperature was the highest in July with 27 °C (Figure 1).

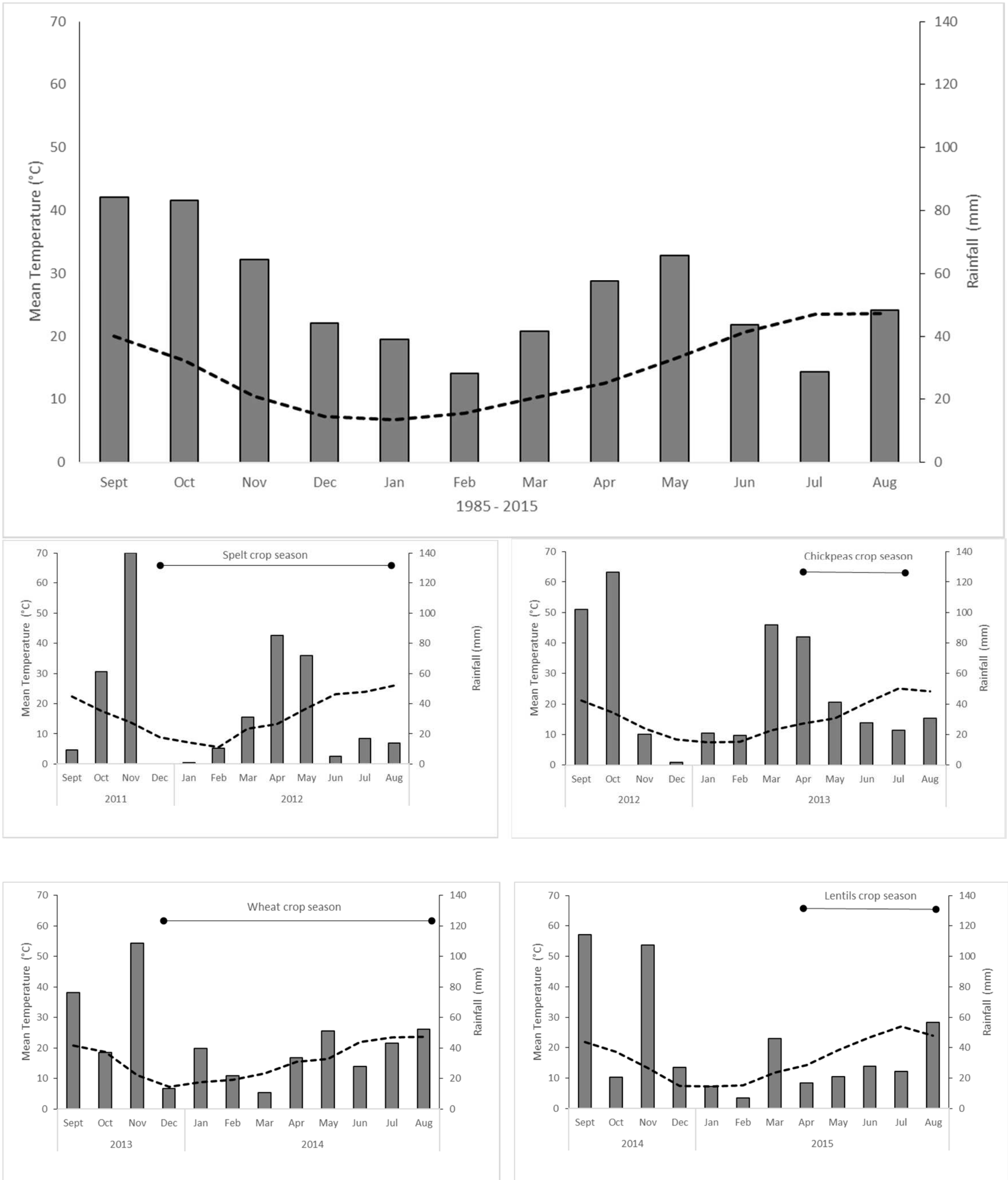

Figure 1. Mean monthly temperature and total rainfall of the area in the last 20 years (top) and during each cash crop growing season of the four year rotation.

Effects of green manure, organic fertilization and tillage strategies on crops biomass, yield and N efficiency parameters

The results of the analysis of variance on the effect of tillage, fertilization and green manure and their interactions on biomass, yield and N efficiency parameters of the four crops in rotation are reported in table 1 and 2. Significant main effects of fertilization in spelt and wheat for all parameters was found (Table 1). As regards the leguminous crops (Table 2), the fertilization revealed a significant effect on N uptake efficiency both of chickpea and lentil. Moreover, green manure had a significant effect on N supply of lentil and on the N uptake efficiency of both legumes, as well as the two-way interaction F × G had a significant effect.

Table 3 reports crops biomass and yield, except yield for lentil, since production failed. No significant differences were recorded between tillage systems for biomass and yield of each crop, as well as no significant differences were recorded between green manure and fertilization treatments for legumes.

By contrast, significant differences were found for both cereals between fertilization treatments. In particular, spelt biomass and yield were significantly higher with F+ by 21% and 15%, respectively, as compared to F-, whereas in wheat biomass and yield were higher by 27% and 36%, respectively.

No significant differences were recorded between tillage systems for cash crops N efficiency parameters (NUE and N uptake efficiency). The same parameters revealed that for cereals the F+ treatment determined lower values than F- (Table 4). More in depth, for spelt crop the NUE and N uptake efficiency were significantly lower in +F by 19% and 86%, than in F-, respectively, whereas for wheat they were lower by 24% and 84%, respectively. As regards the two leguminous crops, N uptake efficiency showed better results without green manure and fertilization, in comparison with G+ and F+ plots. However, in this case the two-way interaction F+ × G+ should be analyzed, which had a significant effect (Figure 2). The best N uptake efficiency results were obtained for the double negative control (G- × F-).

Table 1. Analysis of variance for the effect of tillage and fertilization on biomass (d.m.), yield and N efficiency parameters of spelt and wheat crop.

Treatments	spelt	wheat	spelt	wheat	spelt	wheat	spelt	wheat	spelt	wheat	spelt	wheat
	biomass		yield		N uptake		NUE		N supply		N uptake efficiency	
Tillage (T)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fertilization (F)	***	**	***	**	***	***	***	**	***	***	***	***
T × F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

, * = Significant at the P<0.01 and 0.001 probability levels, respectively. n.s. =not significant.

Table 2. Analysis of variance for the effect of tillage, fertilization and green manure on biomass (d.m.), yield and N efficiency parameters of chickpea and lentil crop.

Treatments	chickpea	lentil	chickpea	lentil	chickpea	lentil	chickpea	lentil	chickpea	lentil	chickpea	lentil
	biomass		yield		N uptake		NUE		N supply		N uptake efficiency	
Tillage (T)	ns	ns	ns	-	ns	ns	ns	-	ns	ns	ns	ns
Fertilization (F)	ns	ns	ns	-	ns	ns	ns	-	ns	ns	***	***
Green manure (G)	ns	ns	ns	-	ns	ns	ns	-	ns	**	***	***
T × F	ns	ns	ns	-	ns	ns	ns	-	ns	ns	ns	ns
T × G	ns	ns	ns	-	ns	ns	ns	-	ns	ns	ns	ns
F × G	ns	ns	ns	-	ns	ns	ns	-	ns	ns	***	***
T × F × G	ns	ns	ns	-	ns	ns	ns	-	ns	ns	ns	ns

*, *** = Significant at the P<0.05 and 0.001 probability levels, respectively. n.s.= not significant.

Table 3. Cash crops biomass and yield (except for lentil). Means (± Std Dev) are presented under different tillage, green manure and fertilizer strategies (P: conventional plough; C: reduced chisel; F+: composted farmyard; F-: no fertilizer; G+: green manure; G-: no green manure).

Treatments	Spelt		Chickpea		Wheat		Lentil	
	Biomass ($t\ ha^{-1}$)	Yield ($t\ ha^{-1}$)	Biomass ($t\ ha^{-1}$)	Yield ($t\ ha^{-1}$)	Biomass ($t\ ha^{-1}$)	Yield ($t\ ha^{-1}$)	Biomass ($t\ ha^{-1}$)	Yield ($t\ ha^{-1}$)
Tillage								
P	6.80a ±1.02	2.33a ±0.19	0.87a ±0.31	0.38a ±0.13	8.00a ±1.51	3.23a ±0.71	0.91a ±0.27	-
C	6.21a ±0.87	2.32a ±0.32	0.84a ±0.29	0.38a ±0.16	7.75a ±1.44	3.16a ±0.71	0.67a ±0.67	-
Green manure								
G+	-	-	0.86a ±0.29	0.39a ±0.15	-	-	0.81a ±0.28	-
G-	-	-	0.85a ±0.30	0.38a ±0.13	-	-	0.76a ±0.28	-
Fertilization								
F+	7.11a ±0.69	2.49a ±0.18	0.76a ±0.28	0.32a ±0.15	8.81a ±1.14	3.68a ±0.53	0.72a ±0.24	-
F-	5.89b ±0.84	2.16b ±0.21	0.95a ±0.27	0.44a ±0.09	6.94b ±1.11	2.71b ±0.48	0.86a ±0.30	-
Means	6.50	2.33	0.86	0.38	7.88	3.20	0.79	-

Notes: For each factor, the mean values in each column followed by a different letter are significantly different between treatments according to SNK.

