**Effects of enhancing soil organic carbon sequestration in the topsoil by
fertilization on crop productivity and stability: evidence from long-term
experiments with wheat-maize cropping systems in China**

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Abstract

Although organic carbon sequestration in agricultural soils has been recommended as a ‘win-win strategy’ for mitigating climate change and ensuring food security, great uncertainty still remains in identifying the relationships between soil organic carbon (SOC) sequestration and crop productivity. Using data from 17 long-term experiments in China we determined the effects of fertilization strategies on SOC stocks at 0-20 cm depth in the North, North East, North West and South. The impacts of changes in topsoil SOC stocks on the yield and yield stability of winter wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were determined. Results showed that application of inorganic fertilizers (NPK) plus animal manure over 20-30 years significantly increased SOC stocks to 20-cm depth by 32-87% whilst NPK plus wheat/maize straw application increased it by 26-38% compared to controls. The efficiency of SOC sequestration differed between regions with 7.4-13.1% of annual C input into the topsoil being retained as SOC over the study periods. In the northern regions, application of manure had little additional effect on yield compared to NPK over a wide range of topsoil SOC stocks (18 - >50 Mg C ha⁻¹). In the South, average yield from manure applied treatments was 2.5 times greater than that from NPK treatments. Moreover, the yield with NPK plus manure increased until SOC stocks (20-cm depth) increased to ~35 Mg C ha⁻¹. In the northern regions, yield stability was not increased by application of NPK plus manure compared to NPK, whereas in the South there was a significant improvement. We conclude that manure application and straw incorporation could potentially lead to SOC sequestration in topsoil in China,

42 but beneficial effects of this increase in SOC stocks to 20-cm depth on crop yield and
43 yield stability may only be achieved in the South.

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45 **Keywords:** Soil organic carbon; yield; yield stability; manure application; straw
46 return; China

1. Introduction

Soil organic carbon (SOC) can play an important role in increasing crop productivity, improving soil fertility (Tiessen et al., 1994), reducing atmospheric carbon dioxide (CO₂) enrichment (Lal, 2004), and providing other ecosystem services, such as improved soil structure and water retention (Fan et al., 2013a). Low SOC stocks could reduce crop yield through effects on soil fertility and significant nutrient loss may also occur as a result of low nutrient buffer or retention capacity. Changes in SOC stocks have been reported extensively on the global (FAO, 2001), regional (Huang and Sun, 2006; Smith, 2004) and plot scales (Zhang et al., 2010), which suggests that society has paid increasing attention to the potential for sequestering organic carbon in soils in an effort to mitigate climate change and promote crop productivity. For example, it has been reported that a 1% increase in SOC content of the topsoil (0-20 cm) could increase cereal yield by 430 kg ha⁻¹ and reduce yield variability by 3.5% (Pan et al., 2009). However, others have argued that claims about the potential benefits of increasing C inputs to the soil must be made with caution because of the uncertainties regarding how much can be sequestered under different climates and soil types (Brock et al., 2011; Manlay et al., 2007; Seremesic et al., 2011). Therefore, it is imperative to quantify the relationships between C inputs, SOC sequestration and crop productivity.

In the last three decades, China has been facing a challenge to ensure crop production is increased while mitigating greenhouse gas (GHG) emissions. China uses only 7% of the world's arable land to feed 22% of the global population (Fan et al., 2010) and produces over 20% and 17% of the world's maize (*Zea mays* L.) and

wheat (*Triticum aestivum* L.) grain, respectively (FAO, 2010). If China intends to maintain the policy of grain self-sufficiency, crop productivity has to be increased without reducing soil fertility including SOC content, one of the indicators for ensuring crop production (D'Hose et al., 2014). Currently, the average SOC concentration in the root zone of croplands (about 10 g C kg⁻¹) in China is much lower than that (25-40 g C kg⁻¹) in Europe and the United States (Fan et al., 2010). Furthermore, SOC losses from China's croplands have been widely reported (Huang and Sun, 2006; Qin et al., 2013; Sun et al., 2010). SOC stocks of agro-ecosystems may be increased by improving agronomic practices. Applications of animal manure and the incorporation of straw in the soil are recognized as SOC-enhancing management options (Lu et al., 2009; Tian et al., 2015). It has been reported that SOC stocks in the top 20 cm of the world's soils increased by 0.24-0.46 Mg C ha⁻¹ yr⁻¹ with a decade of manure application (Gattinger et al., 2012). In addition, it was reported that in Southern China SOC in the topsoil (0-20 cm) increased by 3.8 Mg C ha⁻¹ following manure application for 22 years compared with soils receiving mineral fertilizers alone (Huang et al., 2010). It has also been estimated that an additional 3.8 Tg C yr⁻¹ could be sequestered in soil if all of the straw generated from arable fields in China were returned to the soils (Lu et al., 2009).

It is challenging to quantify the contribution of SOC to either maintaining or stabilizing crop yield, as the contribution could be concealed by other factors given the complex interactions that occur between soil, root systems and canopies (Bingham, 2001). Most studies conducted across the world have suggested that there is some

linear relationship between SOC stocks in the root zone and crop yield and its stability (Bauer and Black, 1994; Beyer et al., 1999; Lal, 2010; Smith, 2004). However, a significant non-linear relationship has been reported between relative crop yield and SOC in the root zone (Lal, 2009), which implies that there is an upper value of SOC stocks in the root zone beyond which there will be no additional benefit on crop yield of increasing SOC stocks (Loveland and Webb, 2003; Krull et al., 2004). For example, crop yields did not increase any further when SOC content in the topsoil exceeded 2 % in the upland soils of Alberta, Canada (Krull et al., 2004). Moreover there could be potential hazards of adding too much C to soils, such as surface crusting, increased detachment by raindrops, decreased hydraulic conductivity (Haynes and Naidu, 1998) and water-repellency (Olsen et al., 1970). Addition of excessive amounts organic materials to soils could also lead to losses in soil nitrogen (N) and/or phosphorus (P), resulting in surface and ground-water pollution (Patrick et al., 2013).