Table 4. Cash crops N efficiency parameters. Means (± Std Dev) are presented under different tillage, green manure and fertilizer strategies (P: conventional plough; C: reduced chisel; F+: composted farmyard; F-: no fertilizer; G+: green manure; G-: no green manure)

Treatment	Spelt		Chickpea		Wheat		Lentil
	NUE	N uptake efficiency	NUE	N uptake efficiency	NUE	N uptake efficiency	N uptake efficiency
Tillage							
P	18.73a ±3.68	0.54a ±0.42	8.23a ±2.24	0.73a ±0.45	17.84a ±3.66	0.62a ±0.46	0.60a ±0.52
C	20.47a ±3.37	0.55a ±0.43	8.91a ±2.80	0.72a ±0.48	17.75a ±3.02	0.63a ±0.47	0.54a ±0.48
Green manure							
G+	-	-	8.36a ±2.27	0.37b ±0.06	-	-	0.28b ±0.10
G-	-	-	8.78a ±2.80	1.08a ±0.40	-	-	0.86a ±0.56
Fertilization							
F+	17.52b ±3.34	0.13b ±0.02	7.43a ±1.80	0.54b ±0.22	15.37b ±2.27	0.17b ±0.01	0.27b ±0.08
F-	21.67a ±2.50	0.95a ±0.07	9.72a ± 2.65	0.91a ±0.56	20.22a ±2.20	1.08a ±0.08	0.88a ±0.55
Means	19.60	0.54	8.57	0.73	17.80	0.63	0.57

Notes: For each factor, the mean values in each column followed by a different letter are significantly different between treatments according to SNK.

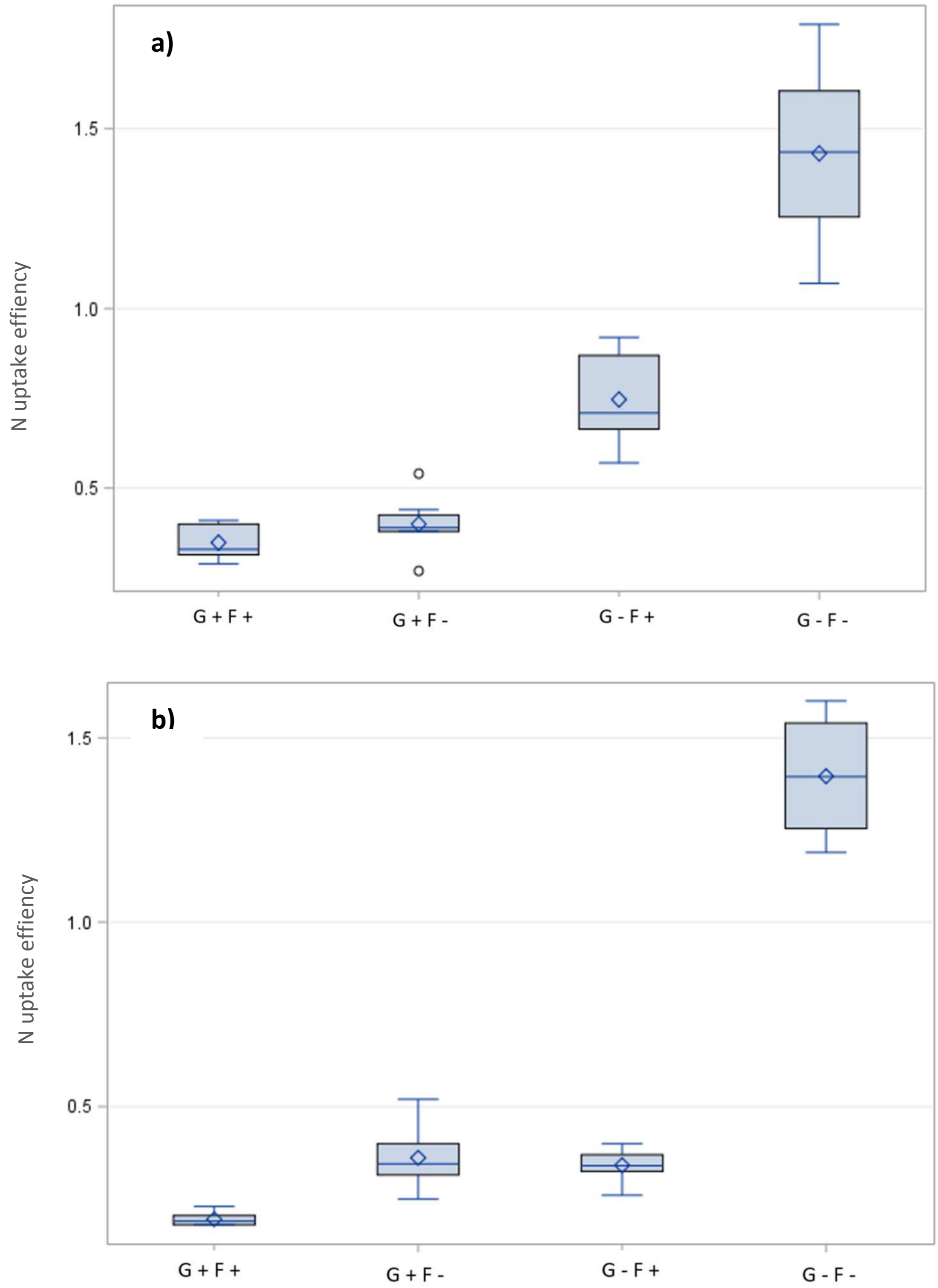

Figure 2. Box plot for the interaction between G (green manure) and F (farmyard manure) on the N uptake efficiency of the legume crops: (a) chickpea and b) lentil.

As regards chickpea, the worst results were found in G+ both in interaction with F+ and F-, whereas G- × F+ showed intermediate value. In lentil, no substantial differences were found among the other interactions, which showed significant lower values (particularly for G+ × F+) as compared to G- × F- (Figure 2).

N balance

In table 5 the soil N surplus/deficit by each treatment combination, over the 4-year experiment, is reported, with means ± standard deviation for each SMN, N uptake and N supply. The chickpea and lentil crops had a different performance: the first legume took up 38-52 kg N ha^{-1}, whereas lentil took up 16-34 kg N ha^{-1}. Despite this difference, the P-G-F- combination showed the highest N uptake, in absolute value, for both crops. The chickpea took up a N rate slightly higher than the N supply for all treatment combinations, except C-G-F+ and C-G-F+. On the contrary, lentil took up less N than the supplied rate, except for P-G-F- that showed a soil N depletion.

The N balance showed that the N surplus/deficit was the highest in G-F+ combination both with P and C tillage. By contrast, the P-G-F+ treatments combination showed the lowest value (deficit of 49.94 kg N ha^{-1}). Moreover, the P-G-F- combination determined a higher N deficit (by more than 100%) compared to P-G-F+. Finally, an intermediate surplus value was obtained by C-G-F+ and C-G-F-.

Discussion

Treatments effect on cash crops biomass, yield and N efficiency

The type of tillage did not affect N uptake when associated with various sources of N (table 3 and 4), unlike Ishaq et al. (2001) results. In particular, NUE values did not show any difference between P and C in all cash crops. This result is in accordance to Montemurro (2009) and in contrast to Lòpez-Bellido and Lòpez-Bellido (2001), which found greater values in conventional than no tillage system.

In general, legumes do not need supplemental N fertilization (Clayton et al., 2004). In particular, chickpea crop can obtain at least some N by N_2 fixation, but the proportion of N fixed is negatively correlated with availability of inorganic N in the soil (Walley et al., 2005). However, in this crop rotation, chickpea took up a N rate slightly higher than the N supply for almost all combinations of treatments (except for C-G-F+ and P-G-F+; table 5), thus suggesting that unfavorable growing conditions for the symbiotic association likely occurred (e.g., reduced soil water availability, high soil mineralization rate, etc.).

Table 5. Soil N surplus/deficit (kg N ha−1) for the whole field experiment divided by treatment combinations (P: conventional plough; RC: reduced chisel; F: composted farmyard; NF: no fertilizer; GM: green manure; NoM: no green manure; SMN: soil mineral nitrogen).

	P G- F-	Std Dev	P G- F+	Std Dev	C G- F-	Std Dev	C G- F+	Std Dev	P G+ F-	Std Dev	P G+ F+	Std Dev	C G+ F-	Std Dev	C G+ F+	Std Dev
2012																
SMN t_0	1643.00	± 116.00	1702	±163.50	1647.00	± 170.70	1698.00	± 102.8	-	-	-	-	-	-	-	-
N uptake	107.30	± 16.85	155.20	± 37.12	95.86	± 20.00	139.30	± 22.45	-	-	-	-	-	-	-	-
N supply	23.55	± 1.44	20.14	± 3.40	22.93	± 2.40	27.07	± 3.00	-	-	-	-	-	-	-	-
2013																
N uptake	46.60	± 18.34	47.90	± 17.56	42.68	± 20.05	40.66	± 11.36	53.17	± 9.07	44.83	± 27.16	51.02	± 8.87	38.14	± 13.90
N supply	43.53	± 1.86	43.07	± 2.81	42.48	± 3.50	43.23	± 2.40	44.31	± 3.14	44.40	± 3.04	42.44	± 0.71	44.81	± 1.48
N after legume	-3.07		-4.83		-0.50		2.57		-8.86		-0.43		-8.58		6.67	
2014																
N uptake	137.90	± 28.94	245.20	± 46.80	131.6	± 18.34	241.9	± 44.79	-	-	-	-	-	-	-	-
N supply	26.56	± 2.10	35.79	± 3.46	26.45	± 1.73	36.41	± 3.49	-	-		-	-	-	-	-
2015																
SMN t_i									1445.00		1361.00		1468.00		1383.00	
SMN t_f	1733	± 90.44	1987.00	±163.30	1759.00	± 142.50	21.88	± 212.70	1726.00	± 142.00	2082.00	± 142.00	1653.00	± 221.50	2118.00	± 178.70
N uptake	21.60	± 2.91	25.03	± 11.07	18.65	± 9.74	16.11	± 6.46	34.00	± 5.90	23.53	± 5.90	18.45	± 7.73	16.03	± 3.32
N supply	27.90	± 2.95	31.39	± 2.22	25.40	± 2.63	26.41	± 3.30	30.91	± 2.84	30.87	± 2.84	28.19	± 3.67	28.55	± 320.00
N after legume	6.30		6.36		6.75		10.30		-3.09		7.34		9.74		12.52	
N surplus/deficit	**-101.86**		**-49.94**		**-59.83**		**185.00**		**269.00**		**728.00**		**186.00**		**754.00**	

The mean values of NUE recorded in this study for cereals were very low, indicating that they require a higher N fertilization rate for optimizing yields. Moreover, the variable rainfall distribution during the cycle could have influenced crop N uptake (figure 1). The lower values of the N efficiency parameters in fertilized than in not-fertilized plots for cereals were probably related to the peculiar characteristics of the compost used (partially decomposed), determining a higher yield in amended plots than those without compost, but a low efficiency in N use in the short term, since N uptake was limited by the slow-release of N by mineralization of compost.