Determining the upper value of SOC stocks would provide guidance for designing management practices to optimise crop yields and mitigate climate change. However, there are few studies to date that have examined the relationship between SOC stocks and crop productivity, and quantified the maximal yield-responsive SOC stocks based on long-term observations (Lal, 2006; 2010). The objective of this research was to analyse datasets from 17 long-term fertilization experiments across China to: 1) determine the impact of different fertilizer application and straw management strategies on SOC sequestration in topsoil (0-20 cm) in different regions; 2) to establish the relationships between SOC stocks to 20-cm depth and crop yield

and yield stability for the different regions; and 3) to determine whether the maximal yield-responsive SOC stocks for enhancing cereal yields differs between regions. The overall aim of the research was to establish whether soil management practices to optimise yield and yield stability should be modified for specific regions.

2. Methods and materials

2.1 Long-term fertilization experiments

Datasets collected at 17 long-term fertilization experiments established between 1979 and 1990 in arable lands across China were used for this study (Fig. 1 and Table S1). Four sites were located in the North East region: Haerbin (HEB), Gongzhuling (GZL-A and -B), and Shenyang (SY); four in the North West region: Urumqi (UM), Zhangye (ZY), Pingliang (PL) and Yangling (YL); five in the North region: Changping (CP), Tianjin (TJ), Yucheng (YC), Zhengzhou (ZZ) and Xuzhou (XZ); and four in the South region: Suining (SN), Chongqing (CQ), Qiyang (QY) and Jinxian (JX).

Climatic conditions, and soil properties at the beginning of the experiments are presented in Tables S1 and S2. Various climatic types are represented in these regions. Annual average temperatures varied from 3.5 °C at the HEB site to 18.3 °C at the CQ site. Annual precipitation ranged from 127 mm at the ZY site (in the arid area) to 1581 mm at the JX site in the humid monsoon climate zone. Annual pan evaporation varied from 990 mm at the CQ site to 2570 mm at the UM site (data from China meteorological sharing service system, <http://cdc.cma.gov.cn/>). Annual evaporation at

the sites in the South region was almost the same as annual precipitation. However, it was much higher than annual precipitation in the other regions (Table S1). Irrigation was applied at the majority of sites in the North and North West (Table S1).

2.2 Management of the field experiments

A detailed description of the cropping systems is shown in Table 1. A monoculture cropping system was practiced at the GZL-A and -B, SY, and PL sites with continuous maize, while a rotation of winter wheat-winter wheat-maize (i.e., two wheat seasons followed by a maize season) at the ZY and UM sites. However, the rotation at site ZY changed once between 1994 and 1997, i.e., three continuous wheat seasons followed by one maize season. A double cropping system was practiced at the other sites; at JX it was maize-maize, at SN and CQ it was wheat-rice (*Oryza sativa* L.), whilst at the remaining sites it was summer maize-winter wheat. At all sites, weeds were removed manually and pesticides were applied when needed to control insect pests and fungal diseases.

Four types of treatments common among all sites were chosen for this study: (i) unfertilized (thereafter, Control); (ii) inorganic N, P and/or potassium (K) fertilizer combined (hereafter referred to as, NP and NPK); (iii) inorganic NP and NPK plus wheat or maize straw returned (hereafter, NPS and NPKS); and (iv) inorganic NP and NPK plus animal manure (hereafter, NPM and NPKM) or 1.5-2.0 times amount of N in manure only or of total N application (hereafter, hNPKM) used in the NPKM treatment. The application rate of inorganic N (urea), P and K fertilizer that could ensure high crop yield at a particular site was set according to recommended rates for

commercial crops in the regions (NP and NPK; Table 1). The inorganic N, P and K fertilizers were applied five to seven days before sowing. The fertilizer-N strategy in the NPM and NPKM treatment differed between sites. At HEB, GZL-B, SY, CP, YC, XZ, ZY, PL, SN, CQ and JX sites the same amount of inorganic N was applied as in the NP and NPK treatments, thus, at these sites the NPM and NPKM treatments received additional N from manure. At GZL-A, TJ, ZZ, UM, YL and QY, the same amount of total N was applied to NP/NPK and NPM/NPKM treatments at a given site, with 30-50% of it from inorganic N-fertilizer when manure was added (i.e., NPM/NPKM). For the NPS and NPKS treatments, maize and wheat straw was chopped and incorporated into the soil (0-20 cm depth) immediately after harvest. N content in straw, was deducted from the total amount of fertilizer-N applied at GZL-A and UM (1995 onwards) so that the total N applied was the same as that received by NP and NPK treatments at these sites. At remaining sites no deduction was made, because the information on N content in straw could not be provided in time for when fertilizers were applied. Micronutrient fertilizers containing boron, zinc, manganese, magnesium and iron were applied across all treatments at the QY site every two to four years to avoid potential deficiencies.

The treatments with large plots ($>100\text{m}^2$, Table S3) did not have replicates except those at the SY, PL, and QY sites. Otherwise, the treatments had two to four replicates (Table S3). At all sites, 100cm depth cement baffle plates were erected along the boundaries of each plot to avoid lateral exchange of water and nutrients between adjacent plots. The treatments were initially randomized at each site and then that

treatment was fixed to the plot in the following years.

2.3 Soil and Plant Sampling and Analysis

Soil samples were collected from the top 20cm soil depth at the beginning of the experiments, and then annually from each plot approximately 15 d after harvest. As the plough depth ranged from 20-25 cm across sites and regions, the depth of soil sampling represents the minimum plough depth for the study. Twenty cores from each plot were collected randomly using a soil auger (5 cm diameter) and the soil from a random four cores combined, which formed five samples for each plot. Air-dried soils (~20 kg, 7 d) were sieved through a 2 mm screen to determine pH (1:1 w/v water) and other soil properties. Sub-samples were crushed to 0.25mm for the measurements of SOC, total N (TN), total P (TP), and total K (TK). SOC content was determined by sulphuric acid-potassium dichromate oxidation (Walkley and Black, 1934). TN was determined by the method described by Bremner and Mulvaney (1965), TP by Murphy and Riley (1962), and TK by Kundsén et al. (1982). Available N was measured following the method of Jackson (1958). Available P was determined by the Olsen-P method (Olsen et al., 1954) and available K by the method of Shi (1976). The contents of TN, TP, and TK in manure were measured following the procedures described by Mahimairaja et al. (1994), Barnett (1994), and Wen et al. (1997), respectively. Soil bulk density was measured by using steel ring with 5-cm diameter, and soil samples were taken at 10 cm depth, dried at 105 °C for eight hours and then weighed (SSSC 2000).