By contrast, the more stabilized fraction of the compost could improve soil fertility and influence N efficiency in the long term (Diacono and Montemurro, 2010). These results are in agreement with Montemurro (2009), who found that N utilization efficiency was not correlated with wheat grain yield.

In chickpea, the best N uptake efficiency results were obtained in plots with neither compost nor green manure (i.e., double negative control) and the worst in plots with green manure irrespective of amendments with compost (figure 2), suggesting that the green manure preceding this legume crop could not be a sustainable solution. Conversely, the fertilizer treatment alone could be used, despite the NUE value probably did not derive from the fertilizer effect only, but also could be due to other biotic and abiotic factors that can influence the success of the legume-Rhizobium association. Results on yield and N efficiency parameters for chickpea are in accordance with Bonfil and Pinthus (1995), which reported that application of 100 kg N ha^{-1} promoted N uptake by chickpea without enhancing final seed yield, maybe because additional fertilizer N encouraged vegetative growth instead of assimilates allocation to seeds.

Also in lentil, all the green manure × fertilization interactions other than the greatest one (G- × F-) had low N uptake efficiency values, confirming that additional fertilization for legumes should be avoided, irrespective of the N source (figure 2). In particular, lentil took up less N than the rate supplied, except in plots managed with mouldboard ploughing only fertilized with green manure (table 5); this might be explained by the better incorporation by tillage (and, then, mineralization) of the cover crop residues in the soil, since this crop is not able to acquire much N_{min}-N from soil layers deeper than 0.6 m, due to the shallow root system (Schmidtke et al. 2004). However, this last result could also have been due to other biotic and abiotic factors.

The N surplus/deficit

Considering the N balance over the whole crops rotation (table 5), as it was expected the N surplus was the highest in the G+ combinations with F+ (both P and C), suggesting that a share of the N by green manure and organic fertilizer remained in soil, with the risk of losses by leaching. Therefore, a possible solution could be to avoid G+ combined with F+ in cereal – legume rotations. Also, this surplus should be considered in a long-term rotation, including a crop able to uptake this mineral N, thus avoiding environmental risks (Montemurro et al. 2010). This last speculation is further supported by the N deficit found in the P-G-F+ combination, that would indicate the possibility of maintaining soil fertility with a slightest soil N depauperation with a single N source (i.e., by F+ only). On the other hand, the P-G-F- combination seemed to be not sustainable, since it determined a higher N deficit, whereas the surplus obtained by C-G-F+ and C-G+F- suggests the needs to better manage the rotation in these two cases, to avoid N loss.

Conclusions

This study assessed the effect of soil tillage, organic fertilizer and green manure on N use in a cereal-legume rotation in organic farming. Different tillage methods did not induce different effects on yield and N efficiency parameters, whereas both organic fertilization and cover crops incorporation into the soil caused significant differences in each cropping cycle, depending on the crop species. Results suggest that legumes do not need supplemental N fertilization and on the whole, since N surplus in the soil was the highest in plots combining green manure with farmyard manure (both with mouldboard ploughing and chiseling), therefore, this combined practice should be avoided in cereal – legume rotations. Moreover, the residual effect over years should be considered when formulating fertilizer requirements in a specific crop rotation.

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References

Amossé C., Jeuffroy M-H., Mary B., David C. 2013. Contribution of relay intercropping with legume cover crops on nitrogen dynamics in organic grain systems. Nutrient Cycling in Agro-Ecosystems 97, (1-3).

Bonfil D.J., Pinthus M.J., 1995. Response of chickpea to nitrogen and a comparison of the factors affecting chickpea seed yield with those affecting wheat grain yield. Exp. Agric. 31, 39–47.

Bremner, J.M. 1996. Nitrogen-total. Methods of Soil Analysis Part 3—Chemical Methods, (methodsofsoilan3), 1085-1121.

Clayton G.W., Rice W.A., Lupwayi N.Z., Johnston A.M., Lafond G.P., Grant C.A., Walley F. 2004. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N_2 fixation and nitrogen partitioning. Can. J. Plant Sci. 84, 79–88.

Crews T.E., Peoples M.B. 2004. Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agricul.., Ecosys., Environ. 102, 279–297.

Davis, S.A., 2010. Cover-crop roller-crimper contributes to weed management in no-till soybean. Weed Sci. 58, 300-309.

Diacono M., Montemurro F. 2010. Long-term effects of organic amendments on soil fertility. A review. Agron. Sust. Develop., 30(2), 401-422.

Gilmour, J.T., Mauromostaukos, A., Gale, P.M., and Norman, R.J., 1998. Kinetics of crop residue decomposition: variability among crops and years. Soil Sci. Soc. Am. J. 62, 750-755.

Hansen, S., Bernard, M.E., Richette, P., Whalen, J.K., Dörsch, P. 2014. Nitrous oxide emissions from a fertile grassland in Western Norway following the application of inorganic and organic fertilizers. Nutr. Cycl. Agroecosyst. 98, 71-85.

Høgh-Jensen H., Loges R., Jørgensen F.V., Vinther F.P., Jensen E.S. 2004. An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. Agricul. Syst. 82, 181–194.

Ishaq, M., M. Ibrahim, and R. Lal. 2001. Tillage effect on nutrient uptake by wheat and cotton as influenced by fertilizer rate. Soil Tillage Res. 62, 41–53.

López-Bellido L., López-Bellido R.J., Castillo J.E., López-Bellido F.J. 2000. Effect of tillage, crop rotation and nitrogen fertilization on wheat under rainfed Mediterranean conditions. Agron. J. 92, 1054-1063. Doi:10.2134/agronj2000.9261054x.

Lòpez-Bellido R.J., Lòpez-Bellido L. 2001. Efficiency of nitrogen in wheat under Mediterranean conditions: effect of tillage, crop rotation and N fertilization. Field Crop Res. 71, 31–46.

Mazzoncini, M., Sapkota, T. B., Bàrberi, P., Antichi, D., & Risaliti, R. 2011. Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content. Soil Till. Res. 114(2), 165-174. Doi: 10.1016/j.still.2011.05.001.

Melero S., López-Bellido R.J., López-Bellido L., Muñoz-Romero V., Moreno F., Murillo J.M. 2011. Long-term effect of tillage, rotation and nitrogen fertiliser on soil quality in a Mediterranean Vertisol. Soil Till. Res. 114(2), 97-107.

Montemurro F. 2009. Different nitrogen fertilization sources, soil tillage, and crop rotations in winter wheat: effect on yield, quality, and nitrogen utilization. J. Plant Nutr., 32 (1), 1-18.

Montemurro F. 2010. Are the organic N fertilizing strategies able to improve lettuce yield, use of nitrogen and N status? J Plant Nutr 33, 1980–1997.

Montemurro F., Ferri D., Tittarelli F., Canali S., Vitti C., 2010. Anaerobic digestate and on-farm compost application: effects on lettuce (*Lactuca sativa* L.) crop production and soil properties. Compost. Sci. Util. 18 (3), 184–193.

Pala M., Ryan J., Zhang H., Singh M., Harris H.C. 2007. Water-use efficiency of wheat-based rotation systems in a Mediterranean environment. Agr. Water Manage. 93(3), 136-144.

Pang, X.P. and Letey, J. 2000. Organic farming: challenge of timing nitrogen availability to crop nitrogen requirements. Soil Sci. Soc. Am. J. 64, 247–253.

Soil Survey Staff 1999. Soil Taxonomy. A basic system of soil classification for making and interpreting soil surveys. Agriculture Handbook 436, USDA.-NRCS. Washington DC.

Schmidtke K., Neumann A., Hof C., Rauber R. 2004. Soil and atmospheric nitrogen uptake by lentil (*Lens culinaris* Medik.) and barley (*Hordeum vulgare* ssp. nudum L.) as monocrops and intercrops. Field Crops Research. 87, 245–256.

Smukler S.M., O'Geen A.T., Jackson L.E. 2012. Assessment of best management practices for nutrient cycling: A case study on an organic farm in a Mediterranean-type climate. Journal of Soil Water Conservation. 67, 16–31.

Souza A., Colozzi-Filho A., Giller K.E., 2003. The soil microbial community and soil tillage. In: El Titi, A. (Ed.), Soil Tillage in Agroecosystems. CRC Press, London, pp. 51–81.

Van Kessel, C Hartley, C. 2000. Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? Field Crops Research 65, 165–181.

Walley F.L., Kyei-Boahen S., Hnatowich G., Stevenson C. 2005. Nitrogen and phosphorus fertility management for desi and kabuli chickpea. Canadian Journal of Plant Science. 85, 73–79.

Walkley A., Black I.A. 1934. An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents, Soil Science. 63, 251–263.Watson C.A., Bengtsson H., Ebbesvik M., Løes A.K., Myrbeck A., Salomon E., Schroder J., Stockdale E.A., 2002. A review of farm-scale nutrient budgets for organic farms as a tool for management of soil fertility. Soil Use and Management. 18, 264–273.