Maize and wheat plants were harvested manually close to the ground with sickles

and all harvested materials were removed from the plots for all the treatments except the NPS and NPKS treatments. Thus, only roots and shed leaf litter were left on the plots. The harvested plants were air-dried, threshed, and oven-dried at 70°C to constant dry weight. For each treatment plot, five samples from the dried straw and grains each were collected for the measurement of nutrient contents. Sampled dry matter was digested with H₂SO₄-H₂O₂, and the concentrations of TN, TP and TK in the digesting solution were measured using the micro-Kjeldahl method, colorimetric analysis and dissolution-flame photometry, respectively (Page et al., 1982). For the NPS and NPKS treatments, the crop residues were chopped and incorporated into the soils (0-20 cm) by disking immediately after harvest each year. Grain yield from a plot was determined from each whole plot.

2.4 Carbon inputs from crops

It was assumed that the annual biomass input from roots, exudation and dead materials by wheat and maize is 30% of aboveground dry matter (Kundu et al., 2007; Zhang et al., 2010), and that 75 and 85% of the total root biomass was in the surface 20 cm of the soils for wheat and maize, respectively (Jiang et al., 2014). 39.9 and 44.4 g C kg⁻¹ (dry matter), as a national average (NCATS, 1994), were used to represent the C contents in dry matter for wheat and maize, respectively. For the NPKS treatment, C contents in maize and wheat straw were set to 12.4 and 27.8 g C kg⁻¹ (fresh weight), respectively (Zhang et al., 2010).

2.5 Statistical analysis

SOC concentration (SOC_C) in the top 20 cm of the soil was converted to SOC stock (SOC_S , Mg C ha⁻¹):

$$SOC_S = SOC_C \times d \times BD \times 10 \quad (1)$$

where d is the soil depth (0.2 m), BD is the soil bulk density (g cm⁻³) and 10 is a unit conversion factor.

SOC_S in a given year can also be expressed as a function of the number of the years since an experiment started (Y_n):

$$SOC_S = aY_n + b \quad (2)$$

where a represents the rate of change in SOC stocks (Mg C ha⁻¹ yr⁻¹) in the top 20 cm depth and b is the SOC stocks at the beginning of the experiment. A similar expression was applied to crop yield (Y_{grain}). However, the slope a represents the rate of change (kg ha⁻¹ yr⁻¹) of crop yields and the intercept b is the initial crop yield. Linear regression was used to fit these functions and the residuals examined to determine the suitability of a linear model. There was no evidence of non-linearity for any of the sites.

As the dataset from long-term experiments in different regions or cropping systems cannot be compared directly, relative crop yield (YR) was used to minimise the influences of crop variety replacement, seasonal variation in weather conditions and changes in agronomic practices during the study period and to make the dataset from individual experiments more comparable. YR was calculated based on the annual

data from the long-term experiments across the four regions.

$$YR = \frac{YE}{Y_{NPK}} \quad (3)$$

where YE is the actual yield of either wheat or maize from a treatment (kg ha^{-1}) at a given site in a given year and Y_{NPK} is the yield of the crop from the NPK treatment at the same site in the same year.

To obtain the maximal yield-responsive SOC_s (SOC_{opt}) for relative crop yield, the linear-plateau model was chosen to quantify the relationship between YR and SOC_s in a given year (Bai et al., 2013; Lal, 2010):

$$YR = \begin{cases} A \cdot SOC_s + B & (SOC_s < SOC_{opt}) \\ YP_{max} & (SOC_s \geq SOC_{opt}) \end{cases} \quad (4)$$

where A means that the relative yield increment per 1 Mg C ha^{-1} SOC increment and B is the relative yield at the initial SOC level in an experiment site; SOC_{opt} (Mg C ha^{-1}) is the upper value of SOC beyond which there is no additional benefit for relative crop yield of increasing SOC stocks further; YP_{max} is the predicted plateau maximum relative yield and fitted with the data. Models were fitted using Genstat (version 16th Edition, 2013, VSN international Trust, Hemel Hempstead, UK).

The stability of grain yield for a treatment was evaluated by its variability (CV , %) (Pan et al., 2009):

$$CV = \frac{Y_{std}}{Y_m} \times 100 \quad (5)$$

where Y_{std} is the standard deviation of the yields of a particular treatment at a site over

the whole experimental period and Y_m is the mean yield for the treatment within the same period. Differences between regions were then compared using values of CV averaged across sites within regions.

The exponential decay equation was used to characterize the relationship between the average SOC_s under a given treatment and yield variation during the experimental period:

$$CV = \beta_0 + \beta_1 e^{-\beta_2 SOC_s} \quad (6)$$

where β_0 is the lower value of CV (lower value of asymptotic line), $\beta_0 + \beta_1$ is the maximum value of CV and β_2 is the rate of decrease of CV.

One-way ANOVA was applied to test the significance of the effects of fertilizer treatments on the average relative yields and SOC storage within the regions. Differences between specific treatments within a region were compared with the least significant differences (L.S.D) at either the 5% or 1% level of probability depending on the analysis. Simple linear regression with groups (Genstat version 16th Edition, 2013, VSN international Trust, Hemel Hempstead, UK) was used to compare the slopes of the relationships between total cumulative C input and the increase in SOC storage between regions. With the exception of fitting linear-plateau models and linear regression with groups (above), all statistical analyses were performed with SPSS v. 17.0 for Windows (SPSS, Inc., 1999, Chicago, USA, www.spss.com). Prior to analysis residuals were checked for homogeneity of variance and normality to ensure that they satisfied the assumptions of parametric tests.

3. Results

3.1 Crop yield and stability

The mean annual rates of change in crop yields over time under the different fertilization strategies at each site are shown in Table 2. At all sites but SY (where there was an increase) crop yield in the control (unfertilized) treatment either did not change or decreased on average over time. The trends for the other treatments were more variable with both positive and negative changes in mean annual yields over the study periods. For application of NPK fertilizer, 37% of the site-crop combinations showed a positive yield trend, whilst 22% showed a negative yield trend. When manure was applied this proportion was 51% (NPKM) and 67% (hNPKM) of site-crops with positive yield trends and only 11 and 6%, respectively, with negative trends.