Discusión general

Discusión general

Capítulo I

Disminución de intensidad de laboreo y control de la flora arvense en cultivos de cereales convencionales de secano

Rendimientos del cereal

Los resultados del experimento de dos años de duración en cultivos de cereales con gestión convencional ponen de manifiesto que el rendimiento del cereal fue significativamente mayor en las parcelas con arado de vertedera con inversión de las capas del suelo comparado con las parcelas con arado de cincel sin inversión del suelo. Este patrón puede estar asociado a diferentes causas, una de ellas es la flora arvense ya que su abundancia fue mayor en las parcelas con arado de cincel (Berner et al., 2008; Peigné et al., 2007). El menor rendimiento del cereal en las parcelas con arado de cincel también puede explicarse por el al aumento de la compactación del suelo que puede limitar el flujo de agua y de nutrientes y en consecuencia, limitar la emergencia del cultivo y el crecimiento de las raíces (Peigné et al., 2007). Woźniak y Soroka et al. (2014) indican que la reducción de la intensidad del laboreo puede dar lugar a condiciones desfavorables para el crecimiento y desarrollo de los cultivos en comparación con el laboreo convencional.

El control de la flora arvense tuvo un efecto significativo sobre el rendimiento del cereal únicamente durante el primer año. El control químico mediante la aplicación de herbicidas favoreció el rendimiento del cereal durante el primer año, sin embargo, no tuvo un efecto significativo en el rendimiento del segundo año. Este efecto puede estar relacionado con la abundancia de la flora arvense, dado que, durante el primer año, el herbicida disminuyó drásticamente la abundancia de la flora arvense. Sin embargo, durante el segundo año, el control en post-emergencia, tanto mecánico como químico, no ejerció un efecto significativo en la biomasa de la flora arvense.

El control mecánico de la flora arvense pudo haber dañado el cultivo durante el primer año del experimento, ya que el rendimiento del cereal fue inferior en las parcelas gestionadas con la grada de púas flexibles que en las parcelas con herbicida y sin ningún tipo de control. La excesiva sequedad del suelo y las adversas condiciones meteorológicas tras el tratamiento dificultaron la recuperación del cultivo. La eficacia del control mediante la grada de púas flexibles durante el segundo año permite obtener rendimientos similares a los que se obtienen con el control químico y al mismo tiempo no afectar la flora arvense. La grada de púas puede ser eficaz siempre y cuando se lleve a cabo de manera adecuada en relación con la fase de crecimiento de la flora arvense y del cultivo, pero también teniendo en cuenta las condiciones climáticas (Pardo et al., 2008). Por otro lado, el cereal puede ser más sensible a las variaciones interanuales de las temperaturas y de la cantidad de precipitación durante la fase de crecimiento que al daño causado por la grada de púas flexibles (Pardo et al., 2008), sobre todo en la región mediterránea, ya que la escasez de agua combinada con las altas temperaturas en primavera puede disminuir significativamente el rendimiento de los cereales.

Flora arvense

La riqueza y abundancia de la flora arvense fueron significativamente mayores en las parcelas con arado de cincel que en las parcelas con arado de vertedera (Peigné et al., 2007) debido a que la germinación y emergencia de las semillas de las especies arvenses se ve favorecida al concentrase en la parte superficial del suelo (Gruber y Claupein, 2009). El aumento de la riqueza de las especies arvenses tras dos años de laboreo mínimo puede ser de especial interés para conservar la diversidad de la flora arvense en los cultivos, ya que puede promover una mayor diversificación de nichos ecológicos manteniendo una alta diversidad de especies pero menor abundancia de cada especie (Murphy et al., 2006; Plaza et al., 2011). Sin embargo, el uso del laboreo mínimo puede aumentar la infestación de la flora arvense, especialmente de las especies perennes, las cuáles pueden afectar el rendimiento del cultivo al ser de difícil control (Gruber y Claupein, 2009). En este experimento, la abundancia de *Convolvulus arvensis* L., una especie perenne muy problemática en los cultivos herbáceos anuales ha incrementado el 96% del primer al segundo año de estudio debido a su relativa tolerancia al laboreo (Gruber y Claupein, 2009).

Los resultados del efecto del tipo de control sobre la flora arvense indican que la aplicación de herbicidas disminuyó drásticamente la abundancia y la riqueza de especies de la flora arvense en comparación con el control mecánico. Sans et al. (2011) sugieren que para preservar la biodiversidad y los servicios ecosistémicos asociados, el objetivo del control de flora arvense no debe ser la completa eliminación, sino la reducción del tamaño de las poblaciones por debajo del umbral de competencia con los cultivos para obtener rendimientos económicamente aceptables. A pesar de que la abundancia de la flora arvense no disminuyó en las parcelas con control mecánico mediante la grada de púas flexible, no se detectó un incremento acumulado de la flora arvense en el segundo año. Los agricultores suelen aplicar herbicidas rutinariamente todos los años porque temen que la infestación de flora arvense sea mayor al año siguiente (Bàrberi, 2002; Peigné et al., 2015), pero en este experimento no encontramos este efecto. En conclusión, a la hora de aplicar cualquier método de control de la flora arvense, es importante hacer un balance entre las pérdidas de cosecha y el coste del tratamiento y el posible daño al cultivo debido a la fitotoxicidad de los herbicidas y el daño mecánico por las grada de púas flexibles (Armengot et al., 2012).

Capítulos II, III y IV

Diseño de sistemas ecológicos sostenibles en cultivos herbáceos extensivos de secano

El análisis del rendimiento de los cultivos de cereales y de leguminosas y la abundancia de la flora arvense a lo largo de los 4 años de la rotación muestra que la fertilización es el factor más importante. Los mayores rendimientos de los cultivos de cereal (espelta y trigo) en las parcelas fertilizadas con estiércol semicompostado ponen de manifiesto que la fertilización es crucial para mantener una producción adecuada, evitar la pérdida de nutrientes en el suelo y mantener la sostenibilidad de los sistemas ecológicos (Fließbach et al., 2007). Sin embargo, el rendimiento del cultivo de garbanzo y la biomasa aérea del cultivo de lenteja disminuyó en las parcelas fertilizadas con estiércol.

El efecto de la fertilización sobre la abundancia de la flora arvense varía en función del tipo de cultivo. La biomasa aérea de flora arvense fue significativamente menor en las parcelas con fertilización durante el cultivo de cereal, mientras que la biomasa de flora arvense tendió a ser mayor en las parcelas fertilizadas durante el cultivo de lenteja. Estos resultados podrían explicarse considerando que, en general, las leguminosas no necesitan fertilización suplementaria con N (Clayton et al., 2003) debido a que pueden obtener el N a través de la fijación de N_2 atmosférico (Walley et al., 2005) y la excesiva fertilización podría haber favorecido el crecimiento de las especies arvenses y en consecuencia haber limitado el crecimiento del cultivo de la leguminosa.

El tipo de laboreo no tuvo efectos significativos en el rendimiento de los cereales (espelta y trigo de invierno) y garbanzo, aunque muchos estudios de regiones templadas señalan que el rendimiento disminuye en los cultivos gestionados con el arado de cincel, sin inversión de las capas superficiales del suelo (Cooper et al., 2016) a causa del efecto combinado de la limitación de nutrientes y la competencia con las especies arvenses (Mäder y Berner, 2011; Peigné et al., 2013). La disponibilidad de nutrientes para los cultivos no parece que se haya visto afectada por el laboreo mínimo en nuestro experimento. La ausencia de diferencias significativas de rendimiento de los cultivos en relación con el tipo de laboreo también ha sido constatada por López-Garrido et al. (2014) en condiciones mediterráneas. Sin embargo, en el último año, la biomasa de lentejas fue mayor en las parcelas con arado de vertedera, coincidiendo con la menor abundancia de la flora arvense en estas parcelas. Diversos estudios a largo plazo señalan que el rendimiento aumenta después de varios años de laboreo mínimo (6 – 7 años) a causa de la mejora de la estructura del suelo que favorece la retención de la humedad del suelo en consecuencia la reducción del estrés hídrico en épocas de escasez de agua (Krauss et al., 2010; Tørresen et al., 2003). Las diferencias entre los diversos estudios en el efecto del laboreo mínimo sobre el rendimiento de los cultivos se explican por la importancia e influencia de factores como la gestión de los cultivos, el tipo de suelo y las condiciones climáticas (Peigné et al., 2013).

La abundancia de la flora arvense no afectó el rendimiento de la espelta y el trigo a causa de la capacidad competitiva del cultivo, que ocasionó que la biomasa no superara el umbral crítico de competencia. Por el contrario, el rendimiento del cultivo de garbanzo y la biomasa del cultivo de lenteja disminuyó probablemente a causa de la abundancia de la flora arvense como refleja el efecto estadísticamente significativo de incorporación al modelo lineal de la biomasa de la flora arvense como variable concomitante. Las leguminosas anuales se caracterizan por ser muy sensibles a la competencia ejercida por las especies arvenses; así, por ejemplo, la lenteja es muy sensible a la competencia debido a su baja estatura, a su lento establecimiento y al limitado crecimiento vegetativo (Ahmadi et al., 2016). El efecto negativo de la flora arvense sobre el crecimiento del garbanzo y la lenteja fue mayor en las parcelas fertilizadas. La rápida liberación de nutrientes, a veces, puede ser ventajosa para la flora arvense ya que suele ser capaz de absorber nutrientes en etapas tempranas del crecimiento con mayor rapidez y eficacia que los cultivos (Bàrberi, 2002). Esto podría ser una explicación de la mayor abundancia de la flora arvense en las parcelas con fertilización y abonos verdes en comparación con el cultivo de leguminosas. En el caso del cultivo de lenteja, el cultivo se agostó a causa de la intensa sequía de primavera y las poblaciones de las especies arvenses proliferaron especialmente en las parcelas con estiércol. Además, la abundancia de la flora arvense en los cultivos de garbanzo y lenteja se explica por el insuficiente control post-emergencia, y pone de manifiesto que la mejora del control de la vegetación arvense en los cultivos de leguminosas es crítico para la viabilidad de la producción ecológica de leguminosas para grano.