When averaged over the experimental period, the yields of wheat relative to the NPK treatment showed a similar pattern in all the regions (Table 3). The highest relative yields were in the manure applied treatments, followed by NPKS. The relative yields were significantly ($P < 0.05$ LSD) greater than the NPK reference in the North West and South, but not the North East and the North indicating that in two out of four regions there was a yield response to application of manure over and above that observed with inorganic NPK. Addition of manure resulted in an improvement in relative yields compared to straw (NPKS) only in the North and South. Yields for the NP treatment were marginally lower than those of the NPK reference in the northern

sites (6-12%; $P < 0.05$ for the North and North West) indicating only a small increase in yield in response to application of K fertilizer. However, the response was greater (12% $P < 0.05$) in the South. Unfertilized controls had yields lower than those for the other treatments.

The relative yields of maize responded in a similar way to fertilizer treatment as those for wheat. By far the greatest benefits to yield from manure additions were found in the South. Smaller ($P < 0.05$) benefits over NPK were found in the North East and North West. The beneficial effects on yield of returning straw to the soil were observed only in the South (Table 3).

Annual yield variation (CV %) was around 20 to 30% for wheat and maize in the North East region, and did not differ between treatments (Fig. 2). In unfertilized controls wheat and maize yield variability in the North and North West was greater (CV approximately 35%) than in the North East ($P < 0.05$), and declined ($P < 0.05$) with the addition of inorganic fertilizer and manure. Thus, the average yield variability of the NPM, NPKM and hNPKM treatments was less (yield was more stable) than that of the control treatment in the North and North West. In the South, yield variability was considerably greater (CV $> 60\%$) than in the northern regions when no fertilizer was applied. Moreover application of NP and NPK did not reduce this variability unlike the response in the northern regions. Applications of manure (NPM, NPKM, hNPKM) reduced yield variability to values comparable to the other regions (CV $\sim 20\%$). By contrast, straw incorporation (NPS, NPKS) did not cause a reduction in yield variability to the same extent as manure.

3.2 Topsoil SOC stocks and C inputs

In order to compare the effects of fertilizer treatments on SOC stocks (i.e. contents), SOC at beginning and end of the experimental period in different regions were compared (Table 4). Average SOC stocks for the Control treatment at the beginning of experiments was the lowest among the treatments in all the regions (19.3-31.9 Mg C ha⁻¹) and the value decreased in the North West and North regions over the whole experimental period, but not in the North East and South. The inorganic fertilizer treatments NP and NPK had relatively small effects on SOC stocks over the experimental period in the North East region. In the other regions, however, SOC stocks in the NP and NPK treatments increased ($P<0.05$). By contrast addition of manure (NPM, NPKM and hNPKM) led to an increase ($P<0.05$) in SOC relative to controls and inorganic fertilizer treatments in all regions ($P<0.05$). Averaged across manure treatments the increases in SOC from beginning to end of experiments were 18, 43, 14 and 23% in the North East, the North West, the North and the South region, respectively. The average SOC stocks from the NPKS treatment at the end of experiments were higher ($P<0.05$) than that at the beginning of the experiments in all the regions. Moreover, the increases in SOC stocks were smaller following straw incorporation than following addition of manure in all regions.

Regional averages of SOC stocks mask considerable variation in SOC dynamics between individual sites. The rates of change in SOC stocks to 20-cm depth under different fertilization strategies at each site are shown in Table 5. With the exception of the CP, YC and YL sites there was either no change (9 sites) or a decrease (5 sites)

in SOC stocks over time in the unfertilized Control treatment. With NPK there was an increase (5 sites), no change (10 sites) or a decrease (1 site) over 20 to 30 years. By contrast, manure application and straw incorporation resulted in an increase ($P<0.05$) of 0.18-1.38 and 0.18-0.62 Mg C ha⁻¹ yr⁻¹ respectively at over 80% of the monitored sites. Further, at sites where there was an increase in SOC stocks over time without fertilizer, the rate of increase with additions of manure and straw were approximately 2 to 7 fold greater.

Average annual C inputs from crops and manure for different treatments in all four regions are shown in Fig. 3. Input of C from crops where only inorganic fertilizer was applied (NP and NPK) was approximately double that of the Control treatment in each of the regions. Furthermore, there were no differences in annual C input from crops between the inorganic fertilizer, manure application and straw incorporation treatments. Total C input to the soil was greater where manure was applied and straw returned to the soil compared with plots receiving inorganic fertilizer only.

In addition, positive linear relationships between change rate of SOC stocks and annual C inputs to the soils over the experimental period were found (Fig. 4). The slopes for the regions ranged from 0.074 to 0.131 which indicated that 7.4% to 13.1% of the annual C inputs could be sequestered in the soils. The slopes differed significantly ($P<0.05$) between regions, with the retention rate of C inputs in the North West being greater than that in the South and North East.

3.3 Responses of crop yield and yield stability to changes in topsoil SOC stocks

Data from individual treatments and sites within a region were combined to examine

the relationship between SOC stocks and relative yield. Significant correlations between SOC stocks and relative yield were found with an apparent maximal yield-responsive SOC stock (SOC_{opt}) above which there may be no increase in relative yield (Fig. 5). YR_{max} determined with the linear-plateau model ranged from 1.14 to 1.43 for wheat and from 1.13 to 2.06 for maize. The greatest relative yields were observed in the South region. The corresponding values of SOC_{opt} were 46.2, 26.5, 21.8 and 34.7 Mg C ha⁻¹ for wheat and 44.4, 28.0, 22.0 and 34.8 Mg C ha⁻¹ for maize in the North East, the North West, the North and the South region, respectively.

Relationships between the variability of wheat and maize yields and the average SOC stocks over experimental period are shown in Fig. 6. There was no correlation in the North East region. However, in the other three regions, with the increase in SOC stocks, the yield variability for both wheat and maize showed a relatively weak (r^2 0.38-0.59) exponential decrease, i.e. yield stability increased with increases in topsoil SOC stocks.

4. Discussion

4.1 Impact of fertilization management on topsoil SOC stocks

Whether or not organic C can be retained in soils depends on the balance between C input and output as influenced by agronomic practices (e.g., fertilization, straw return, rotation, tillage, etc.) and climatic conditions. Results from the present study show that N fertilizer application and organic soil amendments influenced the rate of C sequestration, and that rates differed between regions and sites within regions because of differences in soil type, management and climate which can impact on the retention

of SOC in soils (Gabriel and Kellman, 2011; Gattinger et al., 2012; Lu et al., 2009; Six et al., 2002). Balanced application of inorganic N, P, and K fertilizers increased SOC stocks at four out of five sites in the North, but at only one site in the North West and none in the North East and South regions. The annual rates of change of SOC to 20-cm depth for the regions were broadly consistent with those reported by Lu et al. (2009) when compared at similar application rates of N. By contrast, straw incorporation and application of animal manure led to more consistent increases in SOC stocks at the 20-cm soil depth across sites and regions, with the average rate from manure application ($0.67 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$) being over twice that from straw incorporation ($0.29 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$) for sites where both treatments were included (Table 5).

The slope of the linear relationship between SOC increment and cumulative C input to soils can be considered as the proportion of total C input that is retained in the soils, which ranged from 7.4 to 13.1% between regions. In fact, for a given treatment, estimated annual C inputs differed relatively little between regions (Fig. 3). Moreover, there was only a weak association between the average initial soil C stock of a region and its C retention rate ($P = 0.19$, $R^2 = 0.48$ data not shown), suggesting that the observed differences in retention rate between regions were largely the result of differences in climate or soil properties rather than the initial topsoil SOC stocks. The range of sequestration efficiencies was similar to that observed from the Indian humid subtropical plains at 20-cm depth under a rice-wheat over 20-30 years (7.6-14%, Majumder et al., 2008) and a rice-lentil (*Lens culinaris*) system (9.9%, Srinivasarao et

al., 2012), but lower than that from the temperate region of North America (14-21%, Rasmussen and Collins, 1991) and higher than that from topsoil of subtropical regions under a rice-wheat-jute (*Corchorus olitorius* L.) system (4-5%, Majumder et al., 2007).

4.2 Relative yield response to enhancement of SOC stocks

Investigating the effects of SOC stocks *per se* on yield is difficult, because treatments designed to vary SOC stocks, such as the addition of organic matter, also alter the supply of nutrients alongside effects on soil structure and water holding capacity etc. (Bauer and Black, 1994; Johnston et al., 2009; D'Hose et al., 2014; Loveland and Webb, 2003; Oelofse et al., 2015). Moreover, analysis of relationships using long-term data sets are subject to large variation in yield arising from seasonal variation in weather conditions and changes in agronomic practices during the study period such as the crop rotation. We have minimised these sources of variation by expressing the yield observed under a specific treatment relative to the yield obtained in the same year and site with a full balanced (NPK) inorganic fertilizer supply. When data from the different fertilizer treatments and sites within a region were combined and relative yields plotted against topsoil SOC stocks, apparent maximal yield-responsive SOC stocks (SOC_{opt}) were obtained comparable to that reported by Krull et al. (2004) in a reworking of total biomass yields from sites in arable land of Canada.

In the present study SOC_{opt} values were derived from the fitting of a linear-plateau model to all data including those from unfertilized controls, and thus,

interpretation of the agronomic significance of the SOC_{opt} must be made cautiously.
 The large increase in relative yield from values <1.0 to 1.0 is obtained over a narrow
 range of topsoil SOC stocks in each region and is associated with the application of
 NP and NPK fertilizer. These increases in yield can, therefore, be ascribed to the
 nutritional benefits of fertilizer supply (Oelofse et al., 2015; Pan et al., 2009). In the
 North, and North West, there was a small increase (4-15%) in average relative yield of
 wheat and maize with applications of manure (NPKM and hNPKM, Table 3), which
 was similar to the results reported by Beyer et al., (1999). As the rates of NPK applied
 were based on the recommended fertilizer rates required to satisfy the nutrient
 requirements of commercial crops in the region, the results suggest that manure
 applications may have had small beneficial effects on yield through the supply of
 other macronutrients in the northern regions such as S, micronutrients, or via effects
 on soil physical or biological properties (Zhang et al., 2010). Importantly, the effects
 appeared to occur independently of topsoil SOC stocks over a wide range from
 approximately 18 to >50 Mg C ha⁻¹ (0.72 – >2.0%). In this regard, when the effects of
 NPK supply are taken into account (i.e., when crops are supplied with inorganic NPK
 fertilizer) there appears to be no clear maximal yield-responsive topsoil SOC stocks
 for sustaining the yield of wheat and maize in the North and North West. It was
 reported that wheat and maize yields in plots supplied with animal manure did not
 differ from those given inorganic NPK for 33 years in the North of China (Yang et al.,
 2015). A similar interpretation can be made for the North East even though the
 linear-plateau model yielded a high apparent SOC_{opt} . The high SOC_{opt} results from the

greater variation in the data for this region, especially for unfertilized Controls, and lower coefficient of determination ($R^2 \leq 0.35$).

The concept of maximal yield-responsive SOC stocks has been proposed previously (Loveland and Webb, 2003; Krull et al., 2004), but it has been argued that the quantitative evidence for such an upper value(s) is weak, at least for soils in temperate climates (Loveland and Webb, 2003; Oelofse et al., 2015). Our data for the temperate regions of China support this view. It is worth emphasising too that the range of SOC concentrations (from 0.5% to 1.9%) measured in our soils was well below the threshold of 2% (to 0-20 cm soil depth) generally believed to be critical for maintaining soil functions (Loveland and Webb, 2003). However, our data also indicated that the response in the sub-tropical climate of the South region is appreciably different to the northern regions. In the South there was a marked increase in yield of both wheat and maize with application of manure plus inorganic NPK relative to inorganic NPK on its own (Table 3). Moreover, the relative yield increased with NPKM treatment as SOC stocks increased up to the SOC_{opt} of 35 Mg C ha⁻¹ (Fig. 5). It is conceivable that in the climate, soils and production systems of the South there is a more marked deficiency in the supply of nutrients other than NPK compared to the northern regions and that supply of animal manures and straw, or higher SOC concentrations are required to satisfy these requirements (Zhang et al., 2009, 2010). Alternatively, the restriction to yield may be from some aspect of soil physical quality associated with low SOC stocks (<35 t C ha⁻¹, 1.39 % dry weight) such as soil structure, drainage or soil water retention (Fan et al., 2013a) and that this is alleviated

as SOC is increased by addition of manure.