La incorporación de los abonos verdes antes del cultivo de leguminosa de la rotación favoreció el control de la flora arvense durante su período de crecimiento, pero no afecto significativamente la abundancia de la flora arvense durante el cultivo posterior. Si bien se esperaba encontrar un efecto positivo o neutro del cultivo de cobertura al ser incorporado como abonos verde sobre la abundancia de la flora arvense durante el cultivo posterior, nuestros resultados indican que la abundancia de la flora arvense fue mayor en las parcelas fertilizadas y con abonos verdes durante el cultivo de lentejas. Este resultado sugiere que la introducción de los cultivos de cobertura debe ser cuidadosamente evaluado en función de los cultivos de la rotación y se debe tener en cuenta la posible competencia por los nutrientes y el agua (Plaza-Bonilla et al., 2016).

<u>Cambios en la calidad y la fertilidad del suelo</u>

El principal factor para mejorar la sostenibilidad de los sistemas agrícolas de secano es la fertilidad del suelo debido a que es la base para los procesos que forman parte de la dinámica de los agrosistemas. El aumento de la fertilidad y la conservación del suelo exigen gestionar de forma eficiente la humedad del suelo y el contenido de materia orgánica (Meco et al., 2011). La cantidad de materia orgánica almacenada en el suelo viene determinada por el balance de dos procesos bióticos: la producción de materia orgánica por la vegetación y la descomposición de la materia orgánica por los organismos del suelo, y estos procesos a la vez, dependen de los factores físicos, químicos y biológicos del suelo (Guo y Gifford, 2002). Los resultados obtenidos sugieren que la fertilización es un factor importante en los sistemas ecológicos de secano debido a que contribuye a mantener los niveles de nutrientes del suelo y puede aumentar el nivel de materia orgánica (Alvarez, 2005; Krauss et al., 2010; Maltas et al., 2013). En nuestro experimento de cuatro años, el contenido de SOC, la reserva de carbono y el N fueron mayores en las parcelas con fertilización. La fijación biológica de N_2 atmosférico aporta la mayor parte del nitrógeno incorporado a los ecosistemas terrestres, mientras que la aplicación de estiércol es necesaria para obtener buenos rendimientos y para mantener la sostenibilidad de los sistemas de cultivo (Fließbach et al., 2007; Maltas et al., 2013).

La pérdida global de SOC al final del experimento puede estar relacionada con la acelerada mineralización de la materia orgánica debido a la aplicación de estiércol poco compostado y estabilizado. Diversos estudios han demostrado que la tasa de mineralización del SOC está relacionada con la aplicación de las formas lábiles de SOC, ya que ello induce también la mineralización del SOC nativo del suelo y acelera la descomposición de la materia orgánica del suelo (Molina-Herrera y Romanyà, 2015; Romanyà et al., 2012).

También es importante señalar que la inversión de las capas superficiales del suelo puede reducir la reserva de carbono en la capa superior del suelo, y esto se ve agravado por la falta de fertilización. Las mayores reservas de carbono en las parcelas con arado de vertedera en las capas más profundas se deben a la inversión del suelo, que incorpora materia orgánica y disminuye el SOC en la superficie. Por el contrario, el uso del arado de cincel sin inversión del suelo, puede estratificar el SOC y, en consecuencia, las reservas de carbono del suelo (Cooper et al., 2016). Asimismo, la mayor reserva de carbono en capas más profundas del suelo en comparación con las superficiales, están relacionadas con la mayor densidad aparente del suelo a mayor profundidad (Peigné et al., 2007).

La ausencia de efecto de la incorporación de los abonos verdes en el contenido de SOC podría estar relacionada con la rápida mineralización. Romanyà et al. (2012) señalan que la materia orgánica que ya ha sufrido algún grado de descomposición, como el estiércol semicompostado, es más eficiente en el almacenamiento de SOC en el suelo que la materia orgánica fresca. Además, Masilionyte et al. (2017) señalan que la incorporación de cultivos de cobertura ricos en leguminosas con una baja relación C:N pueden acelerar la descomposición del SOC del suelo. El estado de madurez en que se encuentra la leguminosa en el momento de enterrarla, incide en el aumento de la fertilidad del suelo, si se entierra demasiado verde, los procesos de mineralización primarán sobre los de humificación (Lacasta et al., 2013).

La disminución del 32% de la relación C:N después de los cuatro años del experimento indica que la materia orgánica lábil se descompuso rápidamente, y que la que permanece en el suelo es más recalcitrante y en consecuencia menos disponible (Molina-Herrera y Romanyà, 2015). La tasa de liberación de nutrientes depende en gran medida de la relación C:N, pero también de las propiedades del suelo, las condiciones climáticas y el método de incorporación (Bàrberi, 2002).

Lombrices de tierra y biomasa microbiana del suelo

El mantenimiento de la materia orgánica en el suelo es importante ya que protege el suelo de la degradación, facilita la dinámica de los ciclos biogeoquímicos de los nutrientes, la eficiencia en el uso y conservación del agua del suelo y conserva la biodiversidad edáfica (Meco et al., 2011). Además, la fertilización orgánica favorece a largo plazo las poblaciones de lombrices de tierra debido a la aportación de nutrientes, el incremento de la materia orgánica en el suelo y una mayor producción de restos vegetales, que en última instancia representa mayor cantidad de alimento para las lombrices de tierra. El estiércol semicompostado es considerado uno de los más adecuados para el crecimiento de las poblaciones de lombrices de tierra (Brown et al., 2004; Curry, 2004). Nuestro estudio confirma que la fertilización orgánica mejora el crecimiento y la reproducción de las lombrices de tierra. La biomasa microbiana también aumentó con la fertilización como pone de manifiesto el aumento del carbono microbiano (C_{mic}) y del nitrógeno microbiano (N_{mic}). El aumento global de C_{mic} en todas las parcelas después de los cuatro años de experimento se puede explicar a causa de la aportación de insumos orgánicos al suelo disponibles para la descomposición. La aplicación de fuentes de SOC lábiles promueve la actividad microbiana del suelo y en consecuencia, el aumento de la biomasa microbiana (Molina-Herrera y Romanyà, 2015). Por el contrario, la disminución del N_{mic} podría deberse a que el suelo es pobre en nutrientes y a que los aportes externos de N estaban compuestos mayoritariamente por formas de N no disponible para la descomposición por los microorganismos (Molina-Herrera y Romanyà, 2015).

De manera general, el arado de vertedera influye negativamente en el tamaño de las poblaciones de lombrices de tierra a causa de la intensa perturbación que representa la inversión de las capas superficiales del suelo. Sin embargo, la abundancia de individuos juveniles en las parcelas con arado de vertedera fue significativamente mayor que en las parcelas con arado de cincel. El aumento de la abundancia de lombrices juveniles tras el laboreo está relacionado con la respuesta de las lombrices a la presión creada por el arado y va encaminada a la recuperación del tamaño de las poblaciones (Boström, 1995). El tipo de laboreo también afecta la biomasa microbiana y su distribución a lo largo del perfil del suelo. En las parcelas con arado de cincel la biomasa microbiana tiende a ser mayor en las capas superficiales, y disminuye con la profundidad, mientras que en las parcelas con arado de vertedera la biomasa microbiana se distribuye de forma más homogénea a lo largo del perfil del suelo (Vian et al., 2009).

La pérdida global de SOC después de cuatro años de la rotación y el aumento de la relación C_{mic}/SOC reflejan que los microorganismos del suelo están consumiendo la materia orgánica. La menor proporción de la relación C_{mic}/SOC en las parcelas con arado de cincel y sin fertilización respecto de las parcelas con fertilización se explica por la dificultad de acceso al SOC nativo por parte de los microorganismos a causa de la menor perturbación del suelo que favorece la presencia de zonas del suelo más compactadas (Sparling, 1992; Vian et al., 2009). En cambio, en las parcelas con arado de vertedera el C_{mic}/SOC no varía en relación con la fertilización ya que la inversión de las capas del suelo puede fragmentar la estructura del suelo y hacer accesible el SOC nativo del suelo a los microorganismos (Molina-Herrera y Romanyà, 2015).

La incorporación de los cultivos de cobertura como abonos verdes en la rotación no afecto significativamente la densidad de lombrices ni la biomasa microbiana del suelo. Sin embargo, la biomasa de lombrices aumentó en las parcelas sin abonos verdes. Este resultado, que no se ajusta a la hipótesis inicial que la incorporación de materia orgánica mediante los abonos verdes favorecería a las poblaciones de lombrices de tierra, es difícil de explicar ya que pocos estudios han analizado los efectos del abono verde sobre las lombrices de tierra. Valckx et al. (2011) señalan una tendencia al rechazo de la avena (*Avena sativa* L.) como alimento por parte de las lombrices, lo que sugiere un posible efecto alelopático de la avena afectando a las lombrices de tierra. La presencia de avena en la mezcla de plantas de los cultivos de cobertura podría explicar nuestros resultados, aunque no podemos confirmar un efecto repelente del abono verde sobre las lombrices de tierra.