It can be estimated that the yields of wheat and maize crops in the South region could be increased by 89.6 and 252.4 kg ha⁻¹ respectively for each 1.0 Mg C ha⁻¹ increment in topsoil SOC up to SOC_{opt} . These rates are considerably higher than the 10 – 43 kg ha⁻¹ reported for cereal production in other areas of the world (Lal, 1981; Bauer and Black, 1994; Diaz-Zorita et al., 2002; Pan et al., 2009). Thus, based on average regional yields of wheat (3.1 Mg ha⁻¹) and maize (4.4 Mg ha⁻¹) in the south of China (National Bureau of Statistics of China, 2009), the yield of wheat could be increased by 1.94 and maize by 3.04 Mg ha⁻¹ if SOC stocks was increased to its SOC_{opt} . An additional 198 Mg C ha⁻¹ would need to be retained in soils in the south of China in order to fill the gap between current stock levels and SOC_{opt} . If SOC_{opt} is to be reached in the next 30 years at least 6.6 Mg C ha⁻¹ yr⁻¹ should be applied to the soil (most effectively as animal manure) given that 9% of the C input is sequestered (Fig. 4).

4.3 Yield stability vs. topsoil SOC stocks

Yield stability is an important indicator of agricultural sustainability (Katyal et al., 2001; Lal, 2004; Seremesic et al., 2011). Whether increasing SOC stocks can reduce yield variation is still a matter of debate (Pan et al., 2009; Pan et al., 2006; Yan and Gong, 2010). There is evidence that yield variability increases when crop growth and yield become more dependent on soil fertility to supply the required mineral nutrients (e.g. with zero or unbalanced inorganic fertilizer applications) (Yan and Gong, 2010).

Our data suggest that the extent to which this occurs may depend on the range of SOC stocks or other regional differences in soil and/or climatic conditions. Thus, in the North East there was little difference in the variability of wheat and maize yields between any of the fertilizer treatments (Fig. 2), even though the actual yield of unfertilized Controls was generally ~50-60% of that of crops receiving NPK (Table 3, Fig. 5). This implies that yields of unfertilized crops, although lower, were not more susceptible to seasonal variation in other abiotic and biotic stresses than fertilized crops. Furthermore, variation of wheat and maize yield did not show any clear trend, this may be because of beneficial effects of the high SOC stocks in soils in this region (30 - 45 Mg C ha⁻¹ average for the experimental period, Fig. 6) increasing crop resilience to biotic and abiotic stresses (Fan et al. 2013b), or an inherently lower seasonal variation in climatic conditions or pest problems in this region compared to others. In the North, North West and South, significant negative exponential relationships were found between topsoil SOC stocks and yield variation (Fig. 6). For the North and North West much of the reduction in variability (increase in stability) was associated with the supply of balanced NPK fertilizer (Fig. 2); there was relatively little further improvement with additions of animal manure and increases in SOC stocks above 18 and 25 Mg C ha⁻¹ for the North and North West, respectively. In the South, however, balanced fertilizer applications alone did not substantially reduce yield variability of either wheat or maize and, unlike the northern regions, there may be appreciable benefits for stabilizing yield of applying manure to raise the topsoil SOC stocks above 30 Mg C ha⁻¹.

5. Conclusions

This study shows that whilst there is significant potential for sequestering C in topsoil across several regions of China through straw incorporation and application of animal manure along with NPK fertilizer, there are strong regional differences in the benefits of SOC stocks on the yield and yield stability of wheat and maize. We estimate that if the same amount of manure and straw used in the experiments were applied to the soils in the wheat and maize planted areas, 22.7 and 12.9 Tg C yr⁻¹ respectively could be sequestered in the soils of the four regions studied. Moreover, there was no evidence of the soils reaching saturation. However, in the North, North East and North West, use of animal manure or straw to increase topsoil SOC stocks above current values had relatively little benefit for yield and yield stability relative to the application of NPK fertilizer. In the South, there were substantial yield responses to applications of manure over and above those obtained with NPK and hence there is potential for increasing yield, and improving yield stability, by using manure to increase topsoil SOC stocks to the SOC_{opt} of 35 Mg C ha⁻¹. This could make a significant contribution to improving agricultural sustainability in the region.

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Figure legends

Fig.1 Geographic locations of the long-term experimental sites. North East: Haerbin (HEB), Gongzhuling (GZL-A and -B), and Shenyang (SY); North West: Urumqi (UM), Zhangye (ZY), Pingliang (PL) and Yangling (YL); North: Changping (CP), Tianjin (TJ), Yucheng (YC), Zhengzhou (ZZ) and Xuzhou (XZ); South region: Suining (SN), Chongqing (CQ), Qiyang (QY) and Jinxian (JX).

Fig.2 Average yield variation (%) for wheat and maize for different treatments over the experimental period in each region. In a given region, treatments with a different letter (upper case for wheat or lowercase for maize) are significantly different at $P < 0.01$ as shown by LSD following one-way ANOVA. The error bar in each treatment is the standard deviation. CK is the unfertilized Control. N/A means that there was no NPS treatment in the North east, North and South region.

Fig.3 Annual C inputs from crop residues and manure/straw in different regions. In a given region, treatments with different lower case letter have a significantly different C input by crop biomass at $P < 0.01$ as shown by LSD following one-way ANOVA. Different uppercase letters show significant difference ($P < 0.01$) in total C input between the treatments. The error bar in each treatment is the standard deviation. Data for the NPS treatment sufficient for analysis were available only in the North West region.

Fig.4 Relationships between total cumulative C inputs and the increase in SOC stocks over the experimental period in different regions. n is the number of data points with data pooled from different fertilizer treatments and sites. Linear regression with groups indicated that slopes differed significantly between regions at $P < 0.01$.

Fig.5 Relationship between SOC stocks in the top 20 cm and relative yields of wheat (a) and maize (b) in different regions. n is the number of data points with data pooled from different fertilizer treatments, years and sites. The different symbols indicate the

different fertilizer treatments; relative yield of the NPK reference treatment was 1.0 (symbols not shown).

Fig.6 Relationship between SOC stocks in the 20 cm soil depth and yield variation of wheat and maize. Each value is the yield variation for a given fertilizer treatment at a site calculated over the experimental period and plotted against the average SOC stocks for the treatment over the same period.

Table 1. Cropping rotation and annual inorganic nitrogen (N), phosphorus (P) and potassium (K) fertilizer application rate (kg ha⁻¹) for different treatments at the 17 long-term experimental sites in China.

Region	Site	Cropping system	Experiment duration	Crop	N application rate (kg N ha ⁻¹)				P application rate (kg P ha ⁻¹)	K application rate (kg K ha ⁻¹)
					NP/NPK	NPM/NPKM	hNPKM	NPS/NPKS		
North East	HEB	DC [¶]	1980-2006	Maize/Wheat	150	150	N/A	N/A	75/75	75/75
	GZL-A	MC [‡]	1990-2009	Maize	165	50	74	112	82.5	82.5
	GZL-B	MC	1980-2010	Maize	150	150	150	N/A	75	75
	SY	MC	1979-2012	Maize	120	120	180	N/A	60	60
	CP	DC	1990-2008	Maize/Wheat	150	150	150	150	75/75	45/45
	TJ	DC	1979-2010	Maize	210	105	N/A	210	0	0
				Wheat	285	142.5	N/A	285	95	47.5
	YC	DC	1986-2010	Maize/Wheat	187.5	187.5	187.5	N/A	150/150	75/75
	ZZ	DC	1990-2010	Maize	188	50	282	188	93.8	93.8
				Wheat	165	50	74	165	82.5	82.5
	XZ	DC	1981-2012	Maize	150	150	N/A	N/A	75	112.5
				Wheat	150	150	N/A	N/A	75	112.5
North West	UM	MC	1990-1994	Maize/wheat	99	30	99	99	145.5/145.5	42/42

			1995-2012	Maize/wheat	242	85	152	217	300/300	112.5/112.5
	ZY	MC	1982-1990	Maize	240	240	N/A	N/A	120	120
				Wheat	120	120	N/A	N/A	60	60
			1991-2012	Maize	300	300	N/A	N/A	180	90
				Wheat	150	150	N/A	N/A	90	45
	PL	MC	1979-2012	Maize/wheat	90	90	N/A	90	30/30	0
	YL	DC	1990-2010	Maize	188	56	188	188	94	94
				Wheat	165	50	248	165	82.5	82.5
South	SN	DC	1981-2011	Wheat/rice	120	120	N/A	N/A	60/60	60/60
	CQ	DC	1991-2012	Wheat/rice	150	150	225	150	75/75	75/75
	QY	DC	1990-2012	Maize	210	63	95	210	84	84
				Wheat	90	27	41	90	36	36
	JX	DC	1981-2007	Maize/maize	60	60	N/A	N/A	30/30	60/60

[¶] double-cropping

[‡] mono-cropping

N/A: no data available.

Table 2. Mean annual change in crop yields (kg ha⁻¹ yr⁻¹) over time for the 17 long-term experimental sites in China (n= 20-30).

Region	Sites	Crop	Control	NP	NPK	NPM	NPKM	hNPKM	NPS	NPKS
North	HEB	Wheat	-30.8	33.4	107.5*	74.3	107.9*	78.1*	N/A	N/A
East		Maize	-63.0	185.5*	169.5*	162.6*	175.4*	44.6*	N/A	N/A
	GZL-A	Maize	-14.9	26.5	71.9	N/A	146.8**	107.4*	N/A	121.4**
	GZL-B	Maize	-37.5**	54.3*	87.3**	103.3**	109.4**	72.0*	N/A	N/A
	SY	Maize	23.7*	N/A	27.7**	N/A	38.4**	42.9**	N/A	N/A
North	CP	Wheat	-48.1**	-94.8*	106.6*	N/A	120.1*	124.2*	N/A	72.0*
		Maize	-101.6**	-30.3	75.6	N/A	92.2*	191.7**	N/A	91.1*
	TJ	Wheat	-41.1**	-49.7*	-38.5*	N/A	-60.4**	-36.7*	N/A	-133.8**
		Maize	34.4	-8.3	30.1	N/A	45.7	20.7	N/A	40.3
	YC	Wheat	-21.5	N/A	-59.8	N/A	113.1	N/A	N/A	N/A
		Maize	-218.7*	N/A	-146.6*	N/A	-103.2	N/A	N/A	N/A
	ZZ	Wheat	-26.3*	58.9*	80.3*	N/A	74.8*	53.1*	N/A	36.6
		Maize	-90.7**	121.0*	150.0*	N/A	133.7*	131.9*	N/A	107.8*
	XZ	Wheat	-51.2**	-102.4**	-81.4**	-46.5**	-35.4*	N/A	N/A	N/A
		Maize	-71.4**	-70.3**	-82.2**	-49.9**	-62.4*	N/A	N/A	N/A
North	UM	Wheat	-6.9	103.5*	125.1*	N/A	124.5*	153.5*	N/A	131.3*
West		Maize	-63.5	86.5	194.1*	N/A	116.4	188.5*	N/A	204.4*
	ZY	Wheat	-132.1**	-14.5	21.6	33.2*	66.2*	N/A	N/A	N/A

South	PL	Maize	-321.4**	-53.7	41.1	125.8*	121.4*	N/A	N/A	N/A
		Wheat	-32.1*	-53.5	N/A	-80.6	N/A	N/A	-60.4	N/A
		Maize	53.8	152.5*	N/A	187.3**	N/A	N/A	195.4**	N/A
	YL	Wheat	4.9	102.2*	125.3**	N/A	173.5**	153.8**	N/A	147.8**
		Maize	18.6	5.9	17.3	N/A	31.9	47.7	N/A	5.7
	SN	Wheat	2.9	-25.4	17.7	-35.8	25.2	N/A	N/A	N/A
	CQ	Wheat	-23.7*	-12.5	35.2	N/A	16.59	61.0	N/A	-10.0
	QY	Wheat	-10.4*	-99.1**	-86.7**	N/A	2.4	12.2	N/A	-85.4**
		Maize	-21.7**	-193.5**	-181.4**	N/A	105.0*	17.4	N/A	-197.9**
	JX	Maize	-7.7	-13.3	-16.4	N/A	-9.5	N/A	N/A	N/A
		Maize	11.4	-10.4	25.5	N/A	25.8	N/A	N/A	N/A

Values with * (P<0.05) or ** (P<0.01) indicate a significant linear regression between crop yields and the number of years since the experiment started. N/A: no data available.

Table 3. Relative yield from different treatments relative to the NPK reference treatment averaged over the experimental period in the different regions (n=4 or 5).