El análisis de la diversidad de lombrices de tierra pone de manifiesto que la riqueza de especies es baja, cuatro especies identificadas en el área de estudio, si se compara con otros estudios en climas templados donde la riqueza es entre dos y tres veces mayor (9-13 especies) (Kuntz et al., 2013; Peigné et al., 2009). La comunidad de lombrices está dominada por tres especies, *Aporrectodea trapezoides* (Duges, 1828), *Aporrectodea rosea* (Savigny, 1826), *Allolobophora georgii* (Michaelsen, 1890). La densidad de *A. rosea*, la especie más abundante, fue menor en las parcelas con arado de cincel; algunos estudios señalan que esta especie evita la compactación y el laboreo mínimo, que tiende a crear zonas más compactadas al no perturbar la estructura del suelo (Capowiez et al., 2009; Peigné et al., 2009). Por otro lado, *A. trapezoides* resultó más abundante en las parcelas sin inversión del suelo (con arado de cincel), y esto se debe a que la inversión del suelo transporta la materia orgánica hacia las capas más profundas, y ésta especie, que es anécica, se alimenta de los restos vegetales y del suelo en la el horizonte más superficial del suelo (Guitiérrez-López et al., 2016).

Balance de nitrógeno N

El nitrógeno (N) es uno de los nutrientes más importantes para el cultivo. En los últimos años, la agricultura sostenible ha revitalizado el interés en las rotaciones de cultivos y sus efectos sobre la eficiencia de la utilización de N (Montemurro, 2009). El estudio del balance del nitrógeno en los sistemas herbáceos extensivos ecológicos es esencial debido a que su producción depende tanto de la optimización del uso de los insumos que son aportados mediante las enmiendas orgánicas como de la minimización de la pérdida de nutrientes. Está claro que la fertilización es importante para la fertilidad del suelo, y para la producción de los cultivos de cereal. Por el contrario, las leguminosas no necesitan fertilización suplementaria con N (Clayton et al., 2004), ya que pueden obtener el N a través de la fijación de N_2 atmosférico (Walley et al., 2005). Sin embargo, en esta rotación, el garbanzo absorbió una tasa de N ligeramente superior a la oferta de N, lo que sugiere que probablemente las condiciones fueron desfavorables para el crecimiento del cultivo y por lo tanto para la asociación simbiótica para la fijación de N.

Por otro lado, el sistema de laboreo no afectó la absorción de N, en particular, los valores de la eficiencia del uso de N (NUE) no mostraron ninguna diferencia entre las parcelas con arado de vertedera y arado de cincel en ninguno de los cultivos de la rotación. Este resultado coincide con Montemurro (2009), pero difiere de López-Bellido y López-Bellido (2001), que encontraron valores mayores en el sistema convencional que en el sistema de laboreo mínimo. Los valores medios del NUE en los cultivos de cereal en este estudio fueron muy bajos, lo que indica que estos cultivos requieren una mayor tasa de fertilización para optimizar los rendimientos. Asimismo, también pudo haber influido la variable distribución de las lluvias durante el ciclo de crecimiento que en consecuencia podría haber afectado la absorción de N. Montemurro (2009) demostró que el sistema de laboreo afectó el rendimiento y la absorción de N solamente el año caracterizado por una alta precipitación durante las etapas vegetativas. Por otro lado, la menor eficiencia en el uso de N en las parcelas fertilizadas en comparación con las no fertilizadas en los cereales se debe probablemente a las características del compost utilizado. El estiércol parcialmente descompuesto comporta un mayor rendimiento en las parcelas fertilizadas pero una menor eficiencia del uso del N a corto plazo, ya que su absorción se vio limitada por la lenta liberación de N, durante el proceso de mineralización del compost. Como mencionamos anteriormente en relación a la disminución del SOC, quizás la aplicación de estiércol semicompostado más estable podría mejorar la fertilidad del suelo e influir en la eficiencia del N a largo plazo (Diacono y Montemurro, 2010).

Respecto al cultivo de garbanzo, el sistema más eficiente con relación al uso del N ocurrió en las parcelas sin fertilización y sin abonos verdes, lo que sugiere que la combinación de un cultivo de leguminosas con aportaciones orgánicas y sobre todo el uso de abonos verdes que contenga también leguminosas no es una práctica adecuada. Bonfil y Pinthus (1995) indicaron que la aplicación de 100 kg N ha^{-1} promovió la absorción de N por el garbanzo sin aumentar el rendimiento, quizás debido a que el fertilizante estimuló el crecimiento vegetativo pero no la producción de grano. Del mismo modo, para el cultivo de lenteja, los sistemas menos eficientes resultaron en las parcelas con abonos verdes y fertilización. El estudio confirma que se debe evitar la aportación de insumos orgánicos adicionales en los cultivos de leguminosas.

El balance del N a lo largo de la rotación de cultivos, refleja tal y como se esperaba, que el excedente más alto de N resultó en los tratamientos con abonos verdes y fertilización. Este resultado sugiere que una proporción del N aportado por el abono verde y el fertilizante orgánico permaneció en el suelo, lo que puede acarrear riesgos de pérdidas por lixiviación. El excedente de N debe tenerse en cuenta en una rotación a largo plazo, mediante la incorporación de un cultivo capaz de absorber este N mineral, evitando así riesgos ambientales (Montemurro et al., 2010). Por otro lado, las parcelas con arado de vertedera, sin abonos verdes y sin fertilización tuvieron el mayor déficit de N, demostrando asimismo que no es un sistema sostenible. En ambos sistemas se debe realizar una mejor gestión de la rotación para evitar la pérdida de N ya sea por exceso o por déficit. Estos resultados pueden ser útiles a la hora de planificar la gestión de los agrosistemas con una adecuada fertilización teniendo en cuenta los tipos de cultivos implicados en la rotación, la fertilidad del suelo y las condiciones climáticas de la zona con el fin de optimizar la cantidad y calidad de la producción a largo plazo y reducir los riesgos de contaminación.

Conclusiones

Conclusiones

El estudio de las distintas estrategias para disminuir la intensidad de las actividades agrícolas pone de manifiesto que es necesario llevar a cabo experimentos a largo plazo para poder obtener resultados concluyentes sobre cuáles son las mejores prácticas en los cultivos de secano de la región mediterránea. La valoración de los beneficios e inconvenientes de las prácticas agrícolas requiere el seguimiento a lo largo del tiempo debido a que los cambios en las características del suelo son lentos y el rendimiento de los cultivos varía interanualmente con relación a la disponibilidad de agua y las temperaturas. Además, el efecto de las prácticas agrícolas varía con relación al tipo de cultivo; aspectos como la disponibilidad de nutrientes y la incidencia de las poblaciones arvenses difieren entre los cultivos de cereales de invierno y las leguminosas de primavera.

A continuación se enumeran las principales conclusiones obtenidas, las cuales han sido detalladas en los apartados de discusión y conclusiones de los diferentes capítulos en inglés, y de manera sintética, en los resúmenes en castellano:

- El laboreo mínimo puede afectar el rendimiento de los cultivos si no se realiza un apropiado control de la flora arvense. El laboreo mínimo como el arado de cincel sin inversión de las capas del perfil del suelo, pueden mantener la abundancia y la diversidad de la flora arvense, sin embargo nuestros resultados sugieren que se debe tener precaución con la proliferación de las poblaciones de determinadas especies, especialmente las especies perennes, ya que pueden afectar los rendimientos.

- El control de la flora arvense ya sea químico o mecánico debería estudiarse para cada caso específico, en función del tipo de cultivo y las condiciones locales, ya que a veces los costes del tratamiento y los daños causados al cultivo pueden ser superiores a las pérdidas de rendimientos por la competencia con la flora arvense.

- La fertilización orgánica es muy importante para aumentar y mantener el rendimiento de los cereales en los sistemas agrícolas ecológicos. Por el contrario, los cultivos de leguminosas no necesitan fertilización, e incluso puede ser perjudicial ya que la flora arvense puede beneficiarse de la aportación de nutrientes y competir con las leguminosas, limitando su crecimiento.

- La incorporación de los cultivos de cobertura como abonos verdes en la rotación de cultivos ecológicos, debe ir acompañada de un cuidadoso diseño del sistema teniendo en cuenta las especies a sembrar para evitar los efectos negativos sobre el cultivo posterior.

- La calidad del suelo, estimada mediante el carbono orgánico del suelo, la reserva de carbono, el nitrógeno total, la biomasa microbiana y la abundancia de lombrices de tierra, aumentó con la fertilización. Sin embargo, el uso de estiércol más compostado y estable es importante para mantener y mejorar la fertilidad del suelo a largo plazo.

- El laboreo del suelo ya sea con o sin inversión de las capas del perfil del suelo, puede reducir el contenido de carbono orgánico del suelo y, en consecuencia, la reserva de carbono del suelo, si no se lleva a cabo a una menor profundidad y con una adecuada fertilización que mantenga los niveles de materia orgánica en el suelo.

- En nuestro experimento a medio plazo, el contenido de carbono orgánico del suelo y, en consecuencia, la reserva de carbono en el suelo disminuyeron en todas las parcelas a causa de la aceleración de la descomposición de la materia orgánica por las formas lábiles del estiércol aplicado y a la pobreza de nutrientes del suelo.

- La eficiencia del uso de nitrógeno es importante para establecer sistemas sostenibles a largo plazo, por ello es importante evitar un elevado excedente o un déficit en los balances del uso del nitrógeno en los sistemas ecológicos. En nuestro estudio, los excedentes de nitrógeno asociados a la fertilización y a la incorporación de los abonos verdes indican que se deben evitar ambas combinaciones en las rotaciones de cereales-leguminosas.

- Las leguminosas no necesitan fertilización suplementaria, por ello debe evitarse el exceso de aportes de nitrógeno en los cultivos de leguminosas para mitigar la pérdida del exceso de nitrógeno por lixiviación.

- Para mejorar la eficiencia en el uso de nitrógeno, es importante tener en cuenta el tipo de fertilizante que se utiliza, ya que un fertilizante parcialmente descompuesto puede acelerar la mineralización y en consecuencia disminuir la cantidad de nitrógeno del suelo.

- Los suelos de los cultivos de secano de la región mediterránea se caracterizan generalmente por un pobre contenido de nutrientes y una alta tasa de mineralización de la materia orgánica, por ello es imprescindible mantener los niveles de nutrientes en los suelos independientemente de la gestión que se lleva acabo. Nuestros resultados son variables respecto al efecto de la intensidad de laboreo sobre los cultivos, sin embargo, para diseñar un sistema sostenible es imprescindible conservar la calidad del suelo a largo plazo.