Crop	Treatment	Average relative yields			
		North East	North West	North	South
Wheat	Control	0.63(0.06) b	0.35(0.02) d	0.29(0.01) c	0.52(0.14) e
	NP	0.94(0.05) a	0.94(0.02) c	0.88(0.02) b	0.78(0.06) d
	NPK	1.00 a	1.00 b	1.00 a	1.00 c
	NPM	1.03(0.02) a	1.09(0.02) a	1.04(0.01) a	2.46(0.49) a
	NPKM	1.05(0.02) a	1.11(0.02) a	1.05(0.01) a	2.53(0.52) a
	hNPKM	1.06(0.03) a	1.12(0.03) a	1.07(0.02) a	2.61(0.54) a
	NPS	N/A	1.04(0.02) b	N/A	N/A
	NPKS	1.01(0.04) a	1.07(0.04) a	0.99(0.04) b	1.22(0.06) b
Maize	Control	0.50(0.02) D	0.39(0.03) C	0.43(0.01) C	0.12(0.02) C
	NP	0.94(0.01) C	1.02(0.03) B	0.91(0.02) B	0.59(0.07) C
	NPK	1.00 B	1.00 B	1.00 A	1.00 B
	NPM	1.05(0.01) A	1.10(0.02) A	1.04(0.01) A	2.51(0.26) A
	NPKM	1.06(0.01) A	1.13(0.03) A	1.04(0.01) A	2.52(0.23) A
	hNPKM	1.09(0.02) A	1.15(0.03) A	1.08(0.01) A	2.54(0.22) A
	NPS	N/A	1.02(0.03) B	N/A	N/A
	NPKS	1.01(0.02) B	1.04(0.02) B	1.08(0.03) A	1.27(0.05) B

Numbers in parentheses are standard deviation and within a column treatments followed by a different lowercase letter (wheat) and uppercase letter (maize) are significantly different between treatments at $P < 0.05$ as shown by LSD following one-way ANOVA. N/A: no data available.

Table 4. SOC storage in the top 20 cm soil depth under different treatments in different regions at beginning and end of experimental period (n=4 or 5).

Treatment	Period	Average SOC storage (Mg C ha ⁻¹)			
		North East	North West	North	South
Control	beginning	31.9(1.0) c	19.8(0.5) d	19.3(0.8) d	20.6(0.5) c
	end	29.1(1.3) c	17.9(0.3) e	17.2(1.2) e	18.1(0.6) c
NP	beginning	31.9(1.0) c	19.8(0.5) d	19.3(0.8) d	20.6(0.5) d
	end	32.8(0.9) c	22.4(0.3) c	22.6(0.7) c	22.1(0.5) c
NPK	beginning	31.9(1.0) c	19.8(0.5) d	19.3(0.8) d	20.6(0.5) c
	end	33.6(0.9) c	23.5 (0.3) c	23.8(0.6) c	24.8(0.5) b
NPM	beginning	31.9(1.0) c	19.8(0.5) d	19.3(0.8) d	20.6(0.5) c
	end	42.9(0.9) a	37.5(0.6) a	32.1(0.5) a	33.6(1.3) a
NPKM	beginning	31.9(1.0) c	19.8(0.5) d	19.3(0.8) d	20.6(0.5) c
	end	44.5(1.0) a	38.1(0.6) a	33.8(0.5) a	34.2(1.1) a
hNPKM	beginning	31.9(1.0) c	19.8(0.5) d	19.3(0.8) d	20.6(0.5) c
	end	45.1(1.2) a	39.8(0.7) a	34.2(0.6) a	34.7(1.4) a
NPS	beginning	N/A	19.8(0.5) d	N/A	N/A
	end	N/A	27.2(0.5) b	N/A	N/A
NPKS	beginning	31.9(1.0) c	19.8(0.5) d	19.3(0.8) d	20.6(0.5) c
	end	35.2(1.0) b	27.5(0.6) b	28.6(1.4) b	25.1(0.9) b

Numbers in parentheses are standard deviation and within a column treatments followed by a different lowercase letter (wheat) and uppercase letter (maize) are significantly different at $P < 0.05$ as shown by LSD following one-way ANOVA.

Table 5. Mean rate of change ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$) of the SOC stocks over time at the 17 long-term experimental sites in China (n= 20-30).

Region	Sites	Control	NP	NPK	NPM	NPKM	hNPKM	NPS	NPKS
North East	HEB	-0.13*	-0.10	-0.04	-0.03	-0.08	0.11	N/A	N/A
	GZL-A	-0.09	0.09	0.21	N/A	0.90**	0.83**	N/A	0.18*
	GZL-B	-0.08	-0.14	-0.04	0.32**	0.41**	0.68**	N/A	N/A
	SY	-0.18**	N/A	-0.015	N/A	0.23**	0.30**	N/A	N/A
North	CP	0.29*	0.36**	0.22	N/A	0.48**	0.72**	N/A	0.42**
	TJ	0.15	0.27**	0.26**	N/A	0.61**	0.94**	N/A	0.25**
	YC	0.29**	N/A	0.41**	N/A	1.08**	N/A	N/A	N/A
	ZZ	-0.12*	0.15*	0.12*	N/A	0.42**	0.70**	N/A	0.25*
	XZ	0.002	0.12**	0.08**	0.39**	0.35**	N/A	N/A	N/A
North West	UM	-0.18**	-0.04	-0.15*	N/A	0.75**	1.38**	N/A	0.02
	ZY	-0.29*	-0.08	-0.16	0.24**	0.18*	N/A	N/A	N/A
	PL	0.04	0.14*	N/A	0.50**	N/A	N/A	0.34**	N/A
	YL	0.18**	0.42**	0.46**	N/A	1.09**	1.29**	N/A	0.62**
South	SN	0.01	-0.06	0.02	0.01	0.00	N/A	N/A	N/A
	CQ	-0.23	0.09	0.05	N/A	0.43*	0.65**	N/A	0.37*
	QY	-0.06	0.12	0.17	N/A	0.70**	0.78**	N/A	0.22**
	JX	-0.08	-0.05	-0.08	N/A	0.20	N/A	N/A	N/A

Values with * ($P < 0.05$) or ** ($P < 0.01$) indicate a significant linear relationship between SOC storage and the number of years since the experiment started. N/A: no data available.

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6