Referencias

Referencias[1]

Ahmadi, A.R., Shahbazi, S., Diyanat, M., 2016. Efficacy of Five Herbicides for Weed Control in Rain-Fed Lentil (Lens culinaris Medik.). Weed Technology. 30, 448–455. Doi: 10.1614/WT-D-15-00125.1

Altieri, M., 1999. The ecological role of biodiversity in agroecosystems [WWW Document]. Agriculture, Ecosystems and Environment Doi:10.1016/S0167-8809(99)00028-6

Alvarez, R., 2005. A review of nitrogen fertilizer and conservation tillage effects on soil organic carbon storage. Soil Use and Management. 21, 38–52. Doi:10.1079/SUM2005291

Armengot, L., José-María, L., Chamorro, L., Sans, F.X., 2012. Weed harrowing in organically grown cereal crops avoids yield losses without reducing weed diversity. Agronomy for Sustainable Development. 33, 405–411. Doi:10.1007/s13593-012-0107-8

Bàrberi, P., 2002. Weed management in organic agriculture : are we addressing the right issues ? Weed Research. 42, 177–193. Doi:10.1046/j.1365-3180.2002.00277.x

Berner, A., Hildermann, I., Fliesbach, A., Pfiffner, L., Niggli, U., Mäder, P., 2008. Crop yield and soil fertility response to reduced tillage under organic management. Soil & Tillage Research. 101, 89–96. Doi:10.1016/j.still.2008.07.012

Bonfil D.J., Pinthus M.J., 1995. Response of chickpea to nitrogen and a comparison of the factors affecting chickpea seed yield with those affecting wheat grain yield. Exp. Agric. 31, 39–47. Doi:10.1017/S0014479700024996.

Boström, U., 1995. Earthworm populations (Lumbricidae) in ploughed and undisturbed leys. Soil & Tillage Research. 35, 125–133. Doi:10.1016/0167-1987(95)00489-0

Boutin, C., Jobin, B., Bélanger, L., Choinière, L., 2002. Plant diversity in three types of hedgerows adjacent to cropfields. Biodiversity and Conservation. 11, 1–25. Doi:10.1023/A:1014023326658

Brown, G.G., Edwards, C.A., Brussaard, L., 2004. How earthworms affect plant

[1]Refencias correspondientes a la introducción general, el área de estudio y la discusión general.

growth: burrowing into the mechanisms, in: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, FL, USA. Doi:10.1201/9781420039719.ch2

Bullied, W.J., Marginet, A.M., Van Acker, R.C., 2003. Conventional- and conservation-tillage systems influence emergence periodicity of annual weed species in canola. Weed Science. 51, 886–897. Doi:10.1614/P2002-117

Campiglia, E., Mancinelli, R., De Stefanis, E., Pucciarmati, S., Radicetti, E., 2015. The long-term effects of conventional and organic cropping systems, tillage managements and weather conditions on yield and grain quality of durum wheat (*Triticum durum* Desf.) in the Mediterranean environment of Central Italy. Field Crop Research. 176, 34–44. Doi:10.1016/j.fcr.2015.02.021

Capowiez, Y., Cadoux, S., Bouchant, P., Ruy, S., Roger-Estrade, J., Richard, G., Boizard, H., Capowiez, Y., Cadoux, S., Bouchant, P., Ruy, S., Roger-Estrade, J., Richard, G., & Boizard, H., 2009. The effect of tillage type and cropping system on earthworm communities, macroporosity and water infiltration. Soil & Tillage Research. 105, 209–216. Doi:10.1016/j.still.2009.09.002

Clayton, G., Rice, W.A., Lupwayi, N.Z., Johnston, A.M., Lafond, G.P., Grant, C.A., Walley, F., 2003. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N2 fixation and nitrogen partitioning 84, 79–88. Doi:10.4141/P02-090

Cooper, J., Baranski, M., Stewart, G., Nobel-de Lange, M., Bàrberi, P., Fließbach, A., Peigné, J., Berner, A., Brock, C., Casagrande, M., Crowley, O., David, C., De Vliegher, A., Döring, T.F., Dupont, A., Entz, M., Grosse, M., Haase, T., Halde, C., Hammerl, V., Huiting, H., Leithold, G., Messmer, M., Schloter, M., Sukkel, W., van der Heijden, M.G.A., Willekens, K., Wittwer, R., Mäder, P., 2016. Shallow non-inversion tillage in organic farming maintains crop yields and increases soil C stocks: a meta-analysis. Agronomy for Sustainable Development. 36. Doi:10.1007/s13593-016-0354-1

Crittenden, S.J.J., Eswaramurthy, T., de Goede, R.G.. G.M., Brussaard, L., Pulleman, M.M.M., 2014. Effect of tillage on earthworms over short- and medium-term in conventional and organic farming. Applied Soil Ecology. 83, 140–148. Doi:10.1016/j.apsoil.2014.03.001

Curry, J.P., 2004. Factors affecting the abundance of earthworms in soils, in: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press Boca Raton, Fla, USA, pp. 91–113.

Decaëns, T., Jiménez, J.J., Gioia, C., Measey, G.J., Lavelle, P., 2006. The values of soil animals for conservation biology. European Journal of Soil Biology. 42, S23–S38. Doi:10.1016/j.ejsobi.2006.07.001

Diacono M., Montemurro F. 2010. Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development, 30(2), 401-422. 10.1051/agro/2009040.

Dorado, J., López-Fando, C., 2006. The effect of tillage system and use of a paraplow on weed flora in a semiarid soil from central Spain. Weed Research. 46, 424–431. Doi: 10.1111/j.1365-3180.2006.00526.x.

Ernst, G., Emmerling, C., 2009. Impact of five different tillage systems on soil organic carbon content and the density, biomass, and community composition of earthworms after a ten year period. Eurpèam Journalof Soil Biology. 45, 247–251. Doi:10.1016/j.ejsobi.2009.02.002

European Environment Agency, 2012. Soil erosion. Copenhagen, Denmark.

Fernández-Pascual, M., María, N. D., & Felipe, M. 2002. Fijación biológica del nitrógeno: Factores limitantes. Ciencia y Medio Ambiente.

Fließbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture, Ecosystems and Environment. 118, 273–284. Doi:10.1016/j.agee.2006.05.022

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O’Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M., 2011. Solutions for a cultivated planet. Nature. 478, 337–42. Doi:10.1038/nature10452

Food and Agriculture Organization of the United Nations, 2015. What is conservation agriculture? [WWW Document]. URL http://www.fao.org/ag/ca/1a.html

Freckman, D.W., 1994. Life in the soil: soil biodiversity: its importance to ecosystem processes, in: Report of a Workshop Held at The Natural History Museum, London, England. pp. 1–24.

Gadermaier, F., Berner, A., Fließbach, A., Friedel, J.K., Mäder, P., 2011. Impact of reduced tillage on soil organic carbon and nutrient budgets under organic farming. Renewable Agriculture and Food Systems. 27, 68–80. Doi:10.1017/S1742170510000554

Gliessman, S.R., 2000. Agroecology. Ecological Processes in Sustainable Agriculture. Lewis Publishers, Florida, USA.

Gruber, S., Claupein, W., 2009. Effect of tillage intensity on weed infestation in organic farming. Soil & Tillage Research. 105, 104–111. Doi:10.1016/j.still.2009.06.001

Gutiérrez-López, M., Moreno, G., Trigo, D., Juárez, E. , Jesús, J.B., Díaz-Cosín, D.J.. 2016. The efficiency of earthworm extraction methods is determined by species and soil properties in the Mediterranean communities of Central-Western Spain. European Journal of Soil Biology.73, 59–68. Doi:10.1016/j.ejsobi.2016.01.005

Guo, L., Gifford, R., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology. 8, 345–360. Doi: 10.1046/j.1354-1013.2002.00486.x.

Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. Philosophical Transaction Of the Royal Society. B Biol. Sci. 363, 543–555. Doi: 10.1098/rstb.2007.2169.

Heap, I. 2014. Herbicide resistant weeds. In Integrated Pest Management. Springer Netherlands. pp. 281-301.

Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agriculture, Ecosystems and Environment. 103, 1–25. Doi:10.1016/j.agee.2003.12.018

Instituto Nacinal de Estadística (INE). 2007. Censo Agrario. http://www.ine.es/dyngs/INEbase/es/categoria.htm?c=Estadistica_P&cid=1254735727106

Kassam, A., Friedrich, T., Derpsch, R., Lahmar, R., Mrabet, R., Basch, G., González-Sánchez, E.J., Serraj, R., 2012. Conservation agriculture in the dry Mediterranean climate. Field Crops Research. 132, 7–17. Doi:10.1016/j.fcr.2012.02.023

Krauss, M., Berner, a., Burger, D., Wiemken, a., Niggli, U., Mäder, P., 2010. Reduced tillage in temperate organic farming: implications for crop management and forage production. Soil Use and Management. 26, 12–20. Doi:10.1111/j.1475-2743.2009.00253.x

Kuntz, M., Berner, A., Gattinger, A., Scholberg, J.M., Mäder, P., Pfiffner, L., 2013. Influence of reduced tillage on earthworm and microbial communities under organic arable farming. Pedobiologia (Jena). 56, 251–260. Doi:10.1016/j.pedobi.2013.08.005

Lacasta, C., Maire, N., Meco, R., 2013. Un manejo controvertido : el abono verde en los cultivos herbáceos de secano. La fertilidad de la Tierra, (14), 40-43.

Lacasta, C., Meco, R., 2006. Agricultura ecológica: una alternativa económica y energética para los agrosistemas de cereal en el secano español. Tierras Castilla León 128, 86–99.

Lassaletta, L., Rovira, J., 2005. Agricultura industrial y cambio global. El Ecologista, 45, 52-55.

Lavelle, P., 1996. Diversity of soil fauna and ecosystem function. Biology International. 33, 3–16.

Légère, A., Stevenson, F.C., Ziadi, N., 2008. Contrasting Responses of Weed Communities and Crops to 12 Years of tillage and Fertilization Treatments. Weed Technology. 22, 309–317. Doi:10.1614/WT-07-124.1

Lòpez-Bellido R.J., Lòpez-Bellido L. 2001. Efficiency of nitrogen in wheat under Mediterranean conditions: effect of tillage, crop rotation and N fertilization. Field Crop Research 71, 31–46. Doi: 10.1016/S0378-4290(01)00146-0.

López-Garrido, R., Madejón, E., León-Camacho, M., Girón, I., Moreno, F., Murillo, J.M., 2014. Reduced tillage as an alternative to no-tillage under Mediterranean conditions: A case study. Soil & Tillage Research. 140, 40–47. Doi:10.1016/j.still.2014.02.008

Mäder, P., Berner, A., 2011. Development of reduced tillage systems in organic farming in Europe. Renewable Agriculture and Food Systems:. 27, 7–11. Doi:10.1017/S1742170511000470

Maltas, A., Charles, R., Jeangros, B., Sinaj, S., 2013. Effect of organic fertilizers and reduced-tillage on soil properties, crop nitrogen response and crop yield: Results of a 12-year experiment in Changins, Switzerland. Soil & Tillage Research. 126, 11–18. Doi:10.1016/j.still.2012.07.012

Marshall, E.J.P., Brown, V.K., Boatman, N.D., Lutman, P.J.W., Squire, G.R., Ward, L.K., 2003. The role of weeds in supporting biological diversity within crop fields. Weed Research. 43, 77–89. Doi:10.1046/j.1365-3180.2003.00326.x

Masilionyte, L., Maiksteniene, S., Kriauciuniene, Z., Jablonskyte-Rasce, D., Zou, L., Sarauskis, E., 2017. Effect of cover crops in smothering weeds and volunteer plants in alternative farming systems. Crop Protection. 91, 74–81. Doi:10.1016/j.cropro.2016.09.016

Meco, R., Lacasta, C., Estalrich, E., García Muriedas, G., 1998. La agricultura ecológica en cereales, una alternativa para zonas semiáridas. Congreso de la sociedad española de agricultura ecólogica. pp. 83–93.

Meco, R., Lacasta, C., Moreno, M.M., 2011. Agricultura ecológica en secano: soluciones sostenibles en ambientes mediterráneos. No. 631.586 A3.

Metzke, M., Potthoff, M., Quintern, M., Heß, J., Joergensen, R.G., 2007. Effect of reduced tillage systems on earthworm communities in a 6-year organic rotation. European Journal of Soil Biology. 43, S209–S215. Doi:10.1016/j.ejsobi.2007.08.056

Ministerio de agricultura y pesca, alimentación y medio ambiente, 2011. Superficies y producciones de cultivos. [WWW Document]. URL http://www.magrama.gob.es/estadistica/pags/anuario/2010/ae_2010_13.pdf

Molina-Herrera, S., Romanyà, J., 2015. Synergistic and antagonistic interactions among organic amendments of contrasted stability, nutrient availability and soil organic matter in the regulation of C mineralisation. European Journal of Soil Biology. 70, 118–125. Doi:10.1016/j.ejsobi.2015.09.001

Montemurro F. 2009. Different nitrogen fertilization sources, soil tillage, and crop rotations in winter wheat: effect on yield, quality, and nitrogen utilization. J. Plant Nutr., 32 (1), 1-18.

Montgomery, D.R., 2007. Soil erosion and agricultural sustainability. Proceedings of the National Academy of Sciences of the USA. 104, 13268–13272. Doi:10.1073/pnas.0611508104

Murphy, S.D., Clements, D.R., Belaoussoff, S., Kevan, P.G., Swanton, C.J., 2006. Promotion of weed species diversity and reduction of weed seedbanks with conservation tillage and crop rotation. Weed Science. 54, 69–77. Doi:10.1614/WS-04-125R1.1

Olarieta, J.R., Rodriguez Valle, F.L., Tello, E., 2006. Conservando y destruyendo suelos, transformando paisajes. El factor edáfico en los cambios de uso del territorio (el Vallès, Cataluña, 1853-2004). Areas 25, 76–103.

Pardo, G., Cirujeda, A., Aibar, J., Cavero, J., Zaragoza, C., 2008. Weed harrowing in winter cereal under semi-arid conditions. Spanish Journalof Agriculture Research. 6, 661–670. Doi:10.5424/sjar/2008064-358

Peigné, J., Ball, B.C., Roger-Estrade, J., David, C., 2007. Is conservation tillage suitable for organic farming? A review. Soil Use and Management. 23, 129–144. Doi:10.1111/j.1475-2743.2006.00082.x

Peigné, J., Cannavaciuolo, M., Gautronneau, Y., Aveline, A., Giteau, J.L., Cluzeau, D., 2009. Earthworm populations under different tillage systems in organic farming. Soil & Tillage Research. 104, 207–214. Doi:10.1016/j.still.2009.02.011

Peigné, J., Casagrande, M., Payet, V., David, C., Sans, F.X., Blanco-Moreno, J.M., Cooper, J., Gascoyne, K., Antichi, D., Bàrberi, P., Bigongiali, F., Surböck, A., Kranzler, A., Beeckman, A., Willekens, K., Luik, A., Matt, D., Grosse, M., Heß, J., Clerc, M., Dierauer, H., Mäder, P., 2015. How organic farmers practice conservation agriculture in Europe. Renewable Agriculture and Food Systems. 31, 1–14. Doi:10.1017/S1742170514000477

Peigné, J., Vian, J.-F., Cannavacciuolo, M., Lefevre, V., Gautronneau, Y., Boizard, H., 2013. Assessment of soil structure in the transition layer between topsoil and subsoil using the profil cultural method. Soil & Tillage Research. 127, 13–25. Doi:10.1016/j.still.2012.05.014

Plaza-Bonilla, D., Nolot, J.-M., Raffaillac, D., Justes, E., 2016. Innovative cropping systems to reduce N inputs and maintain wheat yields by inserting grain legumes and cover crops in southwestern France. European Journal of Agronomy. 2011, 1–11. Doi:10.1016/j.eja.2016.05.010

Plaza, E.H., Kozak, M., Navarrete, L., González-Andújar, J.L., 2011. Tillage system did not affect weed diversity in a 23-year experiment in Mediterranean dryland. Agriculture, Ecosystems and Environment. 140, 102–105. Doi:10.1016/j.agee.2010.11.016

Post, W., Izaurralde, R., Mann, L., Bliss, N., 2001. Monitoring and verifying changes of organic carbon in soil. Climate Change. 51, 73–99. Doi: 10.1007/978-94-017-3089-1_4.

Robinson, R.A., Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecolology. 39, 157–176. Doi: 10.1046/j.1365-2664.2002.00695.x.

Romanyà, J., Arco, N., Solà-Morales, I., Armengot, L., Sans, F.X., 2012. Carbon and Nitrogen Stocks and Nitrogen Mineralization in Organically Managed Soils Amended with Composted Manures. J. Environ. Qual. 41, 1337. Doi:10.2134/jeq2011.0456

Romanyà, J., Rovira, P., 2011. An appraisal of soil organic C content in Mediterranean agricultural soils. Soil Use and Management. 27, 321–332. Doi:10.1111/j.1475-2743.2011.00346.x

Safont, G., Chamorro, L., Sans, F., 2007. La reconversión del espacio rural de Gallecs a la agricultura ecológica en la región metropolitana de Barcelona. Consorci del Parc de l'Espai d'Interès Natural de Gallecs.

Sans, F.X., Armengot, L., Bassa, M., Blanco-Moreno, J.M., Caballero-López, B., Chamorro, L., José-María, L., 2013. La intensificación agrícola y la diversidad vegetal en los sistemas cerealistas de secano mediterráneos: implicaciones para la conservación. Ecosistemas. Doi:10.7818/ECOS.2013.22-1.06

Sparling, G.P., 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Soil Research. 30, 195–207. Doi:10.1071/SR9920195

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature. 418, 671–677. Doi:10.1038/nature01014 ER

Tørresen, K.S., Skuterud, R., Tandsæther, H.J., Hagemo, M.B., 2003. Long-term experiments with reduced tillage in spring cereals. I. Effects on weed flora, weed seedbank and grain yield. Crop Protection. 22, 185–200. Doi:10.1016/S0261-2194(02)00145-X

Valckx, J., Cordón, A., Govers, G., Hermy, M., Muys, B., 2011. Pedobiologia - International Journal of Soil Biology Food and habitat preferences of the earthworm Lumbricus terrestris L . for cover crops. Pedobiologia - International Journal Of Soil Biology. 54, S139–S144. Doi:10.1016/j.pedobi.2011.07.004

Vian, J.F., Peigne, J., Chaussod, R., Roger-Estrade, J., 2009. Effects of four tillage systems on soil structure and soil microbial biomass in organic farming. Soil Use and Manag. 25, 1–10. Doi:10.1111/j.1475-2743.2008.00176.x

Walley, F.L., Kyei-Boahen, S., Hnatowich, G., Stevenson, C., 2005. Nitrogen and phosphorus fertility management for desi and kabuli chickpea. Canadian Journal of Plant Science. 85, 73–79.

Woźniak, A., Soroka, M., 2014. Effects of a 3-year reduced tillage on the yield and quality of grain and weed infestation of spring triticale (*Triticosecale* Wittmack). International Journal of Plant Production. 8. Doi:10.22069/ijpp.2014.152